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Phone: +90 362 408 25 15

Fax: +90 362 408 25 15

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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF Origanum acutidens ESSENTIAL OIL AGAINST *Sclerotinia sclerotiorum* (Lib.) DE BARY AND *Phytophthora infestans* (Mont.) DE BARY

Yusuf BAYAR[1]*, Zeynep AKŞİT[2], Samed ŞİMŞEK[3], Ali KANDEMİR[4]

[1]*Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, 40100, Kirsehir, Türkiye*
[2]*Erzincan Binali Yıldırım University, Tourism and Hospitality Vocational School, Department of Hotel, Restaurant and Service, 25240 Erzincan, Türkiye*
[3]*Erzincan Binali Yıldırım University, Çayırlı Vocational School, Department of Medical Services and Techniques, 24500, Erzincan, Türkiye*
[4]*Erzincan Binali Yıldırım University, Science and Art Faculty, Department of Biology, 24002, Erzincan, Türkiye*

Abstract: The aim of this study was to investigate the chemical components and evaluate the antifungal activity of *Origanum acutidens* essential oil. The aerial parts of *O. acutidens* were collected, and the hydrodistillation method was used to extract the essential oil. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the chemical composition of the essential oil. The main components identified were α-terpineol (4.76%), *p*-cymene (7.6%), linalool (14.82%), and carvacrol (49.4%). The essential oils were tested against two pathogens *in vitro* experiments to evaluate their antifungal activity. Different concentrations of the essential oil were applied, and the inhibition of mycelial growth was measured. The results demonstrated that the essential oil exhibited antifungal properties against both pathogens. At a dose of 4.8 µL/Petri dish, the mycelial growth of both pathogens was completely inhibited. However, *Sclerotinia sclerotiorum* showed higher tolerance to the essential oil compared to *Phytophthora infestans.* Furthermore, a dose-effect study was conducted as a part of this research. The LC_{50} values (lethal concentration at which 50% of the pathogens' growth is inhibited) for *P. infestans* and *S. sclerotiorum* were calculated as 0.982 µL/Petri and 1.61 µL/Petri, respectively. The study concluded that the essential oil of *O. acutidens* has the potential to be a natural antifungal agent, particularly against *S. sclerotiorum* and *P. infestans*. However, further research is needed to investigate the mechanisms of action and explore potential applications of this essential oil in managing plant diseases.

Keywords: Origanum acutidens, Essential oil, Antifungal activity, Plant pathogens

***Corresponding author:** Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Kirsehir, 40100, Türkiye
E mail: yusuf.bayar@ahievran.edu.tr (Y. BAYAR)
Yusuf BAYAR https://orcid.org/0000-0001-8393-7218
Zeynep AKŞİT https://orcid.org/0000-0002-0349-0223
Samed ŞİMŞEK https://orcid.org/0000-0001-8451-3425
Ali KANDEMİR https://orcid.org/0000-0001-6217-4282

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1. Introduction

In many agricultural areas around the world, there are significant disease factors that lead to the loss of agricultural products such as fruits and vegetables (Yavuz et al., 2022). Two of these diseases are *Sclerotinia sclerotiorum* (Lib.) de Bary and *Phytophthora infestans* (Mont.) de Bary. *Sclerotinia sclerotiorum* is a necrotrophic phytopathogenic fungus that has been the subject of numerous scientific studies (Otun and Ntushelo, 2020). This pathogen has a wide range of hosts, infecting over 400 species and causing damage to various agricultural products. Due to the durable structures known as sclerotia, *Sclerotinia* species can survive in the soil and their hosts for up to 8 years (Kurt et al., 2011). On the other hand, *Phytophthora infestans* is an oomycete, a fungus-like microorganism that causes late blight, and it is particularly detrimental to potato and tomato plants. It has had significant historical consequences, including the Irish Potato Famine of the 1840s (Blancard, 2012). This pathogen is responsible for numerous epidemics in potatoes and tomatoes, particularly in cool and rainy weather conditions.

Chemical control, specifically the use of synthetic pesticides, is considered the most effective method for combating plant diseases worldwide. However, the extensive use of synthetic pesticides has raised concerns regarding durability issues, environmental problems, and the toxicity and persistence of pesticide residues (Isman, 2000). Consequently, there has been an increased focus on finding alternative methods that are less harmful to human health and the environment, as alternatives to synthetic pesticides.

One such alternative approach involves exploring the effectiveness of naturally occurring compounds found in plants for controlling diseases, pests, and weeds. Vegetable-derived essential oils are among these natural compounds, synthesized as secondary metabolites by aromatic plants (El Ayeb-Zakhama et al., 2017). These essential oils have gained importance as potential alternatives to synthetic pesticides due to their diverse biological effects (Baser and Buchbauer, 2009). Several studies have reported the natural fungicidal properties of essential oils (Bayar, 2018; Affes et al., 2022; Tomić et al., 2023).

The objective of this study was to analyze the chemical components of *O. acutidens* essential oil and evaluate its antifungal activity against the important plant pathogens *S. sclerotiorum* and *P. infestans*.

2. Materials and Methods

2.1. Sample Collection

The aerial parts of *O. acutidens* (1.1 kg) were collected at 32nd kilometers of Gümüşhane/Kelkit road, Türkiye. Systemically identification of the plant samples was carried out by Prof. Dr. Ali Kandemir, at the Biology Department, University of Erzincan Binali Yıldırım. The voucher specimens were deposited under the EBYU 1378 number.

2.2. Essential Oil Extraction

A total of 350 grams of dried aerial parts from *O. acutidens* were blended and subjected to hydrodistillation for 3 hours using a Clevenger apparatus. The extraction process was performed in triplicate. Subsequently, the resulting oils were carefully collected and stored in sealed sample tubes, which were then kept at 4 °C until the analyses were conducted.

2.3. GC-MS Analysis Conditions

GC-MS analyses were conducted utilizing a Thermo Scientific Trace 1310 GC-MS system, which was outfitted with an HP-5MS capillary column (30 m x 0.25 mm and 0.25 µm ID) according to previously published methods (Aksit et al., 2022; Alkan et al., 2021). A carrier gas, helium, was employed at a constant flow rate of 1.2 mL/min in split mode with a ratio of 50:1. The injection site and mass transfer line temperature were both set to 280 °C. The column oven temperature a programmed as initially held at 60 °C for 3 minutes, then increased to 200 °C at a rate of 3 °C/min and held for 0 minutes, and finally ramped up to 240 °C at a rate of 5 °C/min and held for 5 minutes. The mass spectrometer parameters were set as follows: the ion source temperature was maintained at 280 °C, and electron ionization (EI) mode with an ionization energy of 70 eV was employed. Retention indexes (RI) for all components were determined by calculating the retention times using the Van den Dool and Kratz equation, based on a homolog *n*-alkane series (C8-C40). To confirm compound identities, Wiley and NIST2004 MS libraries were utilized. The relative peak area percentages of each compound were calculated based on the peak areas obtained from MS chromatograms.

2.4. Obtaining fungal cultures

In this study, the fungi *P. infestans* and *S. sclerotiorum* were obtained from stock cultures maintained in the Phytoclinical laboratories of Ahi Evran University, Faculty of Agriculture, and Department of Plant Protection. The experiments involved using young fungal cultures derived from these stocks, which were grown on 90 mm petri dishes containing 20 ml of potato dextrose agar (PDA) at a temperature of 25±2 °C for 7 days. The aim was to investigate the in vitro fumigant effect of *O. acutidens* essential oil. To set up the experiment, the prepared PDA was sterilized and cooled to 40 °C before being transferred to 60 mm diameter petri dishes with a depth of 10 mm. Sterile filter paper with a diameter of 5 mm was attached to the lids of the petri dishes containing PDA. Mycelium obtained from 7-day-old fungal cultures was transferred onto the PDA plates. Different concentrations of *O. acutidens* essential oil (0 µl/petri dish for control, and 0.6, 1.2, 2.4, 4.8, and 9.6 µl/petri dish) were applied to the filter papers on the lids of the petri dishes using a micropipette. The petri dishes were then sealed, and the fungal cultures were incubated at a temperature of 25±2 °C for 7 days. At the end of the incubation period, the fungal growth was measured, and the degree of growth inhibition was calculated using the following formula: % Inhibition = [(C − T)/C]× 100, where C represents the mean radial mycelial growth of the pathogen in the control samples, and T represents the mean radial mycelial growth of the treated samples. The experiments were performed with 4 replications and 2 repetitions to ensure statistical reliability.

2.5. Statistical analysis

The significance of differences between treatments in trials was determined through analysis of variance (ANOVA), and means were compared using the Duncan test (Genç and Soysal, 2018). Statistical analyses were conducted using the SPSS 15 computer program.

3. Results and Discussion

3.1. Chemical Composition of Essential Oil of *O. acutidens*

The hydrodistillation process applied to the aerial parts of *O. acutidens* resulted in an essential oil yield of 0.65±0.2% (w/w). This yield was found to be lower compared to the samples collected from Bayburt (Baser et al., 1997) and Sivas (Sökmen et al., 2004), higher compared to Tunceli (Gulec et al., 2014) and Ankara (Cosge et al., 2009) while it was comparable to the samples collected from Erzurum (Kordali et al., 2008) depending on the climate and environmental conditions of collection sites.

The chemical composition of *O. acutidens* is given in Table 1 showing the percentage of each component, retention time (RT), and retention indices (RI). Table 1 shows 27 compounds were identified representing 97.9 % of the essential oil. Oxygenated monoterpenoids (86.64%) were the major class of the oil while

hydrocarbon monoterpenoids constituted 8.1% of the essential oil. *p*-cymene (7.6%), linalool (14.82%), α-terpineol (4.76%), linalyl acetate (%4.16), and carvacrol (49.4%) were the principal components of the essential oil. Previous studies have demonstrated that the carvacrol content in various regions of Türkiye ranged from 61.8% to 87.0% (Baser et al., 1997; Cosge et al., 2009; Gulec et al., 2014; Kordali et al., 2008; Sökmen et al., 2004). However, the current study revealed that the carvacrol content of *O. acutidens* was found as 49.4%. Additionally, this study is the first to report the presence of the carvacrol/*p*-cymene/linalool chemotype in *O. acutidens*.

3.2. Antifungal Activity of Essential Oil of *O. acutidens*

The study investigated the fumigant activity of essential oils obtained from the *O. acutidens* plant against two plant pathogens, *P. infestans* and *S. sclerotiorum.* The results are presented in Table 2-3, which includes the IC_{50} values and the effect of different doses of the essential oil on the mycelial growth of the pathogens. Table 2 shows the inhibitory effect of the essential oil on the mycelial growth of *P. infestans* and *S. sclerotiorum* at various doses. The lowest dose tested, 0.6 µL/Petri dish, did not show any inhibition compared to the control group for both pathogens. However, at a higher dose of 4.8 µL/Petri dish, the essential oil completely inhibited the mycelial growth of both tested fungi. Based on these results, it can be concluded that the essential oil of *O. acutidens* exhibits antifungal properties against *P. infestans* and *S. sclerotiorum*. However, the tolerance of the two pathogens to the essential oil differs. *S. sclerotiorum* showed greater tolerance to the essential oil compared to *P. infestans*, as it required a higher dose (4.8 µL/Petri dish) to completely inhibit its mycelial growth. On the other hand, *P. infestans*, being the more susceptible pathogen, was completely inhibited at a lower dose (4.8 µL/Petri dish) of the essential oil. These findings suggest that the essential oil from the *O. acutidens* plant has potential as a natural antifungal agent, particularly against *P. infestans*, which is known for causing devastating late blight disease in plants. Further studies may be warranted to explore the specific mechanisms of action and the potential application of this essential oil in plant disease management.

Table 1. Chemical composition of *Origanum acutidens* essential oil

RT	RI	Compounds	% Composition
3,68	846	Diketone alcohol	0.19
6,93	952	1-Octen-3-ol	0.53
7,1	969	3-octanone	0.43
7,22	982	α-Myrcene	0.28
8,18	1008	*p*-cymene	7.6
8,3	1019	Limonene	0.14
8,85	1034	*trans*-*β*-ocimene	0.08
9,57	1060	*cis*-linalool oxide	1.98
10,39	1080	Linalool	14.82
11,52	1152	Pinocarveol	0.17
11,68	1125	Verbenol	0.12
12,31	1150	Borneol	4.2
12,62	1165	4-terpineol	1.78
13,02	1172	α-Terpineol	4.76
13,17	1219	Dihydrocarveol	0.55
14,04	1229	Nerol	0.64
14,76	1237	Linalyl acetate	4.16
15,77	1259	Thymol	0.51
16,23	1278	Carvacrol	49.4
17,67	1342	Neryl acetate	1.2
18,17	1358	Geranyl acetate	1.97
19,17	1424	Caryophyllene	0.39
19,66	1441	Aromadendrene	0.41
21,7	1488	Cadinene	0.16
23,03	1572	Spathulenol	0.17
23,14	1580	Caryophyllene oxide	1.23
		Monoterpenes	8.10
		Oxygenated monoterpenes	86.64
		Sesquiterpenes	0.80
		Oxygenated sesquiterpenes	1.32
		Total	97.87

Compounds were listed in order of elution on the HP-5MS column, RT= retention time, RI= retention Indices, Bolded name = Chemotype.

Table 2. Antifungal effects (%) of the *O. acutidens* essential oil

Doses (µL/ Petri dish)	*Phytophthora infestans*	*Sclerotinia sclerotiorum*
Negative Control (0)	0.0±0.00c*	0.0±0.00d
0.6	0.0±0.00c	0.0±0.00d
1.2	53.21±15.60b	8.57±3.15c
2.4	100.00±0.00a	72.08±14.91b
4.8	100.00±0.00a	100.00±0.00a
9.6	100.00±0.00a	100.00±0.00a

*Means in the same column with the same letter were not significantly different by ANOVA (P= 0.05)

Table 3. Lethal concentration values (µL/Petri) of *O. acutidens* essential oil against test microorganisms

Plant	LC Values	Test microorganisms	
		P. infestans	*S. sclerotiorum*
O. acutidens	LC_{50} (µL/ Petri dish)	0.982 µL/ Petri dish	1.61 µL/ Petri dish
	Slope	11.55±4.02	6.77±0.65
	Chi-square	0.06	0.576

LC= effective dose (Lethal concentration).

It appears that the essential oil of *O. acutidens* exhibited higher toxicity towards the plant pathogen *P. infestans* compared to *S. sclerotiorum*. The IC_{50} value, which represents the concentration required to inhibit 50% of the growth of *P. infestans*, was determined to be 0.982 µL/Petri. On the other hand, the LC_{50} value, representing the concentration lethal to 50% of the test organisms, was calculated as 1.61 µL/Petri for *S. sclerotiorum*.

Therefore, based on these values, it can be concluded that the essential oil of *O. acutidens* showed a higher level of toxicity against *P. infestans* compared to *S. sclerotiorum*, indicating its potential as a more effective treatment option against *P. infestans*.

A previous report was reported that the essential oil of *O. acutidens* exhibited antifungal activity against several fungal species, including *Absidia repens, Aspergillus ochraceus*, *Penicillium jensenii, Aspergillus niger, Scopulariopsis chartarum*, and *Cladosporium herbarum* (Çetin et al., 2011). Another study by Kordali et al. (2008) found that *O. acutidens* essential oil demonstrated strong antifungal activity against 17 plant pathogenic fungi. Furthermore, this study also examined the antifungal effects of carvacrol and p-cymene, which are the main components of *O. acutidens* essential oil, on the same fungi. Carvacrol completely inhibited the mycelial growth of all tested fungi, while *p*-cymene exhibited weak antifungal activity against *Fusarium acuminatum* and *Pythium ultimum*, but significantly increased the mycelial growth of *F. culmorum*, *F. equiseti*, *F. nivale, F. oxysporum*, and *Sclerotinia minor*.

In another study by Sökmen et al. (2004), it was found that the essential oil of *O. acutidens* demonstrated remarkable antifungal activity and inhibited the growth of 12 out of 18 tested fungi. Overall, previous studies were suggested that essential oils rich in carvacrol and thymol exhibit the highest antifungal activity against various phytopathogenic fungi. Consequently, the potent antifungal activity of *O. acutidens* essential oil is attributed to its key components.

4. Conclusion

This study primarily focused on investigating the chemical components of the essential oil extracted from *O. acutidens*, as well as its antifungal properties against pathogenic fungi. The emergence of naturally derived pesticides has provided a viable solution to counter the negative impacts associated with synthetic agents, such as the persistence of residues, the development of resistance, and the induction of environmental pollution. Consequently, the utilization of natural pesticides and herbicides offers numerous advantages, including effectiveness, selectivity, biodegradability, and reduced toxicity to towards the environment. Given the well-documented harmful effects of fungicides on both the environment and human well-being, research into the biological activities of essential oils holds great significance.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	Y.B.	Z.A.	S.Ş.	A.K.
C	50	50		
D	25	25	25	25
S		100		
DCP	50	50		
DAI		50	25	25
L	25	25	25	25
W	60	20	10	10
CR		20	20	60
SR	100			
PM	60	40		

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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DETERMINATION OF STRUCTURAL CHARACTERISTICS OF LARGE-SCALE DAIRY FARMS: EXAMPLE OF YOZGAT PROVINCE

Orhan ERMETİN[1]*, Müge ERKAN CAN[2]

[1]*Yozgat Bozok University, Faculty of Agriculture, Department of Animal Science, 66100, Yozgat, Türkiye*
[2]*Çukurova University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, 01330, Adana, Türkiye*

Abstract: This research was carried out to determine the status of dairy cattle farms in Yozgat province, which has significant potential in animal husbandry, to evaluate the positive and negative aspects of the existing barns, and to put forward suggestions for solving the problems. The research was completed by conducting observation and physical measurement studies in 28 dairy cattle farms with more than 50 milking animals, which we classified as large-scale, with the data obtained through questionnaires. Most dairy farms were established in the last ten years and received investment and grant support from various public institutions. It has been observed that the criteria suitable for animal welfare, especially in ventilation and building materials, are not followed by avoiding investment costs in farms established with equity capital. In the dairy farms established by receiving grants and investment support, it was observed that the stables were built in conditions suitable for animal welfare. Still, the herd management system was not established, and the records were not kept healthy because the farm owners and employees of the farms were insufficient in dairy cattle breeding knowledge, which would increase the costs. Although the majority of the examined enterprises did not have structural and technical problems, the absence of a birth partition in 21.43%, an individual calf pen in 25%, a ventilation shaft in 3.57%, and a manure pit slope in 28.57% was seen as an obstacle for these dairy farms to be modern enterprises. It is recommended that the economic sustainability of dairy cattle farms, which is a long-term investment branch, does not only depend on having sufficient equipment in terms of structural features but also on farm owners and employees should have sufficient knowledge and infrastructure on dairy cattle breeding and training should be provided to the relevant people.

Keywords: Large-scale dairy farms, Barn, Dairy cattle, Yozgat

***Corresponding author:** Yozgat Bozok University, Faculty of Agriculture, Department of Animal Science, 66100, Yozgat, Türkiye
E mail: orhan.ermetin@yobu.edu.tr (O. ERMETİN)
Orhan ERMETİN https://orcid.org/0000-0002-3404-0452
Müge ERKAN CAN https://orcid.org/0000-0002-0744-1496

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1. Introduction

The way to create a livestock sector that can compete with the world in milk and meat production is through intensive livestock breeding. According to the farm's milk or meat production purpose, it is necessary to raise animals of qualified breeds with quality feeds in a modern way under hygienic care feeding conditions. In intensive animal breeding, which is done by providing the environmental conditions needed by animals with good genetic characteristics and feeding them with quality feed sources to obtain higher yields, the animals' shelters are planned as tie-stall, loose, or free-stall barns (Kara and Eroğlu, 2015). Türkiye needs to make structural changes in modern animal husbandry to compete with the world, especially the EU. One of the first problems to be solved in this area is the size of the barn. The scale of cattle farms in Türkiye is relatively small compared to other countries. While it is 32.20 heads per farm in the EU, this average is around 4.50 in Türkiye (Kara and Eroğlu, 2018). According to TUIK data for 2021, our country's total number of cattle is 18,036,117 heads, of which 17,850,543 are cattle. 49.44% of our bovine stock is culture breed, 42.81% is crossbreed, and 7.76% is domestic breed. With 20,782,374 tons of milk obtained from 6,580,753 cows milked, approximately 91% of the total milk production is cow's milk, and the average milk yield is 3,158 kg (TUIK, 2021).

Environmental conditions within a shelter play a crucial role in ensuring the well-being of animals. Factors such as ventilation, lighting, temperature, and relative humidity must be carefully considered when constructing shelters, as these factors collectively form what is known as the shelter climate. It is essential to consider the natural behavioral characteristics of animals, including their movements, rest patterns, rumination, and feeding and drinking habits, while also adhering to the principle of achieving maximum benefits at minimum costs during shelter construction. Furthermore, the construction of shelters should incorporate modern features that prioritize animal welfare, consider the dimensions required for the animals, account for their behavior, and adhere to biosecurity guidelines (Uğur, 2014; Mundan et al., 2018).

Production planning in dairy cattle farms; It is the effective use of optimum inputs and operating capacity while determining which production level the farm will achieve optimum profit according to opportunities and targets in a certain period. For successful animal production, dairy farms can focus on increasing milk per animal unit rather than increasing the number of animals and milk. In general, a single herd size will not be economical under all conditions everywhere. According to Göncü and Görgülü (2011), considering intensive operating conditions and cost factors, at least 80 milking capacity should be the starting point, and 176 head milking capacity should be taken into account as the average economic herd size.

2. Materials and Methods

The study examined shelters with over 50 cattle in Yozgat province and the data related to these shelters as the primary material. As a result of the field studies carried out in the study area and the evaluation of the data received from public institutions, the study was carried out in 28 dairy cattle farms that were selected by random sampling method from 43 dairy cattle farms with 50 heads and more dairy cattle and allowed surveys and examinations. Information on the locations of the farms and the number of animals are given in Table 1, and the distribution of the number of animals by age and breed is given in Table 2. 28 farms with and without accessible mattresses; evaluated in terms of farms structure and animal welfare and examined as a modern (qualified) dairy farms. With the data obtained from the dairy cattle farms through face-to-face interviews and surveys between September 01 and December 20, 2022, the collected dairy farms' information was processed by making observations and physical measurements in the barns. The structural and technical characteristics of dairy farms discussed in the study were evaluated. In the study, the numerical values (N) and percentage (%) frequencies of the data obtained from the dairy cattle farms included in the research were presented by calculating.

Table 1. Locations of farms and number of dairy cattle

District	Village	Number of dairy cattle	District	Village	Number of dairy cattle
Akdağmadeni	Oluközü	106	Sarıkaya	Karahallı	120
Boğazlayan	Yenipazar	215		Tepe Dogan	133
Çandır	Büyükkışla	133		Karayakup	165
	Büyükkışla	50		Arpalık	100
Kadışehri	Merkez	100		Yukarı Sarıkaya	78
Merkez	Araplı Çiftliği	80	Şefaatli	Kuzayca	255
	Yazıpınar	84		Kuzayca	80
	Köseyusuflu	50		Kuzayca	60
	Bebek	130	Yenifakılı	Merkez	61
	Kızıltepe	120		Merkez	91
	Kızıltepe	100		Eskioren	115
	Türkmen	80		Fehimli	54
Saraykent	Altınsu	140	Yerköy	Merkez	200
	Ozankasabası	50		Sarıyaprak	185

Table 2. Distribution of cattle by ages and breeds

Current status	Number (Head)	%	Breeds	Number (Head)	%
Milked Cows	1006	32	Holstein	855	27
Dry	554	18	Brown Swiss	290	9
Pregnant Heifer	443	14	Simmental	1914	62
Heifer	368	12	Domestic	30	1
Calf	764	24	Crossbreed	46	1
Total	3135	100	Total	3135	100

3. Results and Discussion

The operating ages of the modern dairy cattle farms studied in the research area vary between 1 and 25 years as of their establishment years widely. 15 farms between 1-5 years (53.57%), 6 farms between 6-10 years (21.43%), 3 farms between 11-15 years (10.71%), 2 farms between 16-20 years (7.14%), and 2 breeders have been operating for 21-25 years (7.14%) (Figure 1). The fact that most dairy farms were established in the last 10 years shows that they benefit from farm investments, grants, and support projects from public resources.

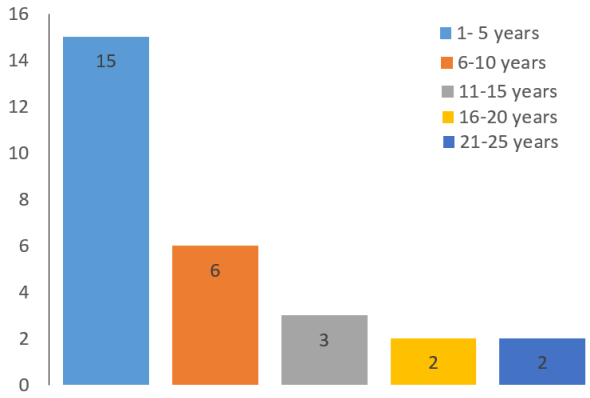

Figure 1. Age of dairy farms by year of establishment.

Of the modern barns examined within the scope of the study, 92.85% (26 farms) are free tie-stall, and 7.15% (2 farms) are tie-stall systems. While the ratio of dairy cattle farms with a free stall system was 89.29% (25 farms), the percentage of dairy cattle barns with tied stall system was 10.71% (3 farms). The common type of tie-stall barn is preferred due to climatic conditions. Mundan et al. (2018) in their study in Şanlıurfa, it was determined that 17.5% of the barns were tie-stall, 82.5% of them were tie-stall type, and free-stall barn system was used in all of them. Their study in Özsağlıcak and Yanar (2022) found that 95.0% of their research had tie-stall barns, 4.8% had free tie-stall barns, and 0.3% had loose barns. 50.6% stated that stone (22.7%), adobe (12.6%), brick (11.6%), pumice (2.2%), and wood (0.3%) were used. Approximately 90% of the modern dairy farms examined within the scope of various studies are free stalls, and it has been reported that animals move freely in such barns and use their rest periods more effectively, and accordingly, the disease levels are lower than the others (Rushen and Pasille, 1999; Öcal, 2020).

In 89.29% (25 farms) of the surveyed dairy cattle barns, there is bedding material; in 3 barns (10.71%), there is insufficient or no substrate material. The employees have stated that the animals are cleaner in the establishments where bedding is used at the stalls, and the disease rates are lower than in the ones that do not use bedding in those establishments. While the base of the shelter is concrete in 27 farms (96.43%), the ground is soil in 2 farms (3.57%). It has been observed that the type of project boards are complied with in the farms built with the support received in public institutions. In contrast, quality materials are not chosen in farms constructed with their resources, especially in ventilation and building materials, avoiding investment costs. Information on the shelter floor, barn width dimensions, wall material, wall thickness, and barn short-side directions are given in Table 3.

While the walls were plastered and clean in 96.43% of the farms (27), it was observed that there was no plaster on the walls in 1 barn (3.57%). While 22 barns (78.57%) have sufficient electricity systems and night lighting is available, it is noted that electricity and energy systems are insufficient, and there is no night lighting in 6 barns (21.43%). This situation can be associated with the distances of the farms to the city networks and their access to the energy lines in terms of their location.

Table 3. Ground, wall, and axis characteristics of shelters

Bedding material	n	%
Rubber	24	85.71
Partially Rubber	1	3.57
Soil + Concrete	2	7.14
Concrete	1	3.57
Wall Material		
Stone	2	7.14
Briquette	7	25.00
Brick	3	10.71
Curtain Concrete	16	57.14
Wall Thickness		
10 cm	2	7.14
20 cm	13	46.43
25 cm	9	32.14
30 cm	3	10.71
50 cm	1	3.57
Barn Width Dimensions		
7m* 20 m-24 m	3	10.71
8m* 38 m-40 m	2	7.14
10 m * 50 m	5	17.86
15 m* 50 m-65 m-66 m	3	10.71
16 m* 67 m-70 m-78 m	4	14.29
25 m* 78 m-82 m-100 m	6	21.43
28 m* 100 m	2	7.14
30 m * 120 m	1	3.57
40 m * 130m	1	3.57
50m * 200 m	1	3.57
Short Side (Shelter Entrance Direction)		
East	7	25.00
South	8	28.57
South West	1	3.57
West	5	17.86
East West	3	10.71
South North	1	3.57
North	3	10.71

When the ventilation conditions were examined, it was seen that there was a ventilation shaft, and the ventilation was sufficient in 25 barns (89.29%), while in 3 barns (10.71%), the required amount of ventilation shafts was not found or the capacity was insufficient. The construction of the roof and the choice of roof materials are also factors that directly affect the ventilation and the environmental conditions inside the shelter. With improperly selected material and faulty roof design, the environmental conditions, especially the temperature and humidity values, will change and become unsuitable for the housed animal. In most barns in Türkiye, the barn wall height is kept short, and the principle of avoiding construction and heating costs has been adopted, but this has brought ventilation problems. Increasing window

areas and effective ventilation are important for improving efficiency, health, and well-being (Öcal, 2020). Other data regarding the chimney and window conditions that determine the ventilation conditions of the farms are given in Table 4.

Table 4. Details on ventilation conditions in shelters

Number of Chimneys	n	%
Along the length	20	71.43
2 pieces	2	7.14
4 pieces	2	7.14
8 pieces	2	7.14
10 pieces	1	3.57
No chimney	1	3.57
Chimney Height		
3 m	2	7.14
4-6 m	10	35.71
6.5-7.5 m	7	25.00
8-10 m	8	28.57
No chimney	1	3.57
Number of windows		
2-10	7	25.00
12-24	6	21.43
30-48	5	17.86
52-80	3	10.71
Along the length	7	25.00
Window opening Direction		
From top to bottom	23	82.14
To the right or left	5	17.86
Barn Height		
3-5.5 m	8	28.58
6-7.5 m	11	39.28
8-10 m	9	32.14
Side Wall Height		
2-4 m	17	60.71
4.5-6 m	7	25
7-14 m	4	14.29
Roofing Materials		
Sheet (Galvanized, isogloss, corrugated, stone, tile top)	13	46.43
Panel material	8	28.57
Roof tile	2	7.14
Wood-tile	2	7.14
Polycarbonate material	1	3.57
Steel material	1	3.57
Styrofoam material	1	3.57

The height of the barn is 6-7.5 m in 39% of the farms, the height of the side wall is 2-4 m in about 61%, 53% of the age of establishment is within 1-5 years, and most of them are plastered, concrete-based, side walls. It has been observed that the walls are made of curtain concrete from one end to the other as a ventilation shaft. The height of the windows from the ground in the barns is between 1.60 m and 3.00 m. Ensuring sufficient window space and chimney space is important for optimum ventilation. It is possible to say that the modern dairy farms we examined are better equipped in terms of structural features compared to traditional family farms. Concrete floors in shelters are the most preferred floor type due to their easy cleaning and longevity, but increasing complaints of animal foot problems can be counted as one of the adverse effects of this flooring type (Manninen et al., 2002). The use of grilled floors in shelters has decreased due to problems with estrus detection, increased foot diseases, and adverse effects on animal welfare. It has been suggested that the ground be designed as a rough structure with different geometries (Haley et al. 2000). It has been recommended that the materials to be used for this design should be used with 12 mm depth, 12-19 mm width, and 9-10 cm spacing. It has been reported that the quality of the ground concrete is also essential (Ondarza, 2003). Foot and udder problems in dairy cattle are among the most important health problems. Failure to prevent these problems causes lameness, yield reductions, and economic losses in the long run (Vermunt and Greenough, 1996).

Animal drinking water and its shape are also among shelters' main planning criteria for breeding and hygiene. While the animal's drinking water frequency is related to its health and productivity characteristics, the drinking conditions are of great importance in terms of comfort and hygiene. In 92.86% of the surveyed holdings (26 farms), animals can freely access water without restrictions or routines. In the remaining 2 farms (7.14%), the animals' drinking water frequency was determined to be 2 times daily.

Among the issues related to planning, yield, and hygiene conditions in shelters are the status and adequacy of special compartments for animals reared in the shelter. The presence of sections suitable for the living conditions of the animals in the shelters, a separate infirmary, and a walking area where the animals can roam freely will also positively affect productivity, animal welfare, and hygiene conditions. These data regarding the shelters in the study area are reported in Table 5.

Among the dairy cattle farms studied in the study area are calf huts, infirmaries, maternity chambers, and walking areas in all farms built with the project. The study observed that the calf compartment was used at the rate of 75%, and accordingly, the areas allocated to the calves were sufficient for group compartments.

Delebe (2022), in his study in Şanlıurfa province, stated that the rate of those who keep each calf in a separate compartment is 4.96%, and the rate of those who keep them freely as a group in a particular place is 95.03%. In the study conducted by Özsağlıcak and Yanar (2022), in the province of Erzincan, 60.3% of the holdings had calves in a separate group section in the same barn, 11.7% in the same barn with the mother, 15.9% in the same barn in individual calf sections and 12% in the same barn. They reported that they were reared in individual calf huts in separate buildings. Kaygısız et al. (2022), in their study in the Andırın district of Kahramanmaraş province, it was observed that in 82% of

the farms, the calves were housed in the same barn with the adult cattle, in 18% of the calves were housed in separate compartments from the adult cattle. Straw and sawdust were used as litter material in the calf compartments in all of the farms. Öcal (2020), in his study on dairy cattle farms in Ankara, reported that the ratio of the birth chamber is 100%, and the infirmary rate is 80% in the dairy cattle farms he examined.

Table 5. Conditions of individual compartments, promenades, and infirmaries in shelters

Birth Partition	n	%
Exist	22	78.57
None	6	21.43
Number of Birth Division		
None	6	21.43
1	18	64.29
2	4	14.29
Birth Partition Area		
None	6	21.43
12-20 m^2	10	35.71
21-40 m^2	9	32.14
41-50 m^2	3	10.71
Individual Calf Pen		
Exist	21	75.00
None	7	25.00
Number of Individual Calf Pens		
None	7	25.00
15-20	11	39.29
25-30	8	28.57
35-40	2	7.14
Cow Alley	N	%
0-2 m^2	4	14.29
160 m^2	1	3.57
350-360 m^2	2	7.14
500-800 m^2	8	28.57
840-1200 m^2	7	25.00
1300-2000 m^2	6	21.43
Sickroom		
Exist	21	75.00
None	7	25.00

Feeder and stall planning is also among the important design criteria in shelters. These criteria directly affect the productivity and welfare conditions of the animal. During the project, mangers and stalls suitable for the type of animal to be raised should be planned; Controls at feeders and stalls should be done regularly. In 21 dairy farms with stalls, the length of the stall is between 150-200 cm, and the width is between 105-143 cm. Details regarding the feeding and stall system and feeder information of the surveyed establishments are given in Table 6.

When the barn equipment and its structural features are examined, it has been observed that most farms have free stalls, their mangers are close to the ground level, and fed with feed mixers twice a day.

Table 6. Barn equipment and structural features

Feeding system	n	%
Feed bunk+feed alley	23	82.14
Classic feed bunk+feed alley	3	10.71
Classic feed bunk	2	7.14
Width of feed bunk		
Headlock width	23	82.14
45-49 cm	1	3.57
51-60 cm	2	7.14
70-75 cm	2	7.14
Length of feed bunk		
Along the feed alley	23	82.14
15-18 cm	2	7.14
35-50 cm	2	7.14
86 cm	1	3.57
Feed bunk front wall height		
10-30 cm	23	82.14
50-60 cm	3	10.71
70 cm	2	7.14
Feed alley length		
None	3	10.71
16-24 m	4	14.29
38-50 m	6	21.43
65-70 m	5	17.86
78-97 m	5	17.86
100-130 m	5	17.86
Feed alley width		
0 cm	3	10.71
60-100 cm	5	17.86
150-350 cm	8	28.57
400-500 cm	8	28.57
550-600 cm	3	10.71
700 cm	1	3.57
Feeding area location		
In the rest area	27	96.43
In the cow alley	1	3.57
Total number of stalls		
Non-stalls	7	25.00
60-80 stalls	7	25.00
100-120 stalls	9	32.14
140-240 stalls	5	17.86
Height of stalls above ground		
Non-stalls	7	25.00
0-5 cm	1	3.57
10-18 cm	8	28.57
19-25 cm	11	39.28
26-30 cm	1	3.57

All the results regarding the characteristics of manure storage in the researched farms are given in Table 7.

It was determined that 71.43% of the barns examined in the study had manure pits, and the same rate of manure cleaning was done using manure scrapers (20 barns-71.43%). For manure cleaning, 1 barn (3.57%) stated that they scraped manure with a tractor shovel, and 7 barns (25%) noted that the workforce was used for manure cleaning. As cattle manure use, farms used manure as soil fertilizer on their own fields (20 farms-

71.43%); (8 farms-28.57%) sold manure to other farms in the soil.

Table 7. Characteristics of manure pits and storage in the farms

Manure pit slope	n	%
No manure pit	8	28.57
%1	6	21.43
%2	8	28.57
%3	3	10.71
%5	2	7.14
%10	1	3.57
Manure emptied time		
No manure pit	8	28.57
Every 1-2 months	5	17.86
Every 2-3 months	7	25.00
3 > months	8	28.57
Width of manure pit		
No manure pit	8	28.57
2.8-3.5 m	4	14.29
4-5 m	4	14.29
6-7 m	7	25.00
8-10 m	4	14.29
13 m	1	3.57
Length of manure pit		
No manure pit	8	28.57
4-5 m	3	10.71
6-7 m	3	10.71
8-10 m	7	25.00
12 m	1	3.57
15-18 m	2	7.14
20-26 m	2	7.14
30 m	2	7.14
Height of manure pit		
no manure pit	8	28.57
2.5-3.5 m	6	21.43
4-5.5 m	13	46.43
10 m	1	3.57
Manure pit base material		
No manure pit	8	28.57
Concrete	18	64.29
Soil	2	7.14

Soyer (2014), in his study in Aydın, reported that 10.4% of dairy cattle farms use manure scrapers, 89.7% do not have an impermeable manure pit, and 87.4% of the farms use manure on their land. In their study in Özsağlıcak and Yanar (2022) also determined that the workforce did manure removal in the barns at 97.5%, automatic mechanical scrapers at 1.5%, and tractors at 1.0%. Yüzbaşıoğlu (2022) reported that 56% of the dairy farms he examined in the province of Tokat did not have a manure storage area. The results of the study were found to be compatible with the literature on this subject.

It has been observed that barns with manure discharge the manure in periods ranging from 1 month to 3 months. Even if these periods seem sufficient for the effective and systematic use of manure, there are risk conditions such as disruption of these processes, increases in the number of manures, or overflow of manure with precipitation. It is a remarkable result that none of the 28 farms examined did not have a drainage system. 96.43% (27 farms) of the holdings, and thus the manures are built on sloping lands. The remaining 1 farm (3.57%) was established on flat ground.

The farms are on sloping lands, and the drainage systems are insufficient; it causes concern that the wastes generated will pollute the water resources and the natural environment by surface flow and deep infiltration in the slope direction. In addition, city mains water is used as a water source in only 6 farms (21.43%), and groundwater is used in the remaining 22 farms (78.57%). This situation highlights the farm's lack of drainage systems and the necessity to question manure storage conditions' adequacy more seriously. The location where the livestock farms and the manures are established is also important. Especially in cases where drainage systems are inadequate and manure storage conditions differ, this importance increases with the risks of runoff and leakage. In this context, the distances of manures to water sources, milking units, and settlements were determined and given in Figure 2, Figure 3, and Figure 4, respectively.

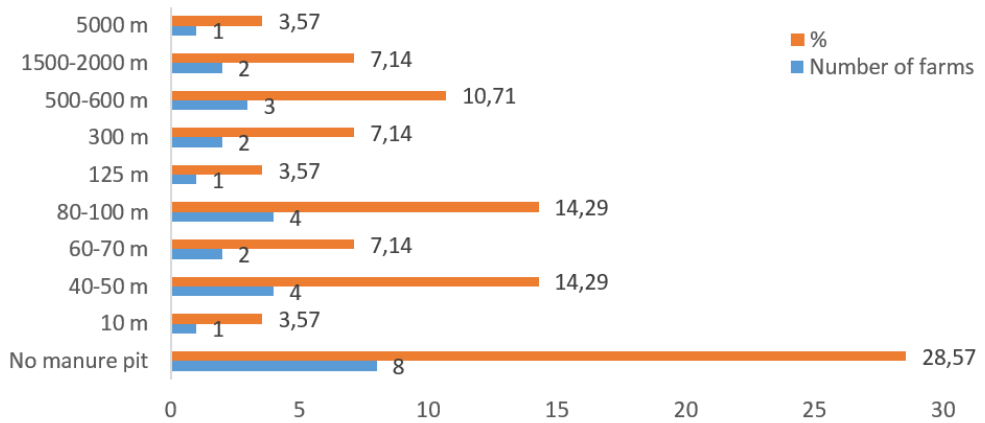

Figure 2. Distances between manure pits and water sources.

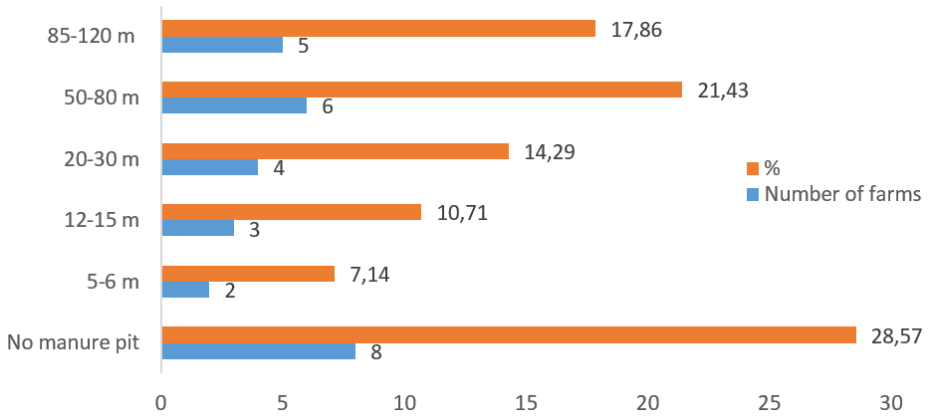

Figure 3. Distances between manure pits and milking units.

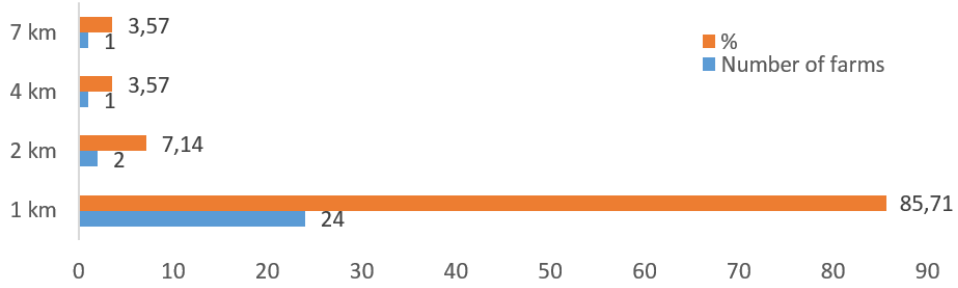

Figure 4. Distances between manure pits and settlements.

In our country, no attention is paid to the disposal, storage, and use of manure by distribution in the field. Liquid manure issues can become an important problem in farms that do not have agricultural land. It should be considered that manures may harm health if they leak into groundwater. Another critical issue is that manure cleaning must be done regularly in the shelter. Manure should be able to be transported out of the shelter with minimal labor and the use of machinery and equipment. If it is known that the daily manure production of dairy cattle weighing approximately 600 kg is 0.05 m^3 or 50 kg, it will be clear how important storage is. For efficient and healthy production, it would be appropriate to take the manure out of the shelter at certain daily intervals (Kırbıyık, 2022).

4. Conclusion

We can show that the studies based on sustainable animal husbandry and animal welfare are still insufficient among the many reasons why the desired efficiency levels cannot be achieved despite the increase in the number of intensification and large-capacity farms thanks to various incentives and grants in dairy cattle breeding in our country. In large capacity barns, which we can classify as modern farms in Yozgat, in farms established by receiving grants from institutions such as TKDK (Agriculture and Rural Development Support Institution), KOP (Konya Plain Project Regional Development Administration), and the Ministry of Agriculture and Forestry, stables are built under conditions suitable for animal welfare. Dairy barns should be designed to accommodate the behavioral needs of animals and produce high-quality products. Moreover, it is necessary to develop new models that consider each region's specific climatic conditions and structural characteristics. As a result, it has been observed that livestock activities in the Yozgat province, which are prevalent, have undergone significant transformations in recent years, transitioning towards modern facilities. To enhance profitability and achieve better milk quality, it may be advisable to encourage the conversion of small-scale enterprises into larger ones. Still, the farm owner and employees are insufficient in these project-based farms because they will increase costs. It was observed that the herd management system was not established, and the records were not kept properly. Making investments without long-term planning of production costs and profit and loss accounts in dairy farms threatens the sustainability of farms. Dairy cattle farming is a long-term line of business, and it isn't easy to convert the investments made for this activity into other investments once the action has started. There is no short-term planning flexibility in dairy farms. Making profitable and sustainable livestock farming is impossible by investing only in barns. It is recommended that relevant farm owners and employees be trained in dairy cattle breeding.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.E.	M.E.C.
C	100	
D		100
S	50	50
DCP	100	
DAI		100
L	50	50
W	50	50
CR	25	75
SR	75	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The survey study was approved by the Ethics Committee of Yozgat Bozok University (protocol code: 32/14 and date: April 20, 2022).

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PRODUCT DEVELOPMENT AND SENSORY EVALUATION OF DARK CHOCOLATE FILLED WITH CHESTNUT HONEY

Gamze DOĞAN[1], İlkay YILMAZ[2]*

[1]*Başkent University, Institute of Social Sciences, Department of Gastronomy and Culinary Arts, 06790, Ankara, Türkiye*
[2]*Başkent University, Faculty of Fine Arts, Design and Architecture, Department of Gastronomy and Culinary Arts, 06790, Ankara, Türkiye*

Abstract: Chocolate is a food that people of all ages love to consume. The sensory quality of chocolate is determined by its appearance, taste, aroma, and flavor. Chestnut honey, on the other hand, is a honey that is rich in amino acids, phenolic compounds, flavonoids, tannins, potassium, magnesium, and bioactive substances produced by the pollen of the chestnut tree and flower nectars, it has high antibacterial activity, and it contains important antioxidant compounds. In this study, it is aimed to develop a new product, which was filled with organic chestnut honey and chocolate, and bring it to the market. Chestnut honey was added to chocolate in varying proportions and subjected to quality grading by trained panelists. In the analysis attended by 10 panelists, the quality criteria of appearance, texture, smell, taste, and general acceptance were evaluated under sub-headings. As a result of the analysis, it was determined that the trained panelists gave 4.5±0.67 points out of 5- point scale above the average in terms of general acceptance, and consequently the favorite product was dark chocolate with 7% chestnut honey. In addition, the consumer taste test was applied to a group of 82 people for the filled chocolate with the highest score in the quality rating test. In the test applied depending on the 5-point scale, it was found that the general appreciation was high with the values of 4.75±0.54. As a result of the findings, it was determined that the bitter feeling of chestnut honey in the mouth was perceived in the obtained chocolate, and this was liked by the consumer ($\bar{X}$ = 4.75). With this study, it can be said that chestnut honey and chocolate provide flavor harmony.

Keywords: Chestnut honey, Dark chocolate, Sensory analysis, Product development

***Corresponding author:** Başkent University, Faculty of Fine Arts, Design and Architecture, Department of Gastronomy and Culinary Arts, 06790, Ankara, Türkiye
E mail: ilkayilmaz001@hotmail.com (İ. YILMAZ)
Gamze DOĞAN https://orcid.org/0000-0001-5743-5804
İlkay YILMAZ https://orcid.org/0000-0001-5938-3112

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1. Introduction

Chocolate was first prepared with various spices and flavorings by Aztecs and Mayans and consumed as a beverage. B.C. It is stated in some sources that chocolate drink was consumed in the civilizations before Aztecs in the 2000s B.C. (Sencer et al., 2018).

Chocolate is a delicious product that has a wide appeal for people from all walks of life, and it is consumed with pleasure (Hastaoğlu and Taşçı, 2021). When the annual chocolate consumption amounts per capita worldwide are examined, it has been determined that it is mostly consumed in Switzerland (9kg). In Türkiye, the annual consumption is determined as 3.1 kg per person.

Chocolate is a product prepared by adding cocoa, cocoa butter, sugar, milk powder, and additives (Hastaoğlu and Taşçı, 2021). According to the Turkish Language Association, chocolate means "candy, milk, peanuts, hazelnuts, etc. in cocoa". It is defined as a kind of sweet food made by adding sugar (TDK, 2023). According to the Turkish Food Codex, Cocoa and Chocolate Products Notification (2017/293) chocolate is defined as "it should contain at least 43% total cocoa solids and at least 26% cocoa butter". Dark chocolate is defined as a product consisting of cocoa products and sugar, containing at least 18% cocoa butter, 14% non-fat cocoa solid, and 35% total dry cocoa solids (TGK, 2017).

There are three main types of chocolate as dark, milk and white chocolate, and it can also be diversified as preparing it with permitted additives and flavorings, milk and dairy products and other food ingredients (Seçuk, 2020). In recent years, ruby chocolate has been added to the assortment of chocolate. Ruby chocolate is produced by a company named Callebaut (2017) and its color is pink. The pink color is obtained by developing pigments in the cocoa beans in the chocolate (Young, 2017).

Dark chocolate contains a high amount of cocoa. It has been determined that the dry weight of the cocoa bean contains approximately 50-57% cocoa butter, 15% fibers, 12-18% polyphenols and high amounts of minerals (Kargın and Güneş, 2017). Dark chocolate has organic compounds, especially flavonoids, polyphenols and catechins. It has been determined that these antioxidants prevent oxidative stress that causes heart diseases, diabetes, and cancer diseases by damaging cells and tissues. In addition, flavonols are known with their providing the expansion of blood vessels, balancing blood pressure and accelerating blood flow, as they provide nitric acid production in the body (Koca, 2011). In

addition, it has been determined that dark chocolate improves brain functions thanks to its components, it increases good cholesterol (HDL) by lowering bad cholesterol (LDL), balances insulin, prevents damage to the brain caused by Alzheimer's and Parkinson's diseases, and it is a store of zinc, phosphorus, and potassium (Sarıgül, 2014). According to a study conducted by Djoussé et al. (2011) in the USA evaluating the clinical effects of chocolate on cardiovascular health among 4970 adults aged 25-93 with heart disease, consumption of 28-34 g dark chocolate at least once a week has a negative effect on the progression of coronary heart disease, and it has been found to be a useful nutrient for the right dose of consumption (Djoussé et al, 2011; Kargın and Güneş, 2017).

Chocolate is a nutritious snack that is consumed fondly in many countries around the world. The main quality elements for chocolates are parameters such as taste, texture, smell, appearance and general taste, and the production method, raw materials used, and storage process affect the quality and degree of appreciation of the chocolate.

Chestnut (*Castania sativa*) honey has a strong herbal aroma and this aroma changes depending on the amount of chestnut oil in its content. The taste is slightly bitter, not too sweet. It is dark in color, sometimes with shades of amber, ranging from brown to almost black. The honey is rich in glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, amino acids and proteins, phenolic compounds, flavonoids, and minerals such as tannins, potassium, magnesium, manganese, and barium (Dağ et al., 2017). It has been stated in many studies that dark honey is rich in antioxidants that provide high therapeutic properties (Pehlivan, 2023). Türkiye is one of the countries with a high beekeeping size due to its variable geographical features, rich vegetation, and honeybee breeds with economic extracts (Güler and Demir 2005). Various unifloral flower honeys are produced from plant sources such as citrus, heather, cotton, sunflower, chestnut, and linden that bees use as nectar source in different geographical regions of our country (Özkök et al., 2021). Chestnut (*Castanea sativa*) honey is produced in the Black Sea, Marmara, and Aegean regions of Türkiye (Sarıkaya et al., 2009). In different studies on Anatolian chestnut honey, it has been found that it contains antioxidant compounds with high antibacterial activity (Ayvaz et al., 2018; Güneş et al., 2017; Sarıkaya et al., 2009). Chestnut (*Castanea sativa*) honey should contain at least 70% of the pollen of the plant it is named after (TGK, 2020). Chestnut honey has the potential to be a complementary product for protective and therapeutic applications of human health (Güneş, 2021).

It is a special working activity carried out in an area with the aim of being a pioneer in the market, producing an existing product more effectively or by improving it, or that has never been produced, but planned to be produced in the future (Şahin and Arabacı, 2017). Today, depending on the flexible structure of demand, businesses must not only sell, but also produce goods considering customer demands, they must find products suitable for the market, promote and sell them. It requires the co-ordination of production and marketing functions (Karayel, 2010). Businesses that want to stay ahead of the competition and grow in the food sector must closely follow the changes in the environment in which their markets are located, and they must develop strategies suitable for this market. One of the strategies that food businesses can choose to gain competitive advantage in both local and global markets is the new product development strategy (Öztürk and Onurlubaş, 2019). Among the main objectives of the businesses in this sector for developing new products can be listed as producing healthier products that can meet the nutritional needs of consumers at a high level, improving food safety, and offering consumers more product options with high preference. These gains can only be achieved depending on the stable progress of the new product development process. Factors influencing product development in businesses are business policies, marketing opportunities, product characteristics, economic factors, and production possibilities (Kobu, 2013). In the food sector, as in all other sectors, what is essential for the success of the new product is that the new product meets customer expectations (Kuşat and Kösekahyaoğlu, 2011). It will be beneficial for public health to produce foods that contain components beneficial to human health and meet customer expectations. Chocolate is a nutritious snack that is consumed fondly in many countries around the world. The main quality elements for chocolates are parameters such as taste, texture, smell, appearance and general taste, and the production method, raw materials used, and storage process affect the quality and degree of appreciation of the chocolate (Hastaoğlu and Taşçı, 2021). The aim of this study is to develop a dark chocolate product containing organic chestnut honey filling and to evaluate consumer acceptance with a sensory analysis method. In this way, a product containing functional components beneficial to human health will be developed, and products liked by the customer will be determined.

2. Materials and Methods

For ganache filling in chocolate production, drop ivory chocolate with 28% cocoa amount produced by Callebaut Company, Danone Tikveşli Gıda ve İçecek San. Trade Inc. Cream containing 35% animal fat, homemade unsalted butter and organic chestnut honey produced in the city of Düzce, Akçakoca district, were used. The German chocolate company Lubeca Dark Drop chocolate, which has 55% cocoa mass, is used for the outside of the chocolate.

In this study, 3 different recipes containing 5%, 7% and 9% chestnut honey in the following amounts were applied while preparing the ganache filling (Table 1).

First, the cream was taken into a saucepan and brought to the boiling point. Butter and chestnut honey were added to it and mixed. It was taken from the stove and added ivory drop chocolate, and then it melted well, it was homogenized with the help of a blender and left to rest.

Table 1. Recipe of the product developed with 5, 7, 9 % chestnut honey (10 pieces)

Products	(%5)	(%7)	(%9)
Ivory Drop Chocolate	35	33	31
Cream	50	50	50
Butter	10	10	10
Chestnut Honey	5	7	9
Total	100.00	100.00	100.00

2.1. Chocolate Production

Bitter couverture drops are used in making chocolate. Drop chocolate is melted and 12 gr. of it is shaped in a polycarbonate mold. In the melting process of drop chocolate, the bain-marie method is applied in a deep bowl on the water that comes to the boiling point, which is a form of manual tempering. The production was carried out in the Chocolate Workshop within the Baskent University Thermopolium Gastronomy Academy. During the production, attention was paid to ensure that the ambient temperature of the workshop did not exceed 18-20 degrees and the humidity not exceeding 55 degrees.

Before starting the production, the polycarbonate mold was cleaned with ethyl alcohol and cotton. Care was taken with a heat gun that the mold temperature was 25-27 degrees. Dark drop chocolate was melted by the bain-marie method until it reached 40-45 degrees. The melted chocolate was poured on a clean and not wet marble counter and tempered by hand with the help of a pallet and scraper. The tempered chocolate at 30 degrees was filled in a squeezing bag. By squeezing into the polycarbonate mold, the entire mold was filled with chocolate. By hitting the marble counter, the air bubbles were removed (Bardakçı, 2022).

The mold was turned upside down and the extra chocolate was poured onto the counter to form the crust of the chocolate. For the chocolate to have smooth edges, the chocolate remaining in the mold was scraped with the help of a scraper and left to stand at room temperature. The chocolates, which started to cool in the mold, were left to cool in the dehumidification cabinet of +12 °C (Figure 1). Since the mold and ganache temperatures should be close to each other, the ganache filled with chestnut honey to be filled into the crust chocolates was brought to 28-29 degrees with the help of heat gun. The ganache was filled into the shells as 1 mm less than the shell height and left to cool.

Figure 1. Chocolates produced with chestnut honey filling.

It was waited for the cooled mold to reach room temperature, and the tempering steps in shell making were repeated, then the base of the chocolate was made. Extra dark chocolate in the mold was cleaned with a scraper and left to cool in the dehumidifier cabinet of +12 ^{o}C. The mold was turned upside down and the chocolates were removed and stored in the cupboard.

2.2. Sensory Analysis

Sensory analysis is the evaluation of symptoms perceived by people with their senses, and it is a quantitative analysis method (Uçan, 2021). The sensory analysis part of the study was carried out as two stages. In the first part, the quality rating test was applied to 10 trained panelists. In the second stage, the consumer taste test, (hedonic) evaluation test, was applied to 82 people.

As a sensory analysis scale, the characteristics of the panel to be applied were determined with reference to the book "Sensory Evaluation in Foods" by Altuğ-Onoğur and Elmacı (2015). In the sensory analysis panels of 3-10 trained, 8-25 semi-trained, and at least 80 untrained panelists, it was stated that 8-25 semi-trained or at least 80 untrained panelists should be used for hedonic tests (Altuğ-Onoğur and Elmacı, 2015). Panelists were asked to neutralize the remaining taste with water or bread. Twelve statements were questioned under 5 main headings in the first part of study. Chocolates in the quality rating test were evaluated considering appearance; brightness, color; tissue, hardness, chewiness / stickiness, softness, odor/flavour; pleasant odor, unpleasant odor, flavor; Undesirable taste was evaluated in terms of mouthfeel, throat sensation, and post-taste impression parameters. In the consumer taste test, a hedonic scale consisting of five statements was used. The quality criteria of appearance, texture, odor, taste, and general appreciation were evaluated by the panelists. This test also has three questions that describe the participants (age, gender, educational background). In both tests, panelists were asked to rate on a 5-point scale as 1 (I like it a lot), 2 (I like it a little), 3 (I neither like it nor dislike it), 4 (I like it a little) and 5 (I like it a lot).

2.3. Data Analysis

The arithmetic averages of the findings obtained from the sensory analysis were taken, and their standard

deviations were calculated. In addition, descriptive statistics were used in the analysis of the obtained data. Whether the consumer taste test used was affected by gender, age and educational status was determined by the Mann- Whitney U test.

3. Results and Discussion

To determine the reliability of the scales used, Cronbah's Apha value was found to be 0.773. This shows that the scales have internal consistency. At the end of the experiments, the averages and standard deviations of the scores given to the characteristics determined in the sensory analysis applied to the chocolates containing different amounts of chestnut honey are given in Table 2. While the brightness of the product containing 9% chestnut honey was found to be high, its color was also found to be high depending on the appearance features. When the texture properties were compared, it was found that chocolate containing 7% chestnut honey had the best softness and chewiness. The most unfavorable chocolate in terms of softness was found to be the one containing 5% chestnut honey. When the odor and aroma were evaluated, chocolate containing 7% chestnut honey was evaluated as fragrant and without unwanted odor. At this stage, the panelists were asked to give a high score to the product without unpleasant odor. On the other hand, chocolate containing 7% chestnut honey received high scores and became the product with the highest acceptability in the taste criteria in which the undesirable taste, the sensation left in the mouth, the sensation left in the throat and the after-taste impression parameters were evaluated. This result was also reflected in the general taste profile and chocolate containing 7% chestnut honey was found to be the product with the highest general acceptability.

When it is to consider the findings in table 2, it is seen that 9% chestnut honey filled chocolate is high under the appearance criterion, with an average value of 4.9 for the gloss and sensory property, and for the color criterion with an average value of 4.8. Hardness and chewiness /stickiness properties under the texture criterion were higher in 7% chestnut honey filled chocolate, while the softness criterion was higher in 9% chestnut honey filled chocolate with an average value of 4.7. In the odor / aroma criterion, it is seen that the 7% chestnut honey filled chocolate has the highest pleasant odor characteristic of 4.6 and an average value of 4.8 undesirable odor. In the flavor criteria, it is seen that the undesirable taste in the mouth, the sensation left in the throat and the after-taste impression properties achieve the highest average values in 7% chestnut honey filled chocolate. Radar chart of dark chocolate filled with chestnut honey in different proportions is given in Figure 2.

Table 2. Sensory analysis findings on dark chocolate with chestnut honey filling in different proportions

	Sensory properties	5% CH* $\bar{X} \pm SS$	7% CH* $\bar{X} \pm SS$	9% CH* $\bar{X} \pm SS$
Appearance	Brightness	3.9±0.78	4.2±1.08	4.9±0.3
	Color	4±0.67	4.2±0.75	4.8±0.4
Tissue	Hardness	4.1±0.49	4.6±0.66	4.3±0.78
	Chewability / Stickiness	4.1±0.66	4.8±0.40	4.5±0.67
	Softness	4.2±0.46	4.5±0.67	4.7±0.46
Smell	Pleasant smell	4.2±0.46	4.6±0.66	4.1±0.83
	Unpleasant smell	4.2±0.64	4.8±0.40	4.1±0.94
Flavor	Undesirable Taste	3.9±0.8	4.6±0.49	3.9±1.04
	Mouth Feeling	3.8±0.78	4.4±0.66	3.9±0.54
	Throat Sensation	3.7±1.25	4.4±1.20	3.7±1.19
	After Taste Impression	3.8±0.64	4.5±0.67	4.2±0.60
General Acceptability	General Acceptability	3.8±0.64	4.5±0.67	4.0±0.63

Results are given as mean ± standard deviation. $\bar{X} \pm SS$, CH= chestnut honey.

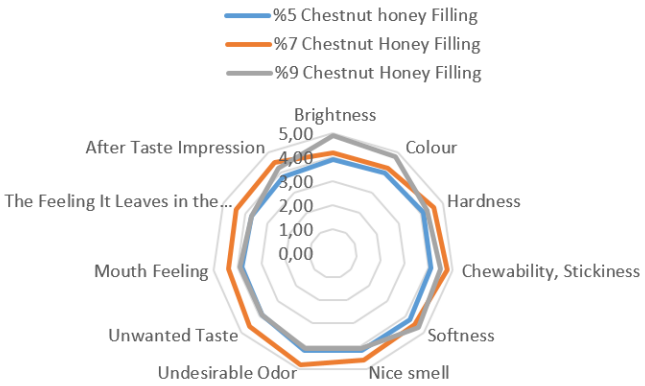

Figure 2. Quality rating test radar chart of dark chocolate with chestnut honey filling in different proportions.

It was determined that the general appreciation criterion was highest in the chocolate filled with 7% chestnut honey with a value of 4.5. It has been determined that the distinctive pungent smell and taste of chestnut honey does not disturb experienced panelists when used at a rate of 7%, and its aroma is appreciated when eaten with chocolate.

As a result of the finding, it was decided to apply the consumer taste test of 7% chestnut honey dark chocolate sample with the participation of 82 people.

3.1. Hedonic Taste Results

The developed product was presented to the consumer taste test, and it was tried to analyze whether the developed product would be accepted by the consumer. For this purpose, considering the quality rating test result, 7% chestnut honey filled dark chocolate was applied to a group of 82 people. The criteria of the hedonic test were determined as appearance, smell, texture, taste and general taste, and then the group consisting of consumers was asked to evaluate.

50% of the panelists participating in the research are men, 50% are women, 35% are graduates, 50% are undergraduates, and 15% are high school graduates. The age range is between 25 and 45.

Considering the factors of gender, age, and educational status in terms of perception of sensory analysis profile in the consumer taste test, it was found that these characteristics of the participants did not affect the result statistically (P>0.05). In Table 3, the mean values with standard deviation of sensory properties of dark chocolate filled with 7% chestnut honey are given. According to the findings, it was seen that the tissue criterion had the highest rate with a mean value of 4.9±0.30. The odor criterion in which the unique smell of chestnut honey is also felt in chocolate achieved the second highest rate with an average value of 4.65. The consumer taste test radar chart is shown in Figure 3.

Table 3. Consumer hedonic test mean and standard deviation values and acceptance rate

Sensory Properties	$\bar{X} \pm SS$*	Acceptance Rate (%)
Appearance	4.60±0.73	92
Smell	4.65±0.65	93
Tissue	4.90±0.30	98
Flavor	4.55±0.80	91
General acceptability	4.75±0.54	95

*Results are given as mean ± standard deviation. $\bar{X} \pm SS$.

When the literature was examined, it was seen that chestnut honey filling was not used for chocolate production in the previous studies. However, when the functional product development studies are reviewed, it is seen that there are chocolate development studies with pomegranate. According to this study, milk chocolate with pomegranate seeds and milk, and white chocolate with pomegranate jelly were evaluated sensorially by 17 volunteer panelists with different tastes and food cultures.

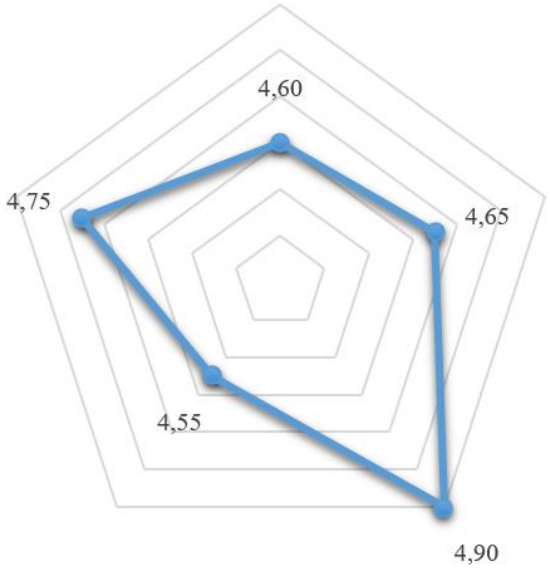

Figure 3. Radar graph of consumer taste test for dark chocolate with 7% chestnut honey filling.

In the sensory evaluation, the color, smell, texture, taste/aroma, and mouthfeel characteristics of the pomegranate chocolates produced were examined, and the scoring was made from 1 to 5, with 5 being the highest. According to the sensory evaluation score made by the panelists, although white chocolate with pomegranate jelly received the highest score in terms of colour, smell and taste/aroma, milk chocolate with pomegranate jelly was the most preferred example in terms of consumption and consumer acceptability. As a result of the evaluations, it has been determined that chocolate with pomegranate is highly appreciated and it will be a preferred product, and the pomegranate chocolates with an average of 17.5% pomegranate seeds or 32.3% pomegranate jelly are more accepted by consumers (Yıldırım et al., 2016). In our study, it is seen that the general taste of chocolate with chestnut honey was appreciated with a score of 4.75 out of 5, which is a score above the average.

In an analysis carried out to be able to predict the chocolate samples with different contents, and to measure their sensory liking by the participants, it was found that smokers among the participants were more successful in detecting the components of chocolate with ingredients such as anise, cardamom or sage (Hastaoğlu, Taşçi, 2021). In our study, smokers were not included in the sensory evaluation. According to another study in which melon, anise, rose, linden, sugar, honey, and sugar flavoring aromas were added to chocolate and evaluated sensorially, it was found that flavoring agents did not have any effect on increasing the perception of sweetness in chocolate formulations (Sencer et al., 2018). Chestnut honey, which was included in our study, did not show any change in the perception of sweetness. A sensory

analysis was carried out in a study in which oatmeal, a functional food rich in dietary fiber and β-glucan, and dried blueberries, which are rich in phenolic substances and contain high antioxidants, were added to chocolate. When the sensory analysis results of the oatmeal and blueberry chocolates were examined, the highest score in terms of "general acceptability" was the control sample, the unadded chocolate, and the lowest score was the chocolate with 40% and 50% added additives. Statistically, the effect of oatmeal and blueberry chocolates on overall acceptability was found to be insignificant (P>0.05) (Üzümcü and Özsisli, 2023). In our study, chocolate containing 7% chestnut honey had the highest results in terms of general acceptability.

4. Conclusion

In this study, a dark chocolate product containing organic chestnut honey filling was developed, and it was presented to the consumer with the sensory analysis method. It was determined that the taste criterion also took the average value of 4.55. According to this result, it is seen that the unique smell and taste of chestnut honey is appreciated by consumers above the average. Appearance criterion, on the other hand, received an average score of 4.60. Here, the panelists stated that they expected a relationship with the image of chestnut honey. It was determined that the general appreciation criterion of the consumer taste test applied to the group consisting of 82 panelists received an average value of 4.75. As a result of the results obtained, it was determined that 7% chestnut honey filled chocolate was the favorite according to the test results on consumers, and a different functional chocolate to consume was obtained. Chocolate is a universally preferred food. With the increasing interest of consumers for products with different contents, their desire to try different products, and the growth of the boutique and industrial chocolate market, products with different contents attract consumers' attention. In this study, the quality rating test of the chocolate, which appeals to people of all ages and from all walks of life and that consumed with pleasure, is diversified and enriched with ingredients in different proportions, and the quality rating test of the components available in it is applied to 10 panelists. As a result of the results obtained, a consumer taste test consisting of 82 people has been conducted. As a result of the sensory analysis carried out in this direction, it was determined that the distinctive sharp smell of chestnut honey and the bitter taste it leaves in the mouth are also quite felt in chocolate. In the evaluation on a scale of 5, it is seen that the general appreciation was appreciated with a score of 4.75 points that was above the average. Chocolate and chocolate products are enjoyed all over the world and in our country. Having considered the additives with functional properties such as chestnut honey, which we use as an additive in our study, and which is rich in phenolic substances, this study and similar ones can be carried out, and the functional property and nutritional quality of chocolate can be increased, thereby positively affecting public health.

The study has shown that chestnut honey-filled dark chocolate will make a great contribution to boutique chocolate producing places, patisseries, hotels, and industrial facility chocolate factories in terms of offering different flavors to consumers and expanding the market area. What is more, it is expected to provide support in terms of introducing a new product to the market. As a result of these results obtained, it has been determined that the product obtained will provide support in terms of bringing it to the market. It can be said that when the bitter taste of chestnut honey is combined with chocolate, it creates a harmonious taste. Recently, one of the popular trends in gastronomy is "Food Pairing", which is a concept translated into our language as 'flavor matching'. This is in fact a method to find out which food will go well with what, and what flavor will emerge as a result. In other words, it can be called the 'art of balanced harmony' between familiar foods. With this study, it can be said that chestnut honey and chocolate provide flavor harmony.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	G.D.	İ.Y.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Aspergillus niger IMPROVES THE NUTRITIONAL COMPOSITION OF APPLE POMACE BY SOLID-STATE FERMENTATION

Aydın ALTOP[1], Emrah GÜNGÖR[1]*, Güray ERENER[1]

[1]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, 55139, Samsun, Türkiye*

Abstract: The effect of solid-state fermentation using *Aspergillus niger* on the nutritional composition of apple pomace was investigated in this study. Apple pomace was milled to 2 mm and sterilized at 121 °C for 15 min before fermentation. *Aspergillus niger* strain (ATCC 200345) was cultured and inoculated to apple pomace 10^4 spores/ml. Raw and fermented apple pomace were analyzed for determination of the crude protein (CP), ash, ether extract (EE), crude fiber (CF), neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and acid-detergent lignin (ADL) content. The CP ($P<0.001$) and ash ($P<0.05$) contents of apple pomace were increased after solid-state fermentation. However, *A. niger* decreased ($P<0.001$) the CF, NDF, ADF, hemicellulose, NFE and EE ($P<0.05$) contents of apple pomace. The ADL content of apple pomace was not affected by solid-state fermentation. The results demonstrated that solid-state fermentation using *A. niger* can improve the nutritional composition of apple pomace.

Keywords: Agricultural by-product, Nutritional enrichment, *Malus domestica*, *Aspergillus niger*

***Corresponding author:** Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, 55139, Samsun, Türkiye
E mail: emrah.gungor@omu.edu.tr (E. GÜNGÖR)
Aydın ALTOP https://orcid.org/0000-0002-3966-300X
Emrah GÜNGÖR https://orcid.org/0000-0003-4380-6162
Güray ERENER https://orcid.org/0000-0002-8025-2560

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1. Introduction

The increasing world population increases the demand for protein foods. Animal production is important in meeting the need for protein. Feed costs in animal production constitute 60-70% of operating costs (Güngör et al., 2017). Therefore, alternative feed sources should be made available for animal nutrition.

Apple pomace is an agricultural waste product from the production process of feed mills. Apple pomace is rich in minerals and carbohydrates, but it has low crude protein (CP) and high crude fiber (CF) content (Beigh et al., 2015). These characteristics limit the use of apple pomace in animal nutrition. Apple pulp can be used in 5% of diets in broiler chicks without adverse effects on growth performance, but higher levels impair the feed conversion ratio (Ayhan et al., 2009). Similarly, Beigh et al. (2015) noted that apple pomace can be used in animal nutrition at limited levels because of its low CP content.

Solid-state fermentation means microbial growth in the insoluble solid substrates immersed in free water (Yasar and Tosun, 2020). Solid-state fermentation can improve the nutritional quality of agricultural residues (Altop et al., 2018). *Aspergillus niger* was used in solid-state fermentation of apple pomace to produce organic acids (Shojaosadati and Babaeipour, 2002) and enzymes (Dhillon et al., 2012). However, recent studies showed that *A. niger* can improve the nutritional composition of apple pomace (Yang et al., 2022). Tosun and Yaşar (2021) demonstrated that *A. niger* increased ash and EE content and decreased the crude fiber content of apple pomace by solid-state fermentation for 3 days. However, there is a lack of study on the effect of solid-state fermentation using *A. niger* for a longer period on the nutritional composition of apple pomace. This study aimed to investigate the effect of *A. niger* solid-state fermentation for 7 days on the main nutritional composition of apple pomace.

2. Materials and Methods

Apple pomace was supplied from a local juice factory in Samsun, Turkiye. *Aspergillus niger* (ATCC 200345) was obtained from the American Type Culture Collection (ATCC). The apple pomace was dried at 75 °C for 48 hours and ground to a size of 2mm. Dried apple pomace was enriched by the nutritional salt (glucose: urea: $(NH_4)_2SO_4$: peptone: KH_2PO_4: $MgSO_4$. $7H_2O$= 4: 2: 6: 1: 4: 1) and sterilized by autoclave at 121 °C for 15 min. *Aspergillus niger* was cultured in Potato-Dextrose Agar and spore suspension was prepared at 10^4 spores/ml. Sterilized apple pomace samples were inoculated by *A. niger* spores and incubated at 30 °C for 7 days. After the incubation period, apple pomace was dried at room temperature 30-35 °C for 6 days to reach 90% dry matter (DM). Unfermented and fermented apple pomace were analyzed to determine the DM, ash, CP, EE and CF contents according to AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid

detergent lignin (ADL) contents were determined according to the methods of Van Soest et al. (1991).

2.1. Statistical Analysis

The experiment was conducted in triplicate, and the results were presented as means and pooled standard error of mean (SEM). Obtained data were analyzed with the Student t test (SPSS 21.0 Statistics). Results were considered significantly different at P≤0.05.

3. Results

Solid-state fermentation increased the CP and ash contents of apple pomace (P<0.001 and P=0.044, respectively). However, fermented apple pomace had a lower CF, NDF, ADF and hemicellulose contents than those of unfermented apple pomace (P=0.004, P<0.001 and P<0.001, respectively). Similarly, *A. niger* decreased the EE and NFE content (P=0.019 and P<0.001, respectively). However, the ADL content was not changed (P>0.05) by solid-state fermentation (Table 1).

4. Discussion

Feed costs in animal production constitute an important part of operating costs (Güngör et al., 2017). Finding alternative feedstuffs to the current feedstuffs will reduce feed costs. Solid-state fermentation can be used to improve the nutritional composition of agricultural wastes obtained at affordable prices (Tosun and Yaşar, 2021). The nutritional composition of apple pomace was improved by solid-state fermentation using *A. niger* for 7 days in the present study. Similarly, Tosun and Yaşar (2021) showed that *A. niger* improved the nutritional quality of apple pomace by increasing ash and EE content and decreasing CF, ADF and NDF content with solid-state fermentation for 3 days. Similar results were taken from the studies on solid-state fermentation of apple pomace using *A. niger* (Zhong-Tao et al., 2009; Yang et al., 2022).

The prices of protein-rich-feed stuffs, which are essential for meeting the protein needs of animals, are higher than other feed raw materials. Therefore, the use of economical feed raw materials in order to meet the protein deficit in the diet of animals makes a significant contribution to reducing feed costs. Apple pomace is used at a limited level in animal feeds due to its low CP content (Beigh et al., 2015). Solid-state fermentation using *A. niger* increased the CP content of apple pomace in this study. Similarly, Yang et al. (2022) noted that *A. niger* elevated the CP, pure protein and amino acid contents of apple pomace in solid-state fermentation. Zhong-Tao et al. (2009) also reported that the crude protein content of apple pomace was increased by solid-state fermentation using *A. niger*. Various microbial enzymes such as proteinase, cellulase and pectinase can be produced by *A. niger* during solid-state fermentation (Zhong-Tao et al., 2009). Similarly, Yang et al. (2022) showed that *A. niger* enriched produced cellulase in apple pomace solid-state fermentation. The increase in CP content of apple pomace can be related to the enzymes produced by *A. niger* during fermentation. Indeed, the results of the study conducted by Yang et al. (2022) demonstrated that the increase in the CP level of apple pomace is related to the cellulase production capacity.

Crude fiber content of a feedstuff is an important factor affecting its digestibility. In general, feeds with low CF content have high digestibility, while feeds with high CF content have low digestibility. The reduction of CF, ADF, NDF and hemicellulose levels in apple pomace by solid-state fermentation in the present study is in accordance with the results of the various studies (Tosun and Yaşar, 2021). Tosun and Yaşar (2021) reported that *A. niger* decreased the CF content of apple pomace in solid-state fermentation. Similarly, solid-state fermentation using *A. niger* reduced the CF level in pomegranate pomace (Gungor et al., 2021a). Yang et al. (2022) reported that *A. niger* can produce cellulase in apple pomace during solid-state fermentation. Decreases in the CF, ADF, NDF and hemicellulose levels can be because of the cellulase activity in apple pomace through fermentation.

Microorganisms can accumulate some minerals in the fermentation medium during solid-state fermentation and increase the ash content of substrate (Xiao et al., 2021).

Table 1. Nutritional composition of unfermented and fermented apple pomace by *Aspergillus niger* in solid-state fermentation

Nutrients (%, dry matter basis)	UAP	FAP	SEM	P
Crude Protein	5.39	17.55	2.721	<0.001
Ether Extract	2.78	2.39	0.100	0.019
Ash	3.34	7.62	1.163	0.044
NFE	62.31	48.33	3.142	<0.001
Crude Fiber	26.14	22.77	0.792	0.004
Hemicellulose	12.78	7.00	1.295	<0.001
NDF	51.78	43.99	1.746	<0.001
ADF	39.00	36.99	0.454	<0.001
ADL	21.52	19.94	0.685	0.294

UAP= unfermented apple pomace, FAP= fermented apple pomace, SEM= standard error of mean, NFE= nitrogen-free extract, NDF= neutral-detergent fiber, ADF= acid-detergent fiber, ADL= acid-detergent lignin.

Tosun and Yaşar (2021) reported that solid-state fermentation increased the ash content of apple pomace starting on the first day. Similarly, the ash level of apple pomace was increased by solid-state fermentation using *A. niger* for 7 days in this study. Increase in the ash content of apple pomace after fermentation can be due to the accumulation of minerals by *A. niger* during fermentation. Gungor et al. (2021b) also reported that *A. niger* increased the ash content of grape pomace in solid-state fermentation.

Aspergillus spp. can enrich the substrates with microbial lipids by producing them during solid-state fermentation and increasing the EE content of the fermentation medium (Hui et al., 2010). Solid-state fermentation using *A. niger* increased the EE content of apple pomace (Tosun and Yaşar, 2021). However, *A. niger* reduced the EE content of apple pomace by fermentation in this study. Similarly, Gungor et al. (2021b) showed that solid-state fermentation using *A. niger* decreased the EE content of grape pomace.

Microorganisms try to meet the carbon needed to sustain their growth mainly from the easily degradable carbohydrates in the fermentation medium (Papagianni, 2007). The sharp decrease in the NFE level of apple pulp may be due to the fact that *Aspergillus niger* may consume the easily digestible carbohydrates in apple pomace and use them for mineral accumulation and production of microbial proteins such as enzymes and mycelium. Similarly, Gungor et al. (2021b) reported a reduced NFE content in *A. niger*-fermented grape pomace compared to unfermented grape pomace. However, *A. niger* increased the total reduced sugar content of apple pomace after solid-state fermentation (Tosun and Yaşar, 2021). The different results may be due to the differences in *A. niger* strains and fermentation conditions.

5. Conclusion

The results of the present study demonstrated that solid-state fermentation using *A. niger* improved the nutritional quality of apple pomace by increasing CP and ash contents with reducing CF, ADF and NDF levels. The optimum fermentation conditions need to be determined to obtain higher quality fermented apple pomace in further studies. In addition, the effect of fermented apple pomace on ruminant and non-ruminant animals can be investigated in future studies.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.A.	E.G.	G.E.
C	40	30	30
D	40	40	20
S	50		50
DCP	30	60	10
DAI	30	70	
L	50	50	
W	40	50	10
CR	40		60
SR	20	60	20
PM	100		
FA	100		

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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EFFECTS OF AGE, CALVING SEASON AND SPECIFIC GRAVITY OF FIRST MILKING COLOSTRUM ON MILK COMPONENTS AND SOMATIC CELL COUNT IN EARLY LACTATION ANATOLIAN BUFFALO (*Bubalus bubalis*) COWS

Hüseyin ERDEM[1]*, İbrahim Cihangir OKUYUCU[1], Hasan DEMİRCİ[1]

[1]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, 55139 Samsun, Türkiye*

Abstract: This study aimed to evaluate the milk components and somatic cell count (SCC) of Anatolian buffalo cows at different stages of lactation (days 15th, 30th, 45th and 60th of lactation) by determining the effects of the age of the cow, calving season and specific gravity of colostrum (SGC) on these milk quality traits. The experiment was conducted using 62 healthy lactating primiparous and multiparous Anatolian buffalo cows at two private farms in Samsun, Türkiye. The SGC values at the second hour (first milking) after birth were measured using a colostrometer, and the milk components were analyzed using a milk analyser. In addition, the SCC in milk was measured with a portable cell counter. The data are divided into three groups: age (≤80 mo and >80 mo), calving season (spring and summer) and SGC (<1.070 g/l and ≥1.070 g/l). The age and calving season of the cows affected some milk components in different lactation periods. The dry matter (DM), solids-non-fat (SNF) and protein percentage of the cows in the group with high SGC (≥1.070 g/l) were higher than those of cows in the group with low SGC (<1.070 g/l) on day 15 of lactation. Similarly, the DM and fat percentage of the milk of the cows in the group with high SGC (≥1.070 g/ml) were higher than those of the milk from the cows in the group with low SGC (<1.070 g/l) on day 45 of lactation. In contrast, on day 60 of lactation, the log SCC value of the milk of cows with high SGC was lower than that of the milk from those with low SGC in other lactation periods. Among the environmental factors in this study, only the SGC can be partially controlled by herd management practices. Management of the dry period, a sensitive period for buffalo cows, has an important effect on increasing colostrum and milk quality at the beginning of lactation.

Keywords: Buffalo cow, Calving age, Calving season, Colostrum specific gravity, Milk components, Somatic cell count

***Corresponding author:** Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, 55139 Samsun, Türkiye
E mail: hserdem@omu.edu.tr (H. ERDEM)
Hüseyin ERDEM https://orcid.org/0000-0001-5273-3925
İbrahim Cihangir OKUYUCU https://orcid.org/0000-0002-2138-6577
Hasan DEMİRCİ https://orcid.org/0000-0001-9227-7442

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1. Introduction

Recently, intensive buffalo farming systems in Türkiye and some European countries (Italy, Romania and Bulgaria) are growing rapidly to satisfy the increasing interest of consumers in buffalo dairy products. This intensification process also increases the required quality of the milk (Napolitano et al., 2019). The quality of milk is important not only to improve the product but also to produce healthy calves with high viability and growth performance.

As in dairy cattle farms, female buffalo calves replace buffalo cows that are culled from the herd for various reasons. In other words, the herd is renewed by choosing healthy heifers over the cows that leave the herd (Erez and Göncü, 2012; Singh et al., 2019). In addition, it is essential to obtain high-quality milk from each buffalo cow every year, which depends on the farm conditions. Although calf management is one of the most important parts of herd management (Okuyucu and Erdem, 2017), the calves are often neglected on buffalo farms (Gupta et al., 2019). Therefore, neonatal calf mortality resulting in economic losses, health problems and poor growth performance are common problems in dairy buffalo farms. Calf mortality is usually high in calves aged 0–90 days, confirming the claim by Khan et al. (2007) that most buffalo calf deaths (87.50%) occur between 0 and 90 days. The calves receive most of their primary nutrients from milk and the rest from roughage and concentrate (Singh and Saini, 2020). Therefore, buffalo calves should consume sufficient amounts of necessary nutrients with milk in the newborn period of their lives for high milk yields in the future. Moreover, these features are important for improving product quality because high-quality milk is characterized by high milk components, low somatic cell (SCC) and microorganism counts (Şahin and Yıldırım, 2012). The SCC is a reliable criterion for detecting udder health, milk quality, hygiene and food safety (Şahin et al., 2017; Erdem and Okuyucu, 2019). Depending on the nature and level of a cow's udder infection, the SCC increases significantly as the first defence response (Aytekin and Boztepe, 2014).

The lactation period in ruminant animals begins with

colostrum secreted before birth, stored in the udder and released from the udder after birth. The colostrum secreted from the udder is rapidly transformed into normal milk in subsequent milkings. It is important for the calf to obtain sufficient passive immunity that the calf receives this unique fluid in sufficient quality and quantity as soon as possible after birth. A large body of authors generally associate colostrum quality with high levels of immunoglobulin (Hoyraz et al., 2015; Erdem et al., 2022a). Several methods can be used to predict colostrum quality in dairy farms. To determine colostrum quality, colostrometers (densimeters) and refractometers are generally used because they are simple, practical and easily applicable. The colostrum densimeter (KRUUSE UK Ltd, Langeskov, Denmark) evaluates the quality of colostrum according to its specific gravity (Erdem et al., 2022b). Determining the quality of colostrum and focusing on the direct and/or indirect effects of milk quality in the later stages of lactation are important in terms of both feeding the calf with high quality colostrum and milk and understanding the quality of the milk offered to the market.

Several factors affect milk components and SCC in buffalo cows, such as parity, lactation period, age, live weight, feeding, calving season, pregnancy, body condition score, lameness, heat stress, milking temperament, and udder health (Boro et al., 2018). Many authors have focused on non-genetic factors affecting milk component values (Misra et al., 2008; Yadav et al., 2013; Patbandha et al., 2015) and SCC (Şahin et al., 2017; Erdem and Okuyucu, 2019). However, reports on the effects of age and calving season on milk components and SCC in early lactation Anatolian buffalo cows are still limited. To the best of our knowledge, no detailed information is available on the effects of SGC value, which is an important indicator in determining colostrum quality on the components and SCC values of milk produced in early lactation Anatolian buffalo cows. Therefore, revealing these effects will provide important information. In this context, this study aimed (i) to evaluate the components and SCC of milk produced at different lactation stages by early lactation Anatolian buffalo cows and (ii) to determine the effects of SGC, age and calving season on the components and SCC of milk from early lactation Anatolian buffalo cows.

2. Materials and Methods

This study was carried out on two dairy buffalo farms from December to November. These farms are situated in the Black Sea region of Türkiye. A total of 62 healthy primiparous and multiparous Anatolian buffalo cows in two intensive condition farms were randomly selected from Samsun province. Moreover, records were taken from the breeders to confirm the ages and calving seasons of the cows.

During the experiment, the buffalo cows were housed in free-stall (an open barning system with a concrete floor) barns and fed a total mixed ration balanced according to their physiological needs. The cows were fed wheat straw, corn silage and concentrate under similar breeding conditions.

To determine the specific gravity of colostrum (SGC) of cows at the first milking, 2 hours after calving, colostrum samples (approximately 0.25 L) were collected from the cows before the calves were allowed to feed. The colostrum samples reached the laboratory approximately 45 min later, transported in containers at 4 °C. These samples were analysed after they were heated to 20–22 °C in a hot water bath. The colostrum quality was determined by a KRUUSE© colostrometer based on the relationship between immunoglobulin (Ig) concentration and specific gravity (Kaygısız and Kose, 2007; Erdem and Okuyucu, 2020).

To evaluate the milk components (DM, fat, protein, lactose, density and freezing point) and SCC of the cows, a milk sample was collected from each buffalo cow during the morning milking on days 15, 30, 45 and 60 of lactation. After machine milking, the milk samples were transferred into 50-ml plastic tubes from the bucket. Then, the milk samples were transported to the laboratory by cold chain (+4 °C). The milk samples were analysed for milk components using a milk analyser (Lactostar, Funke-Gerber, Berlin, Germany). Also, the SCC in milk was measured using a portable cell counter (DeLaval, Tumba, Sweden).

Our study obtained a single value by taking the arithmetic mean of the SGC values (first milking colostrum). The first group consisted of values lower than this average (Group-1: <1.070 g/l), and the values higher than this average were taken as the second group (Group-2: ≥1.070 g/l). Similarly, the data were grouped according to the age of the cow (Group-1: ≤80 mo, Group-2: >80 mo) and calving season (Group-1: Spring, Group-2: Summer). Also, a logarithmic transformation (base 10) was performed on the SCC data to form a normal distribution. The statistical analyses were performed using the general linear model (the linear mixed model)/univariate procedure of SPSS.

To evaluate the effects of age, calving season and SGC values on milk components and log SCC on days 15, 30, 45 and 60 of lactation, the Equation 1 was used:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + \varepsilon_{ijkl} \quad (1)$$

where, Y_{ijkl} = the observation value (DM, fat, SNF, protein, lactose, density, and log SCC), μ= the overall mean, a_i = the effect of age (i= 1, 2), b_j= the effect of calving season (j=1, 2), c_k= the effect of SGC (k=1, 2) and e_{ijkl} = a random error.

All statistical analyses were performed using SPSS 21.0.

3. Results

In our study, the means of the milk components, SCC, and log SCC according to different lactation periods are shown in Table 1. The percentage of DM on days 15, 30, 45 and 60 of lactation periods ranged from

17.05%±0.163% to 17.78±0.147%. The fat percentage during these lactation periods ranged from 7.63±0.134% to 7.83±0.117%. Additionally, the average SNF percentages of the milk were 9.42±0.081%, 9.66±0.082%, 9.96±0.104% and 9.93±0.108%, at the days 15, 30, 45 and 60 of lactation, respectively. The protein percentage on days 15, 30, 45 and 60 of lactation were 4.86±0.023%, 4.88±0.023%, 4.90±0.021% and 4.97±0.032% respectively. The mean lactose percentage on days 15, 30, 45 and 60 of lactation ranged from 4.91±0.023% to 4.99±0.023%. In addition, the milk density values ranged from 1.0269±0.00012 g/l to 1.0273±0.00012 g/l.

In this research, the log SCC, which is considered a trustworthy indicator of the hygienic quality of milk and udder health, ranged from 4.617±0.0296 (47290.3±3177.24 cell/ml) to 4.701±0.0281 (57096.8±3937.8 cell/ml) on days 15, 30, 45 and 60 of lactation (Table 1).

The data presented in Table 2 show that the effect of the age of the cow on all the milk components was statistically insignificant on day 15 of lactation. As shown in Table 2, the effects of the calving season on fat percentage were significant (P=0.002) on day 15 of lactation. The highest fat percentage was found in the summer months (17.2%), compared to the spring months (16.9%). However, the DM, SNF, protein and lactose percentages produced on day 15 of lactation were not affected by calving season. The DM (P=0.044), SNF (P=0.046) and protein (P=0.033) percentages produced on day 15 of lactation were affected by changes in the SGC of buffalo cows at first milking (2 hours after calving), but the fat and lactose were not.

In this experiment, the mean milk component values of buffalo cows on day 30 of lactation according to age, calving season and colostrum SGC groups are shown in Table 3. Except for the protein percentage, the effect of the age group on all the milk components was statistically insignificant on the thirtieth day of lactation; the effect of age on the protein percentage was significantly different on day 30 of lactation (P=0.039). The protein percentage in the older group of buffalo cows (>80 mo) was higher than in the younger buffalo cows (≤80 mo). However, the effect of the calving season and SGC groups on the milk component values of buffalo cows was statistically insignificant for this lactation period.

In the present study, the changes in DM, fat, SNF, protein and lactose values of buffalo cows on day 45 of lactation according to age, calving season and colostrum SGC groups are presented in Table 4. A statistically significant increase in the DM (P=0.048), SNF (P=0.043) and protein (P=0.024) values of the cows with increasing age was seen on day 45 of lactation. The DM, SNF and protein percentage in the milk of cows aged ≤80 mo was lower than those >80 mo in age. However, the effect of the age on the fat and lactose content of buffalo cows was statistically insignificant for this lactation period. Additionally, the effect of the calving season on the milk component values of buffalo cows was statistically insignificant for these lactation periods.

Table 1. Means of milk components, SCC (cell/ml) and logSCC according different lactation periods

Components	15th day	30th day	45th day	60th day
	mean ±SE	mean ±SE	mean ±SE	mean ±SE
DM (%)	17.05±0.163	17.41±0.140	17.78±0.147	17.61±0.139
Fat (%)	7.63±0.134	7.75±0.110	7.83±0.117	7.68±0.100
SNF (%)	9.42±0.081	9.66±0.082	9.96±0.104	9.93±0.108
Protein (%)	4.86±0.023	4.88±0.023	4.90±0.021	4.97±0.032
Lactose (%)	4.93±0.028	4.98±0.026	4.99±0.023	4.91±0.023
Density	1.0273±0.00012	1.0272±0.00001	1.0267±0.00011	1.0269±0.00012
SCC (cell/ml)	49758,1±3727,47	57096,8±3937,8	47290,3±3177,24	50080,65±3302,10
logSCC	4.62±0.032	4.701±0.0281	4.617±0.0296	4.645±0.0285

DM= dry matter, SNF= solids-non-fat, SCC= somatic cell count, SE= standard error.

Table 2. Means milk component values (%) of buffalo cows on day 15 of lactation according to cow's age, calving season and colostrum SGC groups

Components	Cow's age (mo)				Calving season				SGC groups (g/l)			
	≤80 mo	>80 mo	± SEM	P	SP	SU	± SEM	P	G-1	G-2	± SEM	P
DM	17.0	17.1	0.16	0.737	16.9	17.2	0.16	0.873	16.5	17.3	0.16	0.044
Fat	7.7	7.6	0.13	0.857	7.4	7.8	0.13	0.002	7.6	7.8	0.13	0.172
SNF	9.3	9.5	0.08	0.702	9.5	9.2	0.08	0.411	9.2	9.6	0.08	0.046
Protein	4.9	4.9	0.02	0.575	4.9	4.9	0.02	0.844	4.6	4.9	0.23	0.033
Lactose	4.9	5.0	0.03	0.746	4.9	4.9	0.03	0.931	4.9	4.9	0.28	0.966

DM= dry matter, SNF= solids-non-fat, SP= spring, SU= summer, G-1= <1.070 g/l, G-2= ≥1.070 g/l.

Table 3. Means milk component values (%) of buffalo cows on day 30 of lactation according to cow's age, calving season and colostrum SGC groups

Components	Cow's age (mo)				Calving season				SGC groups (g/l)			
	≤80 mo	>80 mo	± SEM	P	SP	SU	± SEM	P	G-1	G-2	± SEM	P
DM	17.4	17.4	0.14	0.933	17.4	17.4	0.14	0.812	17.2	17.5	0.14	0.355
Fat	7.8	7.7	0.11	0.925	7.6	7.9	0.11	0.862	7.7	7.8	0.11	0.750
SNF	9.6	9.7	0.08	0.984	9.7	9.6	0.08	0.535	9.5	9.7	0.08	0.259
Protein	4.8	4.9	0.02	0.039	4.9	4.9	0.02	0.857	4.9	4.9	0.22	0.755
Lactose	5.0	5.0	0.03	0.965	5.0	4.9	0.03	0.245	5.0	5.0	0.26	0.717

DM= dry matter, SNF= solids-non-fat, SP= spring, SU= summer, G-1= <1.070 g/l, G-2= ≥1.070 g/l.

Table 4. Means milk component values (%) of buffalo cows on day 45 of lactation according to cow's age, calving season and colostrum SGC groups

Components	Cow's age (mo)				Calving season				SGC groups (g/l)			
	≤80 mo	>80 mo	± SEM	P	SP	SU	± SEM	P	G-1	G-2	± SEM	P
DM	17.6	18.0	0.15	0.048	17.9	17.7	0.15	0.148	17.4	18.0	0.15	0.027
Fat	7.9	7.8	0.12	0.577	7.8	7.9	0.12	0.531	7.4	8.0	0.12	0.036
SNF	4.8	5.0	0.02	0.043	10.1	9.9	0.10	0.192	10.0	10.0	0.10	0.431
Protein	4.8	5.0	0.02	0.024	4.9	4.9	0.02	0.761	4.9	4.9	0.02	0.307
Lactose	5.0	5.0	0.02	0.583	5.0	5.0	0.02	0.214	5.0	5.0	0.02	0.853

DM= dry matter, SNF= solids-non-fat, SP= spring, SU= summer, G-1= <1.070 g/l, G-2= ≥1.070 g/l.

Table 5. Means milk component values of buffalo cows on day 60 of lactation according to cow's age, calving season and colostrum SGC groups

Components (%)	Cow's age (mo)				Calving season				SGC groups (g/l)			
	≤80 mo	>80 mo	± SEM	P	SP	SU	± SEM	P	G-1	G-2	± SEM	P
DM	17.9	17.4	0.14	0.160	17.7	17.6	0.14	0.244	17.8	17.6	0.14	0.871
Fat	7.8	7.6	0.92	0.520	7.6	7.8	0.92	0.639	7.5	7.9	0.92	0.277
SNF	10.1	9.8	0.11	0.179	10.1	9.8	0.11	0.243	10.2	9.8	0.11	0.265
Protein	5.0	5.0	0.03	0.309	5.0	4.9	0.32	0.350	4.9	5.0	0.32	0.286
Lactose	4.9	4.9	0.02	0.592	4.9	4.9	0.02	0.209	4.9	4.9	0.23	0.447

DM= dry matter, SNF= solids-non-fat, SP= spring, SU= summer, G-1= <1.070 g/l, G-2= ≥1.070 g/l.

On day 45 after calving, the DM and fat percentage of cows in the group with high SGC (≥1.070 g/l) were higher than those of the cows in the group with low SGC (<1.070 g/l) (Table 4). A statistically significant increase in the milk DM (P= 0.027) and fat (P= 0.036) percentage of the cows with increasing SGC of the colostrum produced at first milking (2 hours after calving) was determined on day 45 of lactation. However, the effects of SNF, protein and lactose in the SGC groups were statistically insignificant.

As seen in Table 5, the effect of age, calving season and SGC groups on all the milk components was statistically insignificant on day 60 of lactation.

The changes in milk density values on days 15, 30, 45 and 60 of lactation according to age, calving season and SGC groups are shown in Figure 1. Except for the calving season, the effect of age and SGC groups on all the milk components was statistically insignificant for all lactation periods. However, the density of the milk from buffalo cows calving in the summer months was lower than those calving in the spring months (P<0.05) on days 15 and 30 of lactation (Figure 1b).

In our study, the changes in log SCC on days 15, 30, 45 and 60 of lactation are presented according to age, calving season and SGC groups in Figure 2. The effect of age and calving season on log SCC were statistically insignificant for all lactation periods (Figure 2a,b).

Except for day 60 of lactation, the value during the other periods was higher in cows in the high SGC group (≥1.070 g/l) than in the low SGC group (<1.070 g/l) (Figure 2c). A statistically significant increase was determined in the log SCC value of the buffalo cows with low colostrum SGC during these lactation periods.

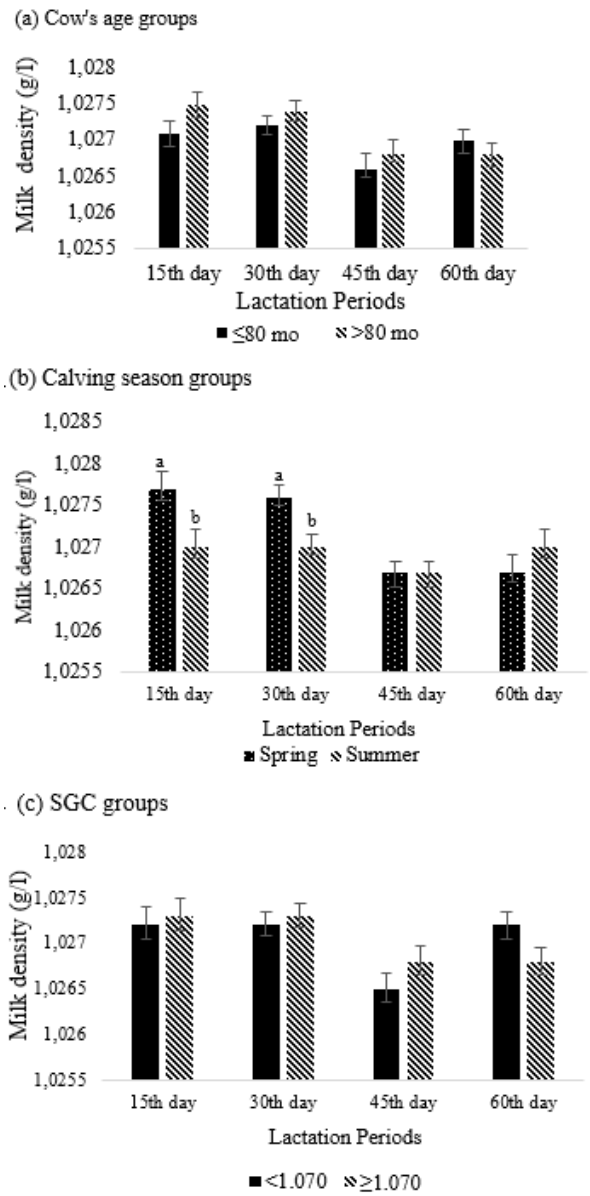

Figure 1. Changes milk density values of buffalo cows according to cow's age, calving season and colostrum SGC groups at different lactation periods

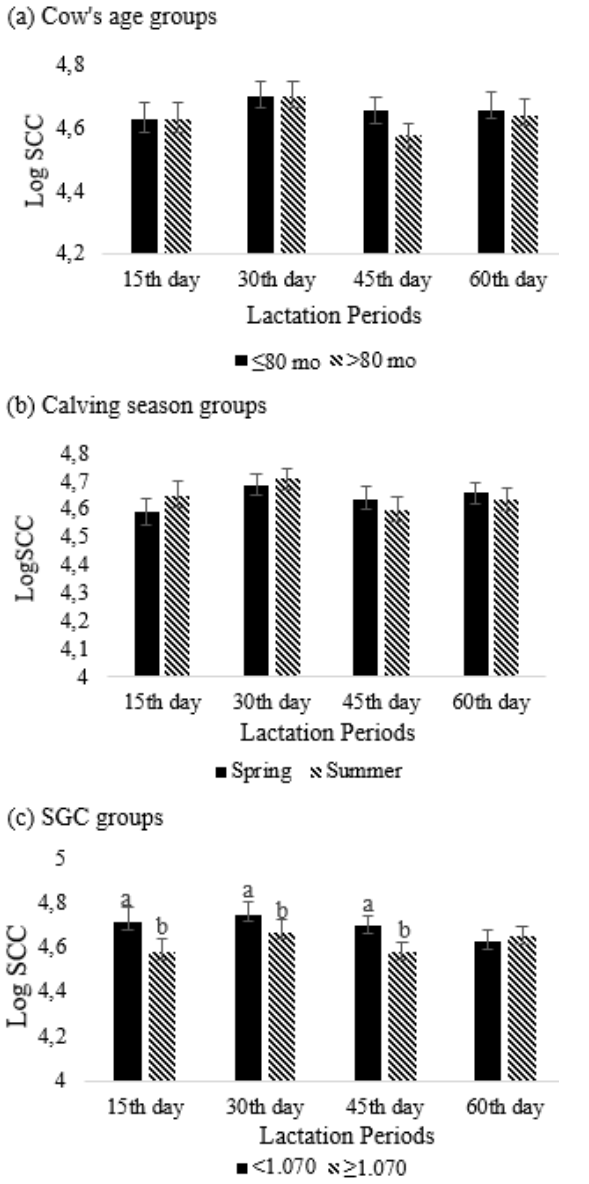

Figure 2. Changes Log SCC values of buffalo cows according to cow's age, calving season and colostrum SGC groups at different lactation periods.

4. Discussion

The DM percentage in this study was lower than that reported in a previous study conducted on Murrah buffalo cows (Sundaram and Harharan, 2013). However, these results were consistent with some studies (Şekerden and Avşar, 2008; Sarfarz et al., 2008) conducted on water buffalo cows but higher than those reported in other studies (Gürler et al., 2013; Şahin et al., 2014). The fat percentage was higher than that reported in some studies (Sarfarz et al., 2008; Tripaldi et al., 2010; Gürler et al., 2013; Şahin et al.,2014) conducted on water buffalo cows. However, the fat percentage in our study was lower than that reported in other studies (Aurelia et al., 2009; Sundaram and Harharan, 2013). Additionally, the SNF percentage of buffalo milk has previously been reported as lower by Şahin et al. (2014).

The protein percentages of the present study were consistent with those reported by Şahin et al. (2014). Conversely, Sarfarz et al. (2008), Tripaldi et al. (2010) and Gürler et al. (2013) reported lower values. The lactose percentage was lower in some studies conducted on water buffalo cows (Sarfarz et al., 2008; Şahin et al., 2014) compared to our results, but higher than that reported in other studies (Gürler et al., 2013; Tripaldi et al., 2010). The milk density value of the present study was consistent with those reported by Şekerden and Avşar (2008). Unlike these results obtained in this study, Şahin et al. (2014) have reported a higher value for milk density. Generally, the mean milk component values were within appropriate ranges for the entire study period. However, the differences observed between the values of some milk components obtained in our study and the results of other studies can be explained by

multiple effects, such as breed, feeding, geographical location, herd management practices, barning systems, body condition score, udder health, heat stress and milking temperament (Damé et al., 2010; Şahin and Yıldırım, 2012; Erdem and Okuyucu, 2019; Boro et al., 2018).

Considering that the SCC in milk produced from a healthy cow is generally <200,000 cells/ml (Aytekin and Boztepe, 2014), the data obtained in this study showed that the SCC value of the milk produced during the early lactation period of Anatolian buffaloes is low. Also, the SCC value was higher than that in a previous study conducted on Anatolian buffalo cows (Şahin et al., 2014). However, the SCC value in this study was lower than that presented in other studies (Şahin et al., 2016; Şahin et al., 2017). It also had a lower SCC value compared to the results of studies in cattle (Atasever and Erdem, 2009; Coban et al., 2009; Stádník and Atasever, 2015). The differences in SCC value of buffalo and cattle are mostly attributed to differences in mammary anatomy and physiology. Several authors stated that buffaloes prevent microorganisms from infecting the udder due to their tight teat sphincter and long narrow teat canal compared to cattle, and because of these differences, buffalo cows have a strong defense mechanism against mastitis (De et al. 2010; Şahin et al., 2017).

In present study, the calving season did not affect protein and lactose content in all lactation periods. However, the highest fat percentage was found in the summer months, compared to the spring months on day 15 of lactation. Contrary to our findings, Patbandha et al. (2015) have reported that the effect of calving season on protein and lactose percentage in Jafarabadi buffalo cows was statistically significant. In an earlier study, Yadav et al. (2013) have reported that the effect of calving season on fat percentage was significantly different. These authors noted that the fat percentage of Murrah buffalo cows calving in the summer months was higher than in the winter months. However, the number of cows calving in the summer months was lower than the number calving in the winter months. The authors associated the lower milk fat ratio of cows calving in summer compared to cows calving in winter with a negative correlation between milk yield and milk fat. These discordances between our findings and results noted previously may be attributed to climatic differences in the geographic regions.

In our study, the effect of the age of the cow on all the milk components was statistically insignificant on day 15 of lactation. Furthermore, the protein (on days 30 and 45 of lactation) DM and SNF (on day 45 of lactation) percentage in the older group of buffalo cows was higher than in the younger buffalo cows. The observed higher DM, SNF, protein content in the cows with age of > 80 mo may be attributable to changes in various physiological mechanisms that support reproductive performance when buffalo cows reach mature equivalents (do Nascimento Rangel et al., 2014; Erdem et al., 2022a). Similarly, Sodi et al. (2008) have observed that the effect of parity on the fat and protein percentages in Murrah buffaloes was not statistically significant. In contrast to Sodi et al. (2008), Sundaram and Harharan (2013) have reported that the effect of parity on DM, fat and protein percentages in Murrah buffalo cows was statistically significant. Observed variations among the obtained findings can be explained by the multi-effects of the variability in locations, dry period management, lactation period, genotype, live weight and barning.

In the our study, as the SGC of buffalo cows at first milking increased, the DM, SNF and protein percentages on day 15 of lactation increased. These findings showed the relationship between SGC and some milk components, a result of the effect of dry period management on colostrum quality (Erdem and Okuyucu, 2020). Hence, sequential effects occurred on milk components in the later stages of lactation. Collier et al. (2012) have demonstrated that changes in the udder histology of cows during the dry period initiated significant changes in the composition of udder secretions. These authors have also reported that the metabolic status of the cow after calving may differ according to changes in feeding conditions and grouping during the dry period. Therefore, the management and feeding of buffaloes in the dry period may directly affect the quality of the products produced in both colostrum and milk yield periods. Considering that the production of good-quality colostrum after calving is associated with a healthy udder and successful dry period management, it is expected that cows producing high-quality colostrum will have high milk components during the lactation period.

The findings obtained in our study showed that the calving season had no effect on the SCC value for all lactation periods. Similar to these findings, studies conducted on Holstein cows (Erdem et al., 2007; Kul et al., 2019) have also reported no differences between the log SCC values for cows calving in the summer months and the log SCC values for cows calving in the spring months. Because the SCC value depends mainly upon season, climatic conditions inside the barn and management in dairy farms, the observed high SCC value in hot-humid climates are attributed to high humidity and ambient temperature (Singh and Ludri 2001). Furthermore, the authors reported that the SCC value increased because the teats were exposed to more microorganisms in hot-humid conditions. Several authors have indicated that the log SCC values for buffalo cows calving in the summer months were higher than for those calving in the spring months (Şahin et al., 2017). Our findings did not support these ideas, as our study failed to evidence calving season has an effect on SCC.

In this study, the differences in cow's age did not affect SCC values for all lactation periods. The findings were consistent with the results of the study conducted on cattle by Singh and Ludri (2001). However, a previous study (Şahin et al., 2017) on Anatolian buffalo cows has

reported that a statistically significant decrease in the log SCC value of the cows with increasing parity was determined. Although several authors reported that the first parity had a higher SCC value than the later parities due to the different responses of the mechanisms supporting defense mechanisms against udder infection in different parities (Muggli, 1995; Şahin et al., 2017), the SCC value was not affected by the cow's age in our study.

As expected, the buffalo age and calving season affected some milk components in different lactation periods. However, the high SGC in the first milking colostrum positively affected some milk components and SCC, which are important milk quality factors in the later stages of lactation. Because the density or specific gravity of colostrum is mainly dependent on basic components (DM, fat, protein, lactose, mineral, vitamins etc.), biologically active elements and bacteriostatic substances such as immunoglobulins, lactoperoxidase, lactenins, lactoferrins, lysozymes and leukocytes (Puppel et al., 2019), differences in milk components and SCC values of early lactating cows can be attributed to differences in colostrum specific gravity. These active biological substances have an important role in both maternal immunity and calf immunity. In order to explain these results, it is important to understand the importance of dry period management. Many authors have reported that the dry period of management (dry period length and feeding), an important part of herd management practices, is closely related to optimal milk production and adequate body reserve of cows before calving. The cumulative information in the literature suggests that low milk yield due to poor dry period management is due to multiple physiological factors (Collier et al., 2012). The most important physiological factors for milk yield in the next lactation are udder involution and cell proliferation in the dry period. Because colostrogenesis begins 15 to 20 days before calving (Collier et al., 2012), poor dry period management may adversely affect the level of immunoglobulins associated with colostrum quality (Erdem and Okuyucu, 2020). Thus, the management of buffalo cows is likely to affect the quality ingredients of the colostrum and milk produced with the onset of lactation. In addition, Pecka-Kiełb et al. (2018) have reported the relationship between feeding cows in the dry period and the quality of colostrum and milk produced from birth. The same authors have observed that colostrum and milk synthesis may be adversely affected by the onset of lactation in cases where the necessary nutrients cannot be provided in the dry period. These statements have been verified by Collier et al. (2012) and Erdem and Okuyucu (2020). When these statements are evaluated as a whole, dry period management has a significant effect on the cellular regeneration of the udder quarters of buffalo cows (Collier et al., 2012), as well as the rumen, due to the concentrated feed consumed during lactation. Therefore, dry period management (optimal dry period duration and care-feeding), a sensitive period for buffalo cows, is an important environmental factor in increasing colostrum and milk quality at the beginning of lactation. The findings herein demonstrate that the dry period is an important part of herd management in buffalo rearing.

5. Conclusion

The findings obtained in our study indicated that age, calving season and specific gravity of the first milking colostrum of different lactation periods affected some milk components. In particular, the specific gravity, considered an important indicator of colostrum quality, has positive effects on some milk components and SCC values in early lactation. As the specific gravity of the first milking colostrum of buffalo cows increased, some milk components increased in the early lactation period, while the SCC value decreased. Taken together, numerous non-genetic environmental factors (including parity, dry period length, feeding, body condition score, age, and calving season) may indirectly or directly affect colostrum and milk quality in buffalo cows. Some of these factors can be controlled by sensitive herd management. Dry period management is the most important of these environmental factors. Therefore, a better understanding of the importance of dry period management, which is closely related to the colostrum and milk quality of buffalo cows, will be beneficial to improve many future yield aspects.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.E.	İ.C.O.	H.D.
C	80	10	20
D	40	30	30
S	100		
DCP	20	10	70
DAI	100		
L	20	60	20
W	50	50	
CR	50	40	10
SR	70	30	
PM	60	20	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Permissions were obtained from the Ondokuz Mayis University Animal Experiments Local Ethics Committee. All procedures performed involving animals were in accordance with the ethical standards approved by the

Ondokuz Mayis University Animal Experiments Local Ethics Committee (protocol code: 2014/20 and date: June 03, 2014).

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Research Article
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THE CRITICAL LEVELS OF BORON FOR GERMINATION AND SEEDLING GROWTH OF MELON

Gamze KAYA[1]*

[1]*The Ministry of Agriculture and Forestry, Provincial Directorate of Eskişehir, 26150, Eskişehir, Türkiye*

Abstract: This study was conducted to determine the effects of boron levels on germination and seedling growth of melon cultivars with different fruit characteristics under laboratory conditions. The seeds of three melon cultivars (Hasanbey 1, Kırkağaç 589, and Toros Sarıbal) were germinated at different boron levels (0, 20, 40, 60, 80, and 100 mg L^{-1}) constituted by sodium borate ($Na_2B_8O_{13}.4H_2O$). Germination percentage, mean germination time, germination index, seedling growth parameters, and seedling dry matter were investigated. The optimum and toxicity levels of boron were calculated by regression analysis. Germination percentage, mean germination time, and germination index were not affected by increasing boron levels. A boron dose of 20 mg L^{-1} promoted root length, shoot length, and seedling fresh weight of melon; however, seedling growth of melon cultivars was inhibited at higher boron levels than 40 mg L^{-1}. Seedling dry weight and dry matter significantly enhanced when the boron levels were increased. Root length was more sensitive to boron than shoot length. Melon cultivars showed different responses to boron levels and the highest seedling growth parameters were obtained from Kırkağaç 589. The optimum boron level for shoot growth was calculated as 12.8 mg L^{-1}, while the inhibitory level of boron for root growth was 65.4 mg L^{-1}. The toxicity of boron on the germination performance was not detected and higher levels than 20 mg L^{-1} inhibited seedling growth of melon.

Keywords: *Cucumis melo* L., Genotype, Sodium borate, Germination, Seedling growth

***Corresponding author:** The Ministry of Agriculture and Forestry, Provincial Directorate of Eskişehir, 26150, Eskişehir, Türkiye
E mail: pascalcik@hotmail.com (G. KAYA)
Gamze KAYA https://orcid.org/0000-0002-9815-2672

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1. Introduction

Seed germination is adversely influenced by several biotic and abiotic stress factors. The major abiotic stresses in the seedbed are extreme temperatures, drought, flooding, salinity, and high heavy metal concentrations. Also, excessive or scarcity of plant nutrients may change the germination performance and subsequent seedling growth. Of these nutrients, boron (B) which is a micronutrient affecting adversely or positively plants, plays a key role in seed formation because healthy flowers cannot be produced, and no seeds develop in case of deficiency in B (Mozafar, 1993; Landi et al., 2019). However, the plants grown under increasing B concentration in the soil produce high B content in seeds, which negatively affected the germination ability (Rerkasem et al., 1997). B concentration in the arable soil changes from 1 to 467 mg kg^{-1}, but its concentration generally varies between 0.5 and 5 mg kg^{-1} (Gupta, 2016). In Türkiye, 46.2% of the agricultural soils suffer from B deficiencies, while 19.4% and 3.3% of the total area had excess and toxic concentrations of B, respectively (Kıllıoğlu, 2022). Increased B concentrations in soils resulted in an increase in uptake by plant tissues and transportation to the seeds. Recently, Kintl et al. (2023) showed a significant negative relationship between B concentration in black medic seeds and germination. Lower B levels have been used as priming agents for improvement in germination while higher levels of B had a toxic effect on seeds (Bonilla et al., 2004; Alamri et al., 2018; Kintl et al., 2023). Nevertheless, irrigation water contains B, which continuously causes the accumulation of B in the soils in arid and semi-arid conditions due to poor drainage; consequently, it can reach toxic levels (Reid, 2007; Tanaka and Fujiwara, 2008; Landi et al., 2019).

Melon (*Cucumis melo* L.) is a warm-season vegetable with juicy and sweet fruit, and it is cultivated under open fields and greenhouse conditions. There are a lot of melon cultivars with several fruit types (Vural et al., 2000) and they are grown on 63.000 hectares, with a fruit production of 1.6 million tons in Türkiye (Anonymous, 2023). Melons are classified as moderately boron-tolerant plants (Maas and Grattan, 1999); however, it depends on genotypic factors and plant growth stages. In the previous studies, the effects of B on plant growth, physiological parameters, and yield of melon were investigated, but germination and early seedling growth performance of melon under boron stress have not been reported (Goldberg et al., 2003; Shalaby and El-Messairy, 2018). This study aimed to determine the toxic and promoter levels of boron for

seed germination and early seedling growth of melon cultivars.

2. Materials and Methods

A laboratory experiment was performed to determine the effect of boron levels on germination and seedling growth of melon cultivars at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2023. In the study, the seeds of melon cultivars with different fruits morphology (Hasanbey 1, Kırkağaç 589, and Toros Sarıbal) and sodium borate (20.9% $Na_2B_8O_{13}.4H_2O$) were used as materials. Some morphological characteristics of melon cultivars were given in Table 1.

The seeds were germinated at six boron levels (0, 20, 40, 60, 80, and 100 mg B L^{-1}) which were prepared from sodium borate (Etidot-67). Distilled water was used for control (0 mg L^{-1}). Four replicated fifty seeds from each melon cultivar were placed between three filter paper sheets with a dimension of 20 × 20 cm and each paper was watered with 7 mL for respective boron solutions. Just after the filter papers were rolled, they were put into a sealed plastic bag. The packages were incubated at 25 °C for 10 days under dark conditions. Two millimeters of radicle protrusion was considered as the germination criterion. Germination percentage (GP), mean germination time (MGT), and germination index (GI) were also calculated with the Equation 1, 2 and 3:

GP (%)= (Germinated seeds at final day / Total seeds) × 100, (ISTA, 2018) (1)

MGT (day)= $\sum$ Dn / $\sum$ n, (ISTA, 2018) (2)

where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test.

GI= (Number of germinated seeds + Days of the first count) + …. + (Number of germinated seeds + Days of the final count), Salehzade et al. (2009) (3)

On the last day, randomly selected ten seedlings from each treatment were sampled to measure root length, shoot length, and seedling fresh and dry weight. The seedlings were dried in an air oven at 80 °C for 24 h.

2.1. Statistical Analysis

The experiment was set up in a two-factor factorial in a completely randomized design with four replicates. The data were analyzed by ANOVA and the differences were compared by the Least Significant Differences (LSD) test at a 5% level. The optimal boron levels for seedling growth were estimated by a quadratic regression equation. The independent variable (boron level) was attained on X-axis and the dependent variable (length) on the Y-axis. The peak value (-b/2a) from the regression equation ($y= -ax^2+bx+c$) in shoot length and the toxic dose of boron which is lead to a 50% reduction in root length was predicted from the linear regression ($y= -ax+b$) (Fallahi et al., 2017; Wang et al., 2020).

3. Results and Discussion

Analysis of variance revealed that there were no significant effects of boron levels on the germination parameters of melon cultivars (Table 2). However, there were statistically significant differences among melon cultivars for germination percentage, mean germination time, and germination index. Kırkağaç 589 possessed the highest germination percentage and germination index, while it had the shortest mean time for germination. Because there was no information on the germination performance of melon seeds under boron stress, the results of the studies conducted with other crops were considered. For instance, Rerkasem et al. (1997) reported no significant drop in the germination of soybean due to boron levels. The findings of Kaya et al. (2023) confirmed no significant changes in the germination percentage of sunflower, soybean, and poppy seeds and increased germination index up to 90 mg L^{-1}. Contrarily, Turhan and Kuşçu (2021) determined significant differences among B levels for germination percentage, index, energy, and mean germination time in watermelon, pepper, and eggplant, although they used lower doses (up to 16 mg L^{-1}) than in the present study. Furthermore, they reported a decrease in germination percentage and germination index, but an increase in mean germination time. This may be explained by using different plant species, or the germination criterion considered.

Boron levels significantly affected the seedling growth parameters of melon cultivars ($P<0.01$) and the interaction of cultivar × boron level was not significant only for seedling dry weight (Table 3). Kırkağaç 589 produced a higher shoot and root length, seedling fresh and dry weight, and dry matter than the other cultivars. An increase was observed in seedling growth at a boron level of 20 mg L^{-1}. They were decreased by increasing boron levels above 40 mg L^{-1}. This result showed a similarity with the findings of Mokhtari et al. (2022), who reported a significant increase in shoot and root length of sugar beet exposed to boron levels up to 1.6 ppm.

Table 1. Some fruit morphological characteristics of melon cultivars

Cultivars	Skin color	Flesh color	Fruit shape of longitudinal section
Hasanbey 1	Green	Greenish white	Round-oval
Kırkağaç 589	Green and black spots on yellow	White	Long elliptic
Toros Sarıbal	Yellow	Light yellow	Long elliptic

Table 2. Effects of boron levels on germination parameters of melon cultivars

Factor	Germination percentage (%)	Mean germination time (day)	Germination index
Cultivar (A)			
Hasanbey 1	87.8^{c}	2.34^{a}	20.1^{c}†
Kırkağaç589	96.3^{a}	2.02^{c}	23.9^{a}
Toros Sarıbal	91.7^{b}	2.11^{b}	22.2^{b}
Boron level (B)			
0 mg L^{-1}	91.3	2.17	21.8
20 mg L^{-1}	91.0	2.15	21.8
40 mg L^{-1}	92.8	2.12	22.5
60 mg L^{-1}	92.2	2.15	22.1
80 mg L^{-1}	92.2	2.15	22.2
100 mg L^{-1}	92.2	2.21	21.9
Analysis of variance			
A	**	**	**
B	NS	NS	NS
A×B	NS	**	NS

**= significant at 1%, NS= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05.

Table 3. Effects of boron levels on seedling growth parameters of melon cultivars

Factor	Shoot length (cm)	Root length (cm)	Seedling fresh weight (mg $plant^{-1}$)	Seedling dry weight (mg $plant^{-1}$)	Dry matter (%)
Cultivar (A)					
Hasanbey 1	6.35^{b}	6.26^{b}	208^{b}	21.0^{c}	11.1^{b}†
Kırkağaç 589	7.34^{a}	6.53^{a}	255^{a}	26.0^{a}	10.9^{b}
Toros Sarıbal	5.67^{c}	6.17^{b}	213^{b}	24.4^{b}	12.4^{a}
Boron level (B)					
0 mg L^{-1}	7.77^{c}	9.73^{b}	272^{b}	22.3^{c}	8.19^{e}
20 mg L^{-1}	9.05^{a}	10.56^{a}	298^{a}	23.1^{bc}	7.76^{e}
40 mg L^{-1}	8.24^{b}	8.13^{c}	265^{b}	23.8^{abc}	9.06^{d}
60 mg L^{-1}	6.13^{d}	4.80^{d}	211^{c}	23.8^{abc}	11.37^{c}
80 mg L^{-1}	4.42^{e}	2.91^{e}	173^{d}	25.0^{a}	11.42^{b}
100 mg L^{-1}	3.11^{f}	1.78^{f}	134^{e}	24.0^{ab}	17.92^{a}
Analysis of variance					
A	**	**	**	**	**
B	**	**	**	**	**
A×B	**	**	**	NS	**

**= significant at 1%, NS= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05.

The interaction of cultivar × boron level on seedling growth parameters was shown in Figure 1. The shoot length of melon cultivars was changed by boron levels. Boron levels of 20 and 40 mg L^{-1} induced the shoot length, but higher levels inhibited it considerably (Figure 1A). Kırkağaç 589 gave the highest shoot length under all boron levels. At the highest boron level, the shoot length was reduced in Hasanbey 1 by 56%, in Kırkağaç 589 by 61%, and in Toros Sarıbal by 62%. Similar results were announced in sugar beet by Mokhtari et al. (2022) who reported an increase in seedling length in sugar beet exposed to boron levels up to 1.6 ppm. Habtamu et al. (2014) recorded a constant reduction in the shoot length of safflower at higher boron levels than 1 mg L^{-1}. The root length was depressed by boron levels except for 20 mg L^{-1} at which it was promoted (Figure 1B). Hasanbey 1 had the shortest length of root at boron levels of 60 and 80 mg L^{-1}, while no significant differences among cultivars were observed at 100 mg L^{-1}. The root growth was more sensitive to boron levels, and it declined by 81%. This result was confirmed by the findings of Habtamu et al. (2014) and Kaya et al. (2023) in safflower. Depending on shoot and root depletion, the seedling fresh weight was changed. Although Kırkağaç 589 had the highest fresh weight at all levels of boron, the minimum decrease was obtained from Toros Sarıbal by 48.8%, followed by Hasanbey 1 by 49.6% and Kırkağaç 589 by 50%. A boron level of 20 mg L^{-1} supported seedling fresh weight, but higher levels caused a significant decline (Figure 1C). Cıkılı et al. (2013) demonstrated a drastic diminish in shoot and root dry weight in cucumber because they grow the plants at maturity under increasing boron levels of 20 mg kg^{-1}. On the other hand, the dry matter of melon seedlings increased under increasing boron levels (Figure 1D). Dry matter indicates the ratio of dry weight to fresh weight,

and it gives a more precise result than dry wights. In the study, a significant enhancement in dry matter was determined in Toros Sarıbal at 40 mg L^{-1} and higher boron levels caused an enhancement in dry matter.

The relationship between boron levels and shoot and root length was calculated by quadratic and linear regression equations, respectively. The peak value of shoot length was calculated at a boron level of 12.8 mg L^{-1} by means of the quadratic equation of $y=-0.0007x^2+0.0179x+8.2771$, with the significant determination coefficient $R^2=0.935^{**}$ (Figure 2). The shoot length gradually decreased at higher boron levels. However, the significant relationship between root length and boron levels was determined in linear regression, and the toxic dose of boron causing a 50% reduction in root growth was identified as 65.4 mg L^{-1} which was calculated by the equation of $y= -0.0943x+11.036$, $R^2= 0.924^{**}$ (Figure 3).

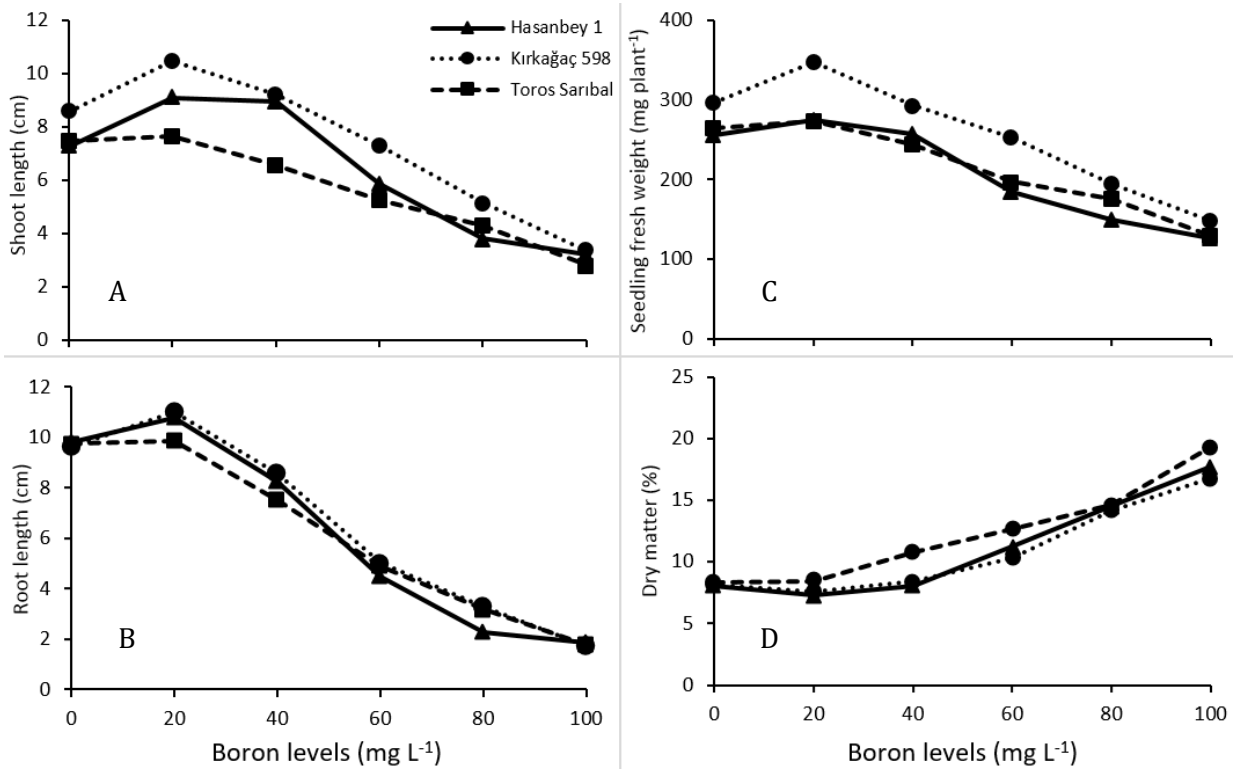

Figure 1. Changes in shoot length (A), root length (B), seedling fresh weight (C), and dry matter (D) of melon cultivars under different boron levels.

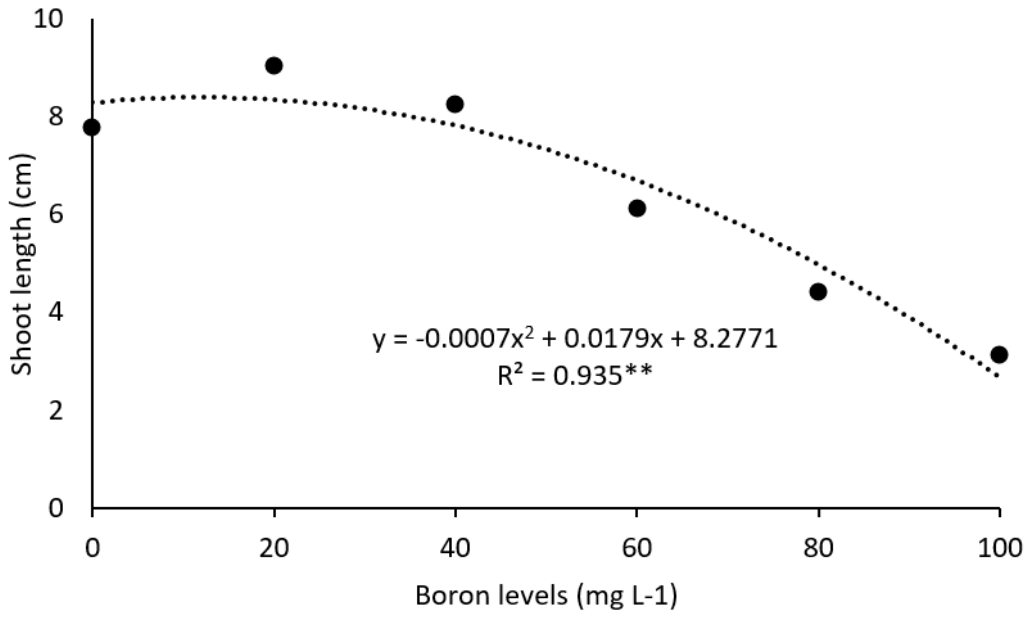

Figure 2. The quadratic regression between boron levels and mean shoot length of melon cultivars.

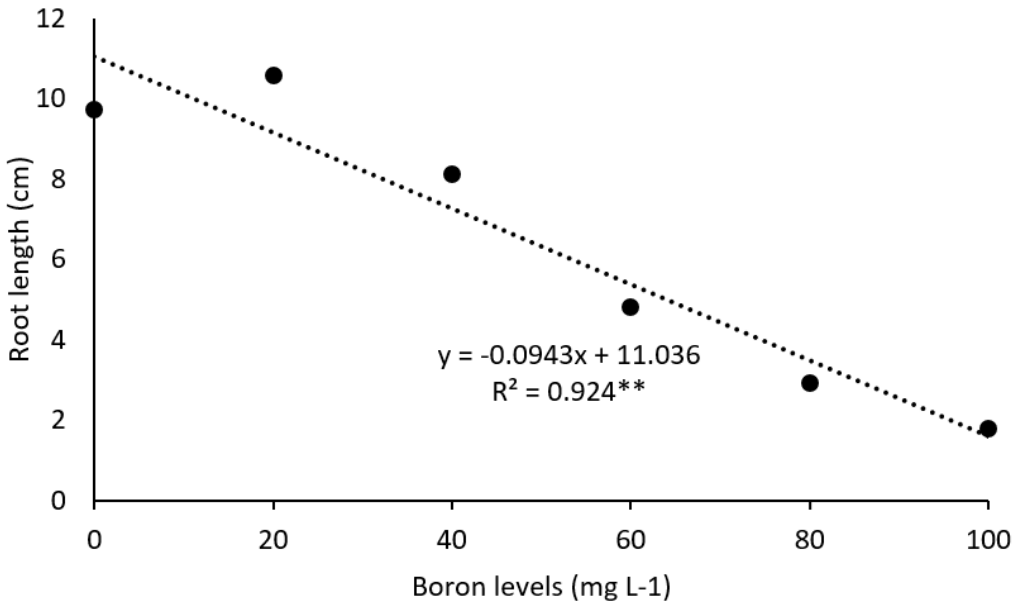

Figure 3. The linear regression between boron levels and mean root length of melon cultivars.

4. Conclusion

The study demonstrated that the germination characteristics of melon cultivars were not adversely influenced by boron levels up to 100 mg L^{-1}. However, increased boron levels depressed the seedling growth, and the inhibitory effects on shoot length were identified at 40 mg L^{-1}. The sensitivity of seedling parts of melon was different and root growth was much more affected by boron levels than shoot growth. It was concluded that the positive effect of boron on seedling growth was up to 12.8 mg L^{-1}, but the detrimental impact was at 65.4 mg L^{-1} during the early seedling development of melon.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	G.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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EXPLORING *I*, *bc-1²* AND *bc-3* GENE LOCUS IN PROMISING COMMON BEAN LINES

Orkun EMİRALİOĞLU[1], Mehmet Zahit YEKEN[1]*, Ali ÇELİK[2], Vahdettin ÇİFTÇİ[1]

[1]*Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Field Crops, 14030 Bolu, Türkiye*
[2]*Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Plant Protection, 14030 Bolu, Türkiye*

Abstract: Common beans (*Phaseolus vulgaris* L.), known as the "poor man's meat", is an internationally important legume crop that appeals to farmers as well as consumers. Many biotic stressors such as bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV) cause significant yield and quality losses in common bean. The most efficient and cost-effective way to lessen of these factors is to develop resistant cultivars. Local genotypes have been cultivated in many areas for years and have varied distinguishing characteristics as a result of spontaneous mutations. Identifying bean germplasm harboring gene sources is critical for developing resistant cultivars against BCMV and BCMNV. For this purpose, a total of 43 promising common bean lines selected from local genotypes cultivated across various regions of Türkiye were subjected to screening using diverse molecular markers (ROC11, SBD-5 and SW-13) to investigate gene sources associated with BCMV and BCMNV. The findings revealed that 21 lines had both *I* and *bc-1²* gene locus. In addition, the *bc-1²+bc-3* gene loci were discovered to be present in the 8 common bean lines. The combination of *I+bc-3* resistance genes, which guarantees immune reaction to BCMV and BCMNV, was found in only one line; YLV-32. These gene sources can be evaluated in marker-assisted breeding to develop modern cultivars resistant to BCMV and BCMNV by breeders.

Keywords: Common bean, Marker, Viral pathogens

***Corresponding author:** Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Field Crops, 14030 Bolu, Türkiye
E mail: yekenmehmetzahit@gmail.com (M. Z. YEKEN)
Orkun EMİRALİOĞLU https://orcid.org/0000-0001-8021-2844
Mehmet Zahit YEKEN https://orcid.org/0000-0003-0490-371X
Ali ÇELİK https://orcid.org/0000-0002-5836-8030
Vahdettin ÇİFTÇİ https://orcid.org/0000-0003-0547-9527

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a diploid plant (2n = 22) and is known to be the most widely grown crop in the grain legume crops (Nadeem et al., 2021). It originates from Latin America and is domesticated independently in the Andean and Mesoamerican centers, which are characterized by large seeds and small seeds (Sperotto and Ricachenevsky, 2017). The seeds of this crop are an important source of protein, minerals and vitamins, playing a vital role in the human diet for lower-income families of the least developed countries of Africa such as Burundi, Democratic Republic of Congo, Rwanda, Uganda and Latin America such as Guatemala, Nicaragua, and El Salvador (Blair, 2013). Türkiye's historical position at the crossroads of the Palearctic region, both culturally and geographically, makes it potentially influential in the dissemination of common bean genotypes from Asia to Europe and Africa. Although Türkiye is not recognized as the primary origin or center of domestication for the common bean, the previous studies indicated a wide diversity in common bean germplasm from Türkiye (Nadeem et al., 2018; Haliloğlu et al., 2022; Özkan et al., 2022; Yeken et al., 2022). These studies revealed that the common landrace collection in Türkiye harbored a substantial amount of genetic diversity. Different local genotypes have been growing by smallholder-farmers in Türkiye for years. The wide genetic diversity of these genotypes constitutes a significant potential source of resistance to biotic stresses such as BCMV/BCMNV.

Viruses belonging to the Potyvirus genus, which is part of the *Potyviridae* family (Çelik et al., 2023a), known as the largest family of plant viruses, pose a significant threat as viral pathogens that severely impact common bean production (Kyle and Provvidenti, 1993). Aphids, seeds, and pollen all play a role in the non-persistent transmission of BCMV/BCMNV (Galvez and Morales, 1989; Silbernagel et al., 2001; El-Sawy et al., 2014; Çelik et al., 2023b). The most influential management technique for these viruses is to combine the use of healthy seeds with resistant cultivars (Drijfhout, 1978; Worrall et al., 2015). *I* gene which is the dominant (Ali, 1950) and six recessive alleles (*bc-u*, *bc-1*, *bc-1²*, *bc-2*, *bc-2²* and *bc-3*) spreaded throughout four loci, providing resistance to BCMV/BCMNV (Drijfhout, 1978). Hence, the *I* gene and *bc*-genes have been performed to obtain virus-resistant common bean cultivars in breeding programs. Containing *I+bc-3* or *I+bc-2²* gene combinations, which are known to give resistance to the majority of BCMV/BCMNV strains, have been employed in the

development of novel common bean cultivars (Drijfhout, 1994; Kelly, 1997).

In breeding programs, molecular markers linked to resistance genes for BCMV/BCMNV are commonly utilized to efficiently select desired gene combinations. This approach is more cost-effective and time-efficient compared to greenhouse screening methods (Deligoz et al., 2021). In literature, various specific markers associated with resistance genes were reported such as SW-13 SCAR (Sequence-characterized Amplified Regions) marker for *I* gene, ROC11 SCAR marker for *bc-3* gene, and SBD-5 SCAR marker for the *bc-1²* gene by Melotto et al. (1996), Miklas et al. (2000) and Johnson et al. (1997), respectively. These markers were used to discover desired genes to develop common bean varieties resistant to BCMV/BCMNV in diverse investigations (Deligöz ve Sökmen, 2013; Pasev et al., 2014; Yeken et al., 2018; Palacıoğlu et al., 2020; Deligöz et al., 2022). While previous studies have identified desirable genes for BCMV/BCMNV in various genetic resources within Türkiye, conducting a comprehensive investigation across different germplasm collections is of utmost importance. Thus, the objective of this study was to examine the presence of *I*, *bc-1²*, and *bc-3* genes in promising common bean lines selected from local genotypes in different provinces of Türkiye. The findings will support further breeding studies for developing new common bean cultivars resistant to BCMV/BCMNV.

2. Materials and Methods

2.1. Plant Material

The forty-three common bean lines selected as promising according to yield and yield components from local genotypes which were collected from different provinces of Türkiye in projects previously conducted projects supported by TUBITAK (Project ID: 115R042 and 109O163) were used as material in the study. The Cornell 49–424, Perry Merrow, (MDRK) Michigan Dark Red Kidney, BMN-RMR-13 (Reg. Number: GP-252, PI 642019, USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD; Pastor-Corrales et al., 2007) were included in the study as control. These genotypes obtained from the United States Department of Agriculture Research Service (USDA-ARS). The information of the common bean lines used in the study was presented in Table 1.

Table 1. The passport data of common bean lines used in the study

No	Lines	Collection Site	District	Altitude(m)	Coordinates
1	Balıkesir-3	Balıkesir	Manyas	428	40° 5'58.61"N/27°56'16.65"E
2	Balıkesir-4	Balıkesir	Manyas	30	40° 7'16.68"N/27°51'15.26"E
3	Balıkesir-19	Balıkesir	Sındırgı	1051	39°18'52,7"N/28°32'53,1"E
4	Bilecik-7	Bilecik	Pazaryeri	876	39°59'12.52"N/ 29°51'7.17"E
5	Bingöl-8	Bingöl	Merkez	1154	39°02'06.1"N/ 40°27'14.4"E
6	Bingöl-12	Bingöl	Merkez	1542	39°02'25.2"N/ 40°29'08.1"E
7	Bingöl-23	Bingöl	Kiğı	1489	39°17'11.8"N/ 40°20'04.0"E
8	Bingöl-25	Bingöl	Solhan	1176	38°54'29.4"N/ 40°56'39.9"E
9	Bingöl-50	Bingöl	Yedisu	-	-
10	Bitlis-5	Bitlis	Hizan	1629	38°13'35.5"N/ 42°25'14.4"E
11	Bitlis-68	Bitlis	Mutki	1303	38°29'08.1"N/ 41°48'17.4"E
12	Bitlis-123	Bitlis	Güroymak	1615	38°30'09.8"N/ 42°07'10.5"E
13	Bitlis-124	Bitlis	Güroymak	1615	38°30'09.8"N/ 42°07'10.5"E
14	Bursa-3	Bursa	Yenişehir	377	40°10'18.45"N/29°37'15.12"E
15	Bursa-4	Bursa	İnegöl	327	40°4'18.83''N/29°26'47.76''E
16	Bursa-21	Bursa	Kestel	435	40° 7'39.19"N/ 29°21'9.43"E
17	Bursa-22	Bursa	Kestel	360	40°10'2.02"N/ 29°18'58.01"E
18	Bursa-23	Bursa	Kestel	360	40°10'2.02"N/ 29°18'58.01"E
19	Bursa-24	Bursa	Orhaneli	487	39° 48' 8.96''N/29° 2' 7.45''E
20	Çanakkale-2	Çanakkale	Yenice	320	39°57'6.22"N/ 27°10'54.75"E
21	Çanakkale-4	Çanakkale	Biga	25	40°14'38.70"N/ 27°22'17.65"E
22	Çanakkale-6	Çanakkale	Biga	25	40°17'26.36"N/ 27°25'14.56"E
23	Çanakkale-8	Çanakkale	Bayramiç	100	39°44'15.48"N/ 26°41'34.82"E
24	Düzce-2	Düzce	Merkez	859	40°42´28.42"N/31°13´33.16"E
25	Düzce-3	Düzce	Merkez	859	40°42´28.42"N/31°13´33.16"E
26	Elazığ-11	Elazığ	Maden	1266	38°30'43.2"N/ 39°33'06.4"E
27	Hakkâri-8	Hakkâri	Merkez	2096	37°36'02.0"N/ 43°41'24.1"E
28	Hakkâri-18	Hakkâri	Merkez	1135	37°29'45.6"N/ 43°34'58.7"E
29	Hakkâri-51	Hakkâri	Merkez	1601	37°25'15.7"N/ 43°33'45.9"E
30	Hakkâri-76	Hakkâri	Merkez	1135	37°29'45.6"N/ 43°34'58.7"E

Table 1. The passport data of common bean lines used in the study (continue)

No	Lines	Collection Site	District	Altitude(m)	Coordinates
31	Malatya-20	Malatya	Doğanşehir	1410	38°03'22.1"N/ 37°44'60.0"E
32	Malatya-25	Malatya	Doğanşehir	1235	38°06'54.4"N/ 37°54'54.7"E
33	Malatya-44	Malatya	Akçadağ	1158	38°15'20.6"N/ 37°55'37.5"E
34	Muş-24	Muş	Hasköy	1350	38°41'04.4"N/ 41°40'25.8"E
35	Muş-48	Muş	Bulanık	1550	39°03'41.7"N/ 42°19'04.2"E
36	Tunceli-8	Tunceli	Pertek	-	-
37	Van-17	Van	Çatak	1702	38°05'51.7"N/ 43°15'37.3"E
38	Van-19	Van	Çatak	1629	38°01'34.2"N/ 43°09'10.9"E
39	Van-49	Van	Gürpınar	1748	38°19'09.1"N/ 43°24'09.6"E
40	Van-64	Van	Bahçesaray	1702	38°06'15.3"N/ 42°51'00.7"E
41	Yalavo-14	Yalova	Çiftlikköy	125	40°39´33.41"N/29°24´36.19"E
42	Yalova-28	Yalova	Merkez	362	40°33'12.70"N/29°12'52.17"E
43	Yalova-32	Yalova	Merkez	428	40°33'38.32"N/29°19'34.07"E

2.2. DNA Extraction

Total DNA was extracted by employing a buffer solution consisting of 125 mM, Tris-HCl pH 8.0, 0.8 M NaCl, 25 mM EDTA pH 8.0, 1% sarcosyl, 1% CTAB, 0.5% sodium disulphite, and 2% PVP-40 (K29-32) following the DArT DNA isolation methodology effectively isolating the genetic material (http://www.diversityarrays.com). After diluting the total DNA to a concentration of 20 ng/L, its quality and concentration were evaluated using a DS-11 FX+ spectrophotometer (DeNovix Inc., USA).

2.3. The Analysis of SCAR Markers

PCR amplifications were carried out in a 20 µL reaction volume, combining 10x PCR buffer, 0.3 µM primer 0.2 µM dNTPs, 1.5 mM $MgCl_2$, 10-20 ng DNA, and 1U Taq DNA polymerase. Amplification reactions were conducted utilizing a T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The temperature profile for PCR amplification in SCAR markers can be found in Table 2. The amplified fragments were resolved on a 1.4% (w/v) agarose gel in 1x TAE buffer. Subsequently, the gel was stained with ethidium bromide, and the fragments were visualized under UV light and documented using the G:BOX F3 gel documentation system (Syngene, Synoptics Ltd., Cambridge, UK). The sizes of the PCR products were determined by using the GeneRuler 100 bp Plus DNA ladders (Thermo Scientific) as reference standards. The presence (+) or absence (-) of amplification products associated with resistance genes was determined in this study.

3. Results and Discussion

Using SCAR markers tightly linked to the resistance genes to BCMV/BCMNV, the forty-three common bean lines were screened for resistance to these viruses. The dominant *I* gene in common bean lines was analyzed using SCAR marker, SW-13. Out of the 43 common bean lines tested, 22 gave the expected product of 690 bp with SW-13 marker, which is related to the *I* gene, as similar to BMN-RMR-13 control (Figure 1). The gene-specific products were not determined in 21 common bean lines and MDRK control (Table 3). The results of the PCR test with SBD-5 marker SBD-5 gave a positive product with 1250 bp for the *bc-1²* gene in all lines (except for Yalova-32). The positive and negative products were also obtained from Perry Marrow and Cornell 49–424, respectively. On the other hand, SCAR marker ROC11 known to be related to the recessive *bc-3* gene was also analyzed in all lines. Findings revealed that 34 common bean lines gave the 350 bp amplified product with this marker, as similar to Perry Marrow (Figure 1; Table 3).

Negative signals were also determined in 9 common bean lines (Balıkesir-19; Bingöl-8; Bingöl-23, Bingöl-50; Bursa-22, Bursa-23, Hakkari-76, Malatya-44 and Yalova-32) and Cornell 49–424 control. The absence of signal reveals the presence of *bc-3* in this marker. Thus, these lines had the recessive *bc-3* gene. The same DNA fragments of the expected size of SW-13, SBD-5 and ROC11 markers were obtained in previous studies. For instance, Yeken et al. (2018) investigated *bc-1²*, *I* and *bc-3* genes using molecular markers in 43 common bean cultivars. Findings revealed that 32 cultivars had the dominant *I* gene, while 40 cultivars contained the *bc-1²* gene. In another study, Palacıoğlu et al. (2020) investigated resistance genes in 39 common bean cultivars using SW-13, SBD-5, ROC11 and eIFE4 markers. They determined the *I+bc-1²* genes in most of the cultivars, and the *bc-3* gene in three cultivars.

SCAR markers were utilized to investigate the presence of the dominant *I* and recessive *bc-1²* genes in a set of 204 dry bean breeding lines (Deligöz et al., 2021). Very recently, Deligöz et al. (2022) screened *I+bc-1²* genes in 58 common bean genotypes using SBD-5 and SW-13 markers, respectively. Out of the tested genotypes, 31 had both the dominant *I* gene and *bc-1²* specific products. In the current study, the combinations of *I+bc-1²* and *bc-1²+bc-3* resistance genes were detected in screened 21 and 8 lines, respectively. However, it was observed that no common bean landrace possessed all three resistance genes.

Table 2. The sequences and PCR conditions of primers associated with resistance genes

Primers	Gene Locus	Marker	Primer sequences (5′–3′)	Band Length	PCR Conditions	References
SW-13	*I*	SCAR	CACAGCGACATTAATTTTCCTTTC CACAGCGACAGGAGGAGTTTA	690	95°C 4 min, 94°C 10 s, 60°C 40 s, 72°C 2 min 35 cycles, 72°C 5 min	Melotto et al. (1996)
SBD-5	*bc-1²*	SCAR	GTGCGGAGAGGCCATCCATTGGTG GTGCGGAGAGTTTCAGTGTTGACA	1250	95°C 4 min, 94°C 10 s, 65°C 40 s, 72°C 2 min 35 cycles, 72°C 5 min	Miklas et al. (2000)
ROC11	*bc-3*	SCAR	CCAATTCTCTTTCACTTGTAACC GCATGTTCCAGCAAACC	350/420	95°C 4 min, 94°C 10 s, 65°C 10 s, 72°C 30 s 35 cycles 72°C 5 min	Johnson et al. (1997)

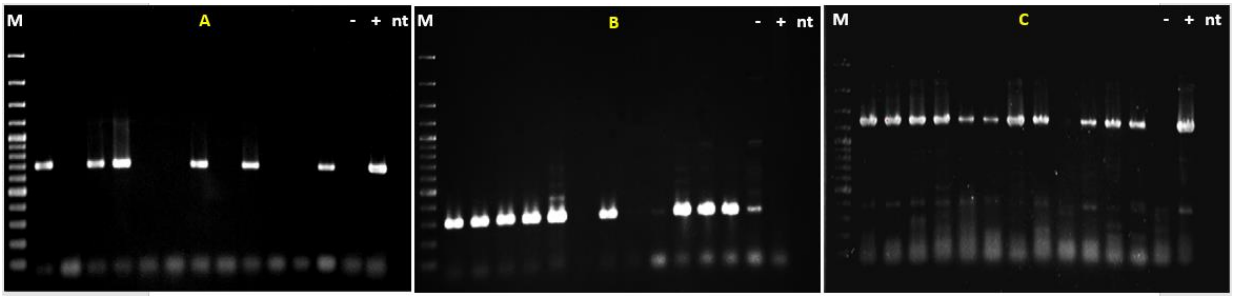

Figure 1. Band profiles obtained as a result of amplification of genes linked to resistance to BCMV/BCMNV in common bean with specific primers. (A: specific PCR product obtained as a result of amplification of dominant *I* gene with SW-13/690 bp marker, MDRK (-), BMN-RMR-13 (+), B: specific PCR product observed by amplification of *bc-3* gene with ROC11/350 bp marker, Perry Marrow (-), Cornell 49–424 (+), C: amplification of *bc-1²* gene with SBD-5/1250 bp marker the resulting specific PCR product, Cornell 49–424 (-), Perry Marrow (+), M: 100 bp DNA ladder).

Table 3. Resistance sources of common bean lines to BCMV and BCMNV

Lines	*I* (SW-13/690 bp)	*bc-3* (ROC11/350-420 bp)	*bc-1²* (SBD-5/1250 bp)	*I+bc-1²*	*bc-3+bc-1²*
Balıkesir-3	+	-	+	*	
Balıkesir-4	+	-	+	*	
Balıkesir-19	-	+	+		*
Bilecik-7	+	-	+	*	
Bingöl-8	-	+	+		*
Bingöl-12	-	-	+		
Bingöl-23	-	+	+		*
Bingöl-25	-	-	+		
Bingöl-50	-	+	+		*
Bitlis-5	+	-	+	*	
Bitlis-68	+	-	+	*	
Bitlis-123	+	-	+	*	
Bitlis-124	+	-	+	*	
Bursa-3	-	-	+		
Bursa-4	+	-	+	*	
Bursa-21	+	-	+	*	
Bursa-22	-	+	+		*
Bursa-23	-	+	+		*
Bursa-24	+	-	+	*	
Çanakkale-2	-	-	+		
Çanakkale-4	-	-	+		
Çanakkale-6	+	-	+	*	
Çanakkale-8	-	-	+		
Düzce-2	+	-	+	*	
Düzce-3	-	-	+		

Table 3. Resistance sources of common bean lines to BCMV and BCMNV (continue)

Lines	*I*	*bc-3*	*bc-1²*	*I+bc-1²*	*bc-3+bc-1²*
	(SW-13/690 bp)	(ROC11/350-420 bp)	(SBD-5/1250 bp)		
Elazığ-11	-	-	+		
Hakkâri-8	+	-	+	*	
Hakkâri-18	-	-	+		
Hakkâri-51	-	-	+		
Hakkâri-76	-	+	+		*
Malatya-20	+	-	+	*	
Malatya-25	+	-	+	*	
Malatya-44	-	+	+		*
Muş-24	+	-	+	*	
Muş-48	-	-	+		
Tunceli-8	+	-	+	*	
Van-17	-	-	+		
Van-19	+	-	+	*	
Van-49	+	-	+	*	
Van-64	+	-	+	*	
Yalavo-14	+	-	+	*	
Yalova-28	-	-	+		
Yalova-32	+	+	-		

In contrast, previous studies conducted by different researchers have consistently demonstrated that the combination of the dominant *I* gene and the recessive *bc-3* gene, each offering distinct mechanisms of resistance and provides comprehensive and potentially durable resistance to strains of BCMV and BCMNV (Melotto et al., 1996; Pastor-Corrales et al., 2007; Pasev et al., 2014). In the current investigation, only one landrace, YLV-32, exhibited the combination of the recessive *bc-3* gene and the dominant *I* gene, providing an immune response to BCMV and BCMNV. Therefore, YLV-32 has potential as a parental source in marker-assisted selection for plant breeding purposes. In a previous study, Palacıoğlu et al. (2020) noted that no common bean cultivars have both *I* and *bc-3* resistance genes in tested cultivars. This finding revealed that local genetic resources provide an excellent source of various genes and could be played a key role in plant breeding to develop new common bean cultivars resistant to BCMV and BCMNV. In this context, more in-depth investigations in different common bean germplasm of Türkiye will reveal the genetic richness and provide significant contributions to developing modern resistant cultivars to BCMV and BCMNV.

4. Conclusion

BCMV and BCMNV are the most important viral pathogens that significantly mitigate common bean production. The most economical and efficient way of virus control is to use resistant plants. To develop resistant plants for these viral pathogens, local genetic resources have potential reservoirs of novel alleles. In this study, 43 common bean lines derived from local genotypes in different provinces of Türkiye were evaluated for gene sources associated with BCMV and BCMNV by using molecular markers (ROC11, SBD-5, and SW-13). The *I*, *bc-3* and *bc-1²* resistant genes were found in the numbers of 22, 9, and 42 common bean lines, respectively. The combination of *I+bc-3* resistance genes, which guarantees immune reaction to BCMV and BCMNV, was found in only one line; YLV-32. This unique material could be used as a parent to develop new common bean cultivars in breeding studies.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.E.	M.Z.Y.	A.Ç.	V.Ç.
C	25	25	25	25
D	25	25	25	25
S				100
DCP	40	30	30	
DAI		50	50	
L	20	40	40	
W		60	40	
CR		50	50	
SR		50	50	
PM	25	25	25	25
FA	25	25	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this

study because of there was no study on animals or humans.

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BIBLIOMETRIC ANALYSIS OF NEXT-GENERATION SEQUENCE APPLICATIONS IN LIVESTOCK

Selçuk KAPLAN[1], Yasin ALTAY[2]*

[1]*Tekirdag Namık Kemal University, Faculty of Agriculture, Department of Agricultural Biotechnology, 59030, Tekirdağ, Türkiye*
[2]*Eskisehir Osmangazi University, Faculty of Agriculture, Department of Animal Science, 26160, Eskisehir, Türkiye*

Abstract: Bibliometric analyzes are widely used in many fields. However, there are still insufficient bibliometric studies evaluating animal science studies from different perspectives. Therefore, we performed the comprehensive bibliometric analysis of 335 documents scanned in the Web of Science (WoS) database in next-generation sequence applications in livestock between 2009 and 2023. According to the analysis results, this field has been increasing interest recently. The fact that the studies (45.07% of total) were carried out by international large research groups with the participation of many researchers shows that the collaborative working culture in this field is developed. BMC Genomics, Animals and Frontiers in Genetics are among the most preferred journals in studies in this field, and 14, 10 and 10 articles have been published, respectively, to date. The number of citations per article indicates the high impact of the articles published in this field. It has been determined that the three most frequently used keywords in next-generation sequence studies in the field of livestock are "identification", "diversity" and "expression". Overall, studies about next-generation sequence applications in livestock seem to be very popular among the scientific community in recent years.

Keywords: Bibliometrics, Next-generation sequence, Genome, SNP, Livestock

***Corresponding author:** Eskisehir Osmangazi University, Faculty of Agriculture, Department of Animal Science, 26160, Eskisehir, Türkiye
E mail: yaltay@ogu.edu.tr (Y. ALTAY)
Selçuk KAPLAN https://orcid.org/0000-0003-1101-2296
Yasin ALTAY https://orcid.org/0000-0003-4049-8301

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1. Introduction

DNA is the fundamental source of life and has the heredity information of all known organisms, from prokaryotes to eukaryotes (Dahm and Banerjee, 2019). James Watson and Francis Crick discovered the double helix structure of DNA in 1953 (Watson and Crick, 1953). The discovery of DNA structure has led to significant changes in biology and genetics (Hood and Galas, 2003; Waters, 2008). In the late 1970s, Frederick Sanger and colleagues developed a new DNA sequencing method called The Sanger Dideoxynucleotide Sequencing Method (Sanger et al., 1977). Frederik Sanger was awarded the second Nobel Prize in Chemistry in 1980 with this method. This method has become a standard method in clinical genetics (Totomoch-Serra et al., 2017). Moreover, the Sanger method has been widely used in forensic and medical sciences (Alex et al., 2020). However, a critical limitation of the Sanger method is high cost, low analytical sensitivity, sequencing fidelity and limited read length (Schuster, 2008; Altimari et al., 2013).

The human genome project was completed in 2003 with a budget of approximately $3 billion over 15 years using the Sanger Sequencing method (Olson, 2002; Lallar and Phadke, 2016). However, the Human Genome Project has led to a search for new technologies due to its high cost and long duration (Van Dijk et al., 2014). Therefore, The National Human Genome Research Institute (NHGRI) has launched a funding program to reduce the cost of human genome sequencing to $1,000 over ten years (Chan, 2005; Schloss, 2008). Thus, high-throughput sequencing technologies have been developed following technological developments in the last two decades (Kim et al., 2014).

The first Next Generation Sequencing (NGS) technology was developed and introduced in 2005 (Margulies et al., 2005). To date, commercial companies such as Illumina and Roche have introduced many different Next Generation Sequencing (NGS) technologies to remove the limitations of Sanger sequencing (Morozova and Marra, 2008; Tipu and Shabbir, 2015). Despite the widespread use of the Sanger Method in DNA sequencing for the last 30 years, the NGS technology developed in 2005 started a new era in DNA sequencing (Voelkerding et al., 2009). While the human genome can be sequenced in one day with NGS technologies, it took about ten years to draft it using the Sanger Sequence method (Behjati and Tarpey, 2013). Today, with the widespread use of NGS technologies, there has been a significant decrease in DNA sequencing costs (Goodwin et al., 2016; Park and Kim, 2016). Thus, NGS technologies can be used in many fields, such as personalized medicine, cancer genomics, forensics and clinical microbiology (Rasheed, 2020).

The discovery of NGS allowed rapid and high throughput whole genome sequencing of individuals (Pareek et al.,

2011; Schneeberger and Weigel, 2011). Therefore, NGS technology has significantly influenced genomics approaches that address the entire genome or defined regions (Koboldt et al., 2013). In this regard, genome-wide association studies (GWASs) that have been ongoing since 2007 play a significant role in determining the individual-specific differences of single nucleotide polymorphisms (SNPs) (Pouladi et al., 2016; Young, 2019). In recent years, it has been seen that GWAS studies with NGS have increased to elucidate the genomic architecture associated with important yield traits in livestock (Bordbar et al., 2020; Jiang et al., 2014). Moreover, these high-throughput technologies and approaches can effectively predict animal phenotype and breeding values for genomic selection (Goddard and Hayes, 2009; Toghiani et al., 2017).

The term "bibliometrics" was described by Pritchard in 1969 (Pritchard, 1969). Bibliometrics is a qualitative and quantitative analysis of scientific literature (Osareh, 1996). Today, the bibliometric study is one of the valuable tools used to understand the growth and trends of the scientific literature (Akhavan et al., 2016; Zahra et al., 2021). The bibliometric analysis uses various metrics such as publications, citations and journals to evaluate a particular field of study (Mishra et al., 2018; Thanuskodi, 2010).

In this study, we aimed to identify current trends and approaches to the Next-Generation Sequence applications in livestock with bibliometric analysis.

2. Materials and Methods

2.1. Materials

The material of this study consists of 335 documents scanned in the Web of Science (WoS) database in animal husbandry between 2009 and 2023 (Table 1). In obtaining the data from the Web of Science (WoS) database, appropriate keywords and journals related to the subject of animal husbandry were identified, in addition to the "next-generation sequence" and "livestock" keywords. The data were downloaded in plain text format (Full Record and Cited References) from the Web of Science (WoS) database and re-checked for suitable subjects.

Table 1. Document structure of primary data

Document Types	Number
Article	278
Article; Book Chapter	2
Article; Data Paper	1
Article; Early Access	1
Article; Proceedings Paper	4
Meeting Abstract	1
Proceedings Paper	1
Review	44
Review; Book Chapter	3

2.2. Bibliometric Analysis

Bibliometric analysis is a sociometric and network analysis method that reveals the social network of scientific studies in a particular field (Önder and Tirink, 2022). This method helps to determine the effectiveness of qualitative and quantitative research methods. As a result, it can influence the trends, policies, collaboration areas, and funding support for future research on the relevant topic. Therefore, the bibliometric analyses performed in this study have reached significant insights for future studies in this field.

2.3. Data Analysis

The data was formatted using R software's "convert2pdf" package (R Core Team, 2016). For statistical analysis related to the topics covered, both the "bibliometrics" package in R software and the "bibloshiny" application were utilized (Aria and Cuccurullo, 2017). The document structure of primary data is given in Table 1.

3. Results and Discussion

The primary data shows articles are the most common document type among 335 documents. Articles constitute 83% of all documents. It is seen that the second most common document type after articles are reviews, which constitute 13% of all documents. Moreover, the remaining document types such as book chapter and proceedings paper make up a small percentage of the total documents. According to the comprehensive bibliometric data analysis of the whole data, the data shows an overall upward trend in articles published each year. Between 2009 and 2011, the production of articles was relatively low, and it is seen that the total number of articles was 11. However, there has been a rapid increase in article production, with 23 articles published in 2012. It is seen that the number of articles published per year peaked at 44 articles in 2021. In 2023, it is seen that there are only 6 articles as of April, but data for the whole year is not available yet. The number of articles published between 2009 and 2023 is given in Figure 1.

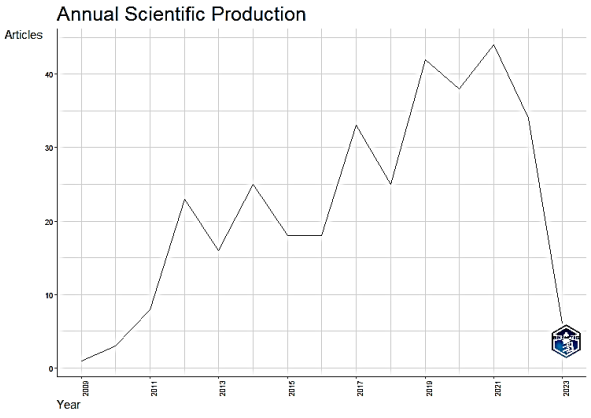

Figure 1. Number of articles published between 2009 and 2023 of next-generation sequence applications in livestock.

The collaborative studies about next-generation sequence applications in livestock are given in Figure 2. The analysis revealed that 2323 authors have contributed to this field. Among them, it is seen that only 9 authors published a single-authored paper. Thus, it demonstrates that collaborative research is widespread in this field. The 8.3 authors per article indicate that large research groups generally conducted the study. Moreover, the international co-authorship constitutes 45.07% of the articles produced, which shows intense participation in the research from different countries.

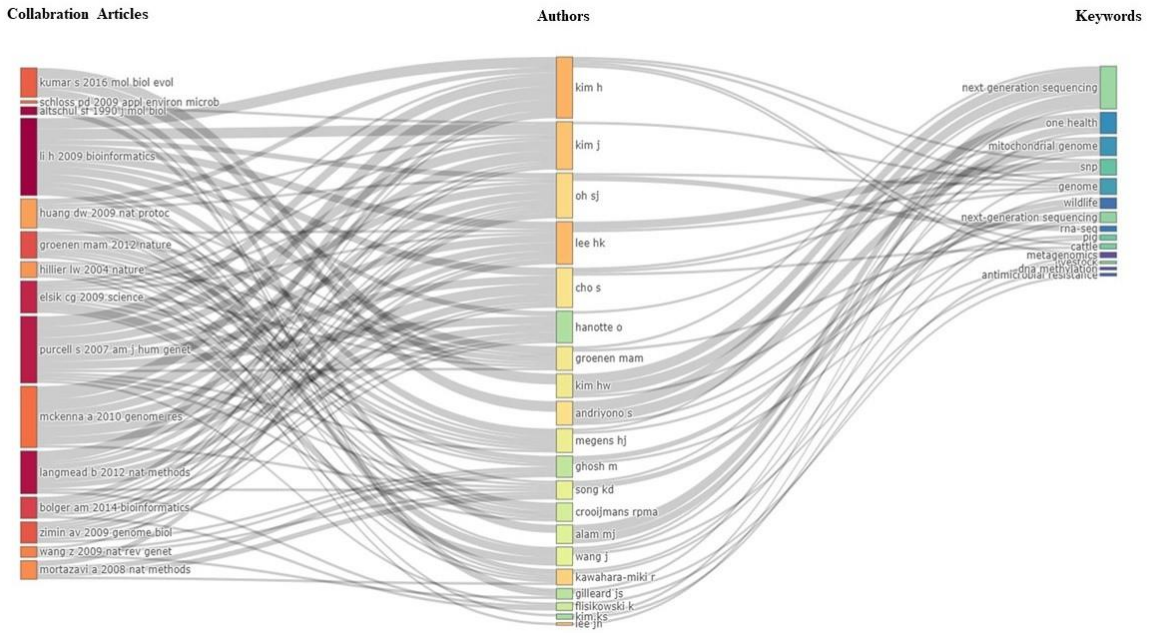

Figure 2. Collaborative studies about next-generation sequence applications in livestock.

Figure 3 provides information about the most published journals. Among the published articles, BMC Genomics ranks first with 14 articles. Frontiers in Genetics, Animals, PLOS ONE and Livestock Science follow BMC Genomics with 10 articles each. The most cited local sources are given in Figure 4. Analysis revealed that among the cited articles, PLOS ONE ranks first with 757 articles. Afterwards, BMC Genomics and Nature 423,371 received the most citations, respectively. According to the analysis results, PLOS ONE and BMC Genomics are among the most preferred journals in next-generation sequence applications in livestock.

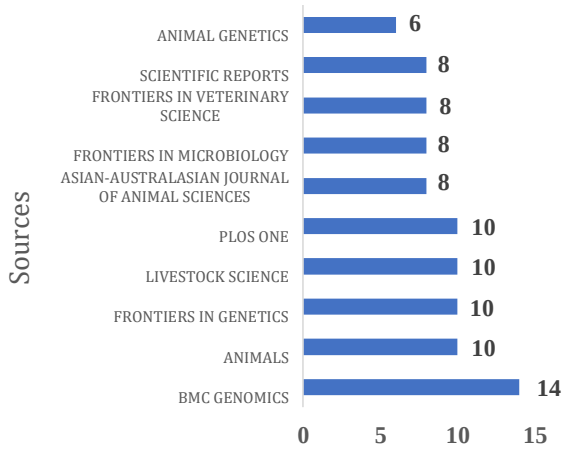

Figure 3. The most published articles in journals about next-generation sequence in livestock.

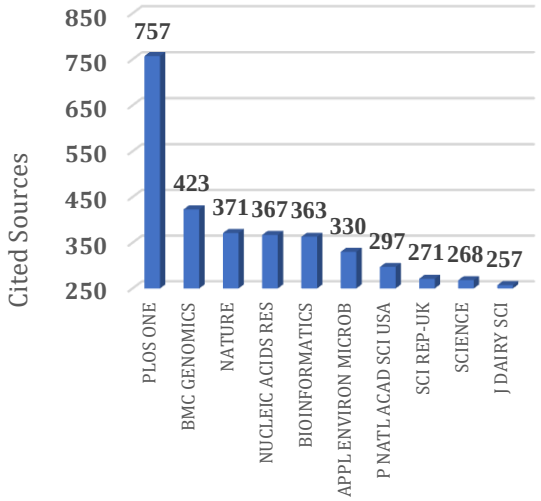

Figure 4. The most cited journals about next-generation sequence in livestock.

Figure 5 provides information about the number of articles published by various authors. According to data, Kim H and Kim HW have the highest number of articles among the most relevant authors in this field. Kim Y is the author with the 3rd most publications in this field.

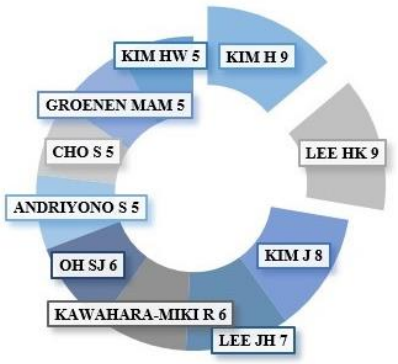

Figure 5. The most productive authors about next-generation sequence in livestock.

The articles published in different institutes from 2009 to 2023 are given in Figure 6. In this context, The National Institute of Animal Science had no articles between 2009 and 2011. However, an increasing graph is observed in the number of articles until 2019 in other years. 17 articles were published in The National Institute of Animal Science in 2023. Seoul National University has the highest number of articles in total. 38 articles were published in this university in 2023. There are no publications in Tokyo Agricultural University between 2009 and 2012. The publications that started in 2013 reached 14 articles in 2017. In 2023, the number of articles was determined as 14. There were no publications in the University of Bern between 2009 and 2016. However, the number of articles increased continuously in the following years, reaching 14 publications in 2021. The number of articles published remained stable in 2022 and 2023, with 14 publications. Although Edinburgh University appeared to have the least number of articles in the first years, the number of articles published has increased continuously from 2012 to 2023, reaching 18. There was no article published in the University of Illinois until 2012. However, there was an increase in the number of publications in the following years, and 16 articles were published every year from 2019 to 2023.

Figure 7 provides information about the trending topic keywords that appeared in scientific studies during each year between 2012 and 2022. According to the analysis results, "identification" was used 41 times in scientific studies conducted between 2014 and 2019 and became the most used keyword. In the studies conducted between 2016 and 2021, the keyword "diversity" was used 34 times and became the 2nd most used. The "expression" keyword was used 23 times in studies between 2014 and 2020 and became the 3rd most used keyword. Considering the keywords used, it is seen that they are generally related to gene research in this field.

The country-based collaborative studies are given in Figure 8. The analyses reveal that the most collaborative scientific studies in the world are done in the United States of America (USA), China and Germany. However, it was determined that the least collaborative work was done in Japan. When the collaborative work of countries with each other is evaluated, it is seen that the USA carries out collaborative work with all countries. It is also seen that Germany, France and China are working together. So, there is strong scientific cooperation between these countries. Much collaborative work is also between England and USA based on their scientific cooperation.

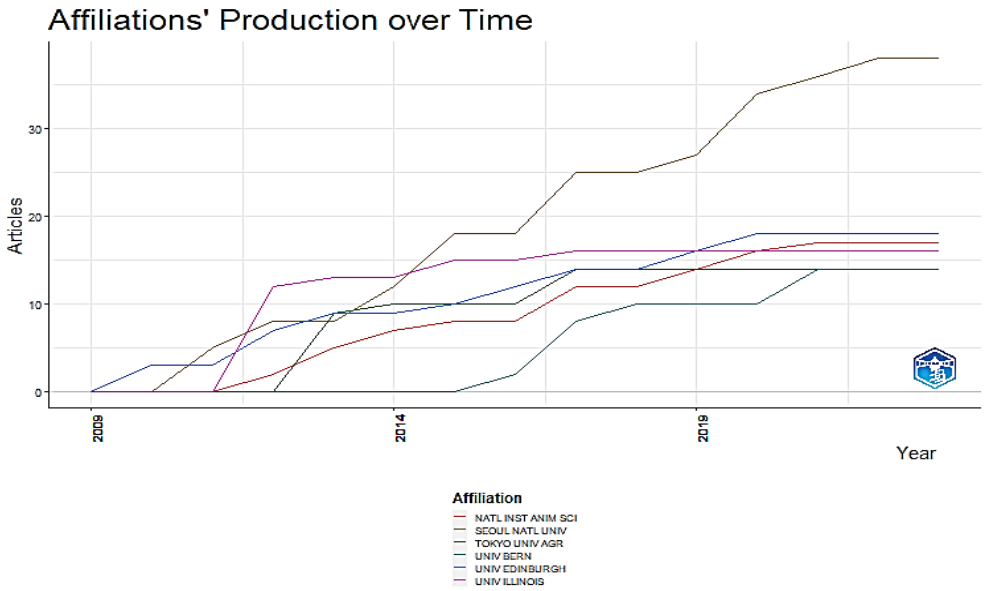

Figure 6. Scientific research institutions that publish the most articles about next-generation sequence in livestock.

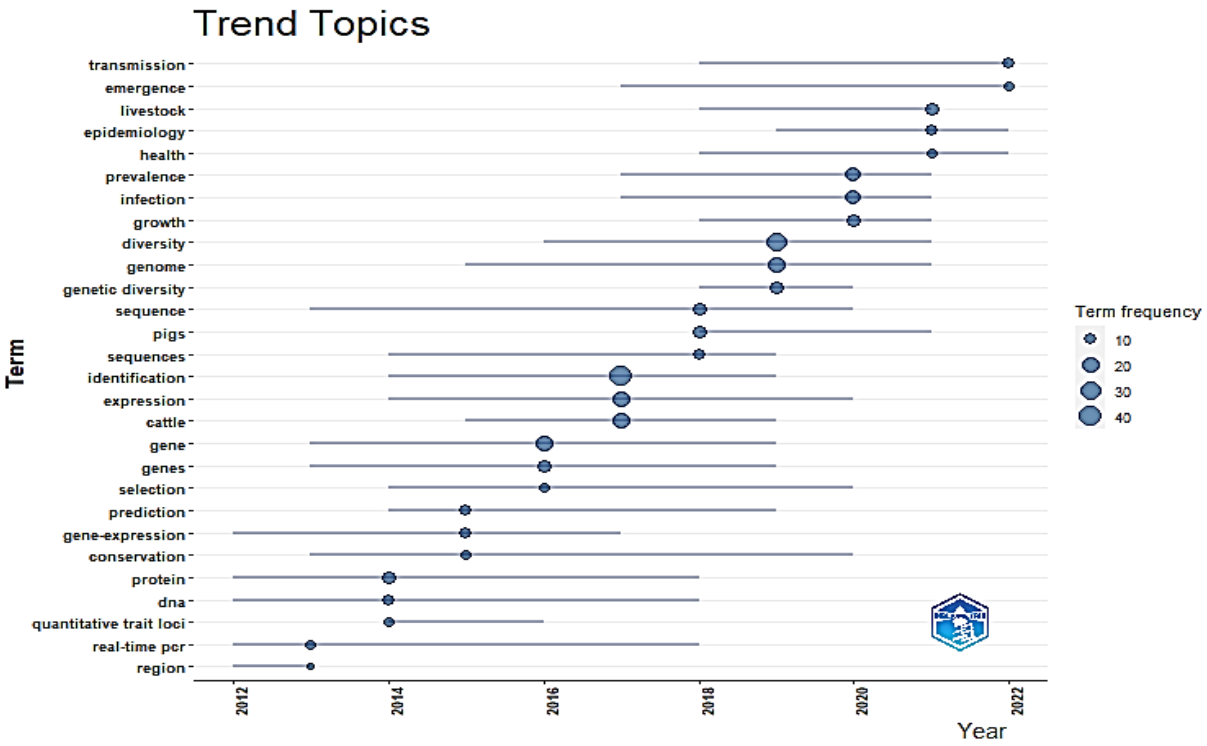

Figure 7. Trend topics keywords in scientific studies about next-generation sequence in livestock.

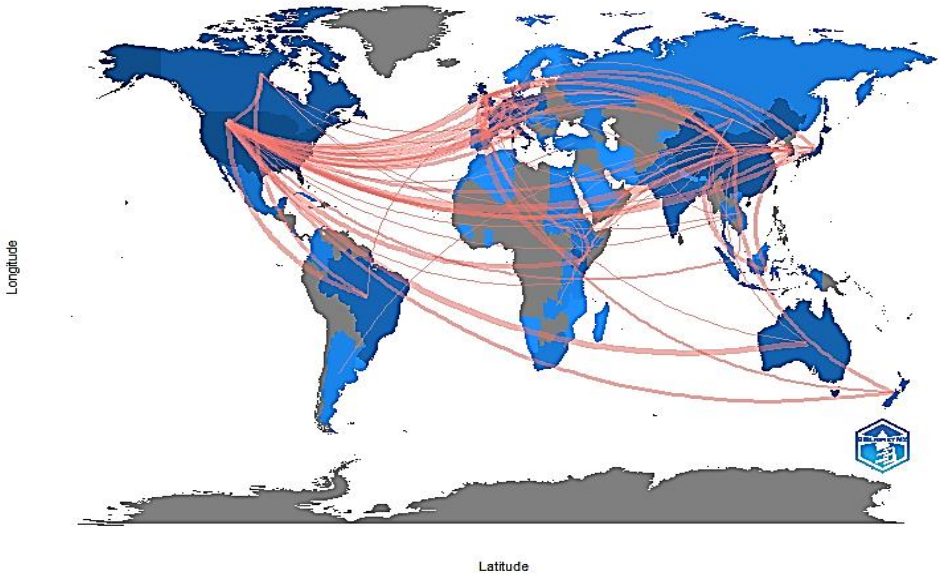

Figure 8. Country-based collaborative studies.

4. Conclusion

The mean age of the document in this study is 5.34 years, indicating that there have been recent new studies and trends in this field. The annual increase in documents is 13.65%, which shows that there has been an increasing demand for studies over the years. 23.03 citations to each document show the scientific impact of these studies. The fact that only 9 of the documents produced by 2323 authors are published documents shows that collaborative studies based on scientific cooperation are common in this field. Moreover, the number of authors per document produced, an average of 8.3, indicates that large research groups generally carry out the studies. Overall, this study presents an in-depth overview of the latest studies on next-generation sequence applications in Livestock. Thus, this perspective is expected to guide future studies in this field.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	S.K.	Y.A.
C	50	50
D	50	50
S		100
DCP	20	80
DAI	20	80
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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DETERMINATION OF YIELD AND YIELD COMPONENTS OF POPCORN (*Zea mays* L. everta) GENOTYPES

Fuat AL[1], Elif ÖZTÜRK*[1], Hasan AKAY[1], İsmail SEZER[1]

[1]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Field Crops, 55200, Samsun, Türkiye*

Abstract: This research was conducted in Samsun Bafra conditions in 2021, using a total of 19 different genotypes of popcorn, which differ from other corn subspecies in terms of grain size and usage area, and have gained popularity for snacking purposes. The experiment was set up in a randomized complete block design with three replications. The study examined plant height, main stem diameter, leaf number, cob length, cob diameter, cob row number, number of kernels per cob, cob weight, kernel weight, and grain yield of the popcorn genotypes. It was determined that there is a statistically significant relationship between these examined parameters and genotypes. According to the results of the experiment, plant height in popcorn genotypes ranged from 128.33 to 207.00 cm, main stem thickness varied between 11.91 and 20.20 mm, leaf number per plant ranged from 8.33 to 12.33, cob length varied from 11.60 to 20.33 cm, cob diameter ranged from 27.63 to 36.49 mm, cob row number varied between 12.00 and 15.67, and the number of kernels per cob ranged from 405.67 to 772.67. The grain yield values ranged from 183.05 to 482.43 kg/da. The research determined that the average grain yields of populations and lines were higher than the overall average of popcorn cultivars. When considering grain yield and yield parameters, the TBCM-6 line stood out. According to the biplot analysis, it was observed that the P2 and P3 populations, along with the TBCM-3, TBCM-4, TBCM-6, TBCM-23, TBCM-44, and TBCM-62 lines, excelled in terms of yield and yield components.

Keywords: Popcorn, Genotype, Biplot, Yield

***Corresponding author:** Ondokuz Mayıs University, Faculty of Agriculture, Department of Field Crops, 55200, Samsun, Türkiye
E mail: elif.ozturk@omu.edu.tr (E. ÖZTÜRK)
Fuat AL https://orcid.org/0009-0005-4409-8956
Elif ÖZTÜRK https://orcid.org/0000-0001-9723-6092
Hasan AKAY https://orcid.org/0000-0003-1198-8686
İsmail SEZER https://orcid.org/0000-0002-8407-7448

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1. Introduction

Maize (*Zea mays* L.), which belongs to the *Maydeae* tribe of the *Gramineae* family, commonly known as the grass family, is a crop that is included in warm climate cereals and can be grown in almost every region of the world (Shaw, 1988; Öner, 2011). In terms of cultivation area, maize ranks second among cereals worldwide, but it holds the first position in terms of yield and production per unit area (Akın, 2022). Maize is one of the economically important cereal crops with versatile usage. Globally, 60% of maize production is used in animal feed, 27% as raw material, 11% in food production, and 4% for other purposes (Anonim, 2016; Mut et al., 2022). In Türkiye, 35% of the produced maize is used for human consumption, 30% for animal feed as silage, and 20% in the feed industry (Gençtan et al., 1995). Maize used for human consumption contributes to approximately 11% of a person's daily caloric needs (Çıldır and Çanakçı, 2006). However, this percentage may vary among developed and developing countries.

Türkiye is one of the countries in the Old World where maize was first cultivated. As a result, various maize cultivars from different subspecies can be found in almost all regions of our country, especially in coastal areas (Sezer and Yanbeyi, 1997; Öner, 2011). Popcorn is one of these subspecies of maize. Popcorn plants differ from other maize subspecies in terms of grain size and usage. Compared to dent, flint, and sweet corn, popcorn has shorter plant height, smaller leaves, cobs (ears), and kernels, and is tougher. The popcorn production area in our country is around 8-10 thousand hectares (Akın, 2022). The Aegean and Mediterranean regions are the main areas for agriculture and production in our country. Approximately half of the popcorn production in our country is in Kahramanmaraş, followed by Adana, Mersin, and Aydın provinces (Öztürk et al., 2019).

In recent years, the increase in production quantity in regions where popcorn farming is practiced has contributed to its growing popularity. One of the main factors that has boosted the popularity of popcorn is its status as a popular snack consumed in cinemas and while watching movies. However, due to the advancements in television technology, online streaming platforms, and other technological innovations, interest in cinema theaters has declined. As a result, the consumption of popcorn in cinemas has decreased while household consumption has increased (Ziegler, 2001). Additionally, popcorn is considered a beneficial dietary product due to

its content of vitamins, minerals, high carbohydrates, low calories, low fat, satiating properties, and its ability to absorb stomach acid (Ülger, 1998; Dickerson, 2003; Özkan, 2007).

Popcorn, which is increasingly becoming popular and experiencing a rise in consumption both globally and in our country, has become a financially rewarding product in the market. Therefore, by expanding popcorn farming in ecologically suitable regions, farmers can have a good income opportunity. This study was conducted in Samsun Bafra conditions with the aim of determining the yield and certain yield parameters of specific popcorn genotypes.

2. Materials and Methods

The research was conducted at the Ondokuz Mayıs University Bafra Agricultural Application and Research Center in 2021. The Agricultural Application and Research Center of Ondokuz Mayıs University is located in the Bafra plain, approximately 2 km southwest of the Bafra district, and 42 km west of Samsun province. The soil analysis results of the study area were performed at the Department of Soil Science and Plant Nutrition of Ondokuz Mayıs University Faculty of Agriculture. Soil tests indicated the site had the following conditions: organic matter 2.65%, sandy clay soil, 44.55%, available N (nitrogen), 0.16, available P_2O_5, 118.26 ppm, K, 340 Ca, 15.91 meq/100 g, and soil pH, 7.40. The foundation year's average temperature and total precipitation values were 19.9 ^oC and 471 mm (Table 1).

A total of 19 genotypes were used in the research, consisting of 3 cultivars (Baharcin, SH9201, R997), 5 populations (P1, P2, P3, P4, P5), and 11 lines (TBCM-3, TBCM-4, TBCM-6, TBCM-19, TBCM-23, TBCM-32, TBCM-41, TBCM-44, TBCM-49, TBCM-62, TBCM-109). The trial was established on May 20, 2021, following a randomized complete block design with three replications. The plot length was 5 meters, with 4 rows and a 2-meter gap between blocks. The planting distance was set at 70 cm between rows and 18 cm within rows. The seeding depth was adjusted to 5-6 cm. Based on soil analysis results, 22 kg cultivars of pure nitrogen in the form of ammonium sulfate and 8 kg of phosphorus (P_2O_5) per hectare were applied (Özkan and Ülger, 2011). Harvesting was carried out on October 15 of 2021.

In the study, measurements were taken for plant height (cm), main stem diameter (mm), leaf count (number/plant), cob (ear) length (cm), cob diameter (mm), cob row count, grain count per cob, individual cob weight (g), cob grain weight (g), and grain yield (kg/da) (Öktem, 1996; Sezer and Yanbeyi, 1997; Kara and Kırtok, 2006; İdikut et al., 2009).

The statistical analysis of the data were performed using the JMP statistical software program. Significance testing of the differences between group means was conducted using the Tukey test. Biplot, Pearson correlation, and Cluster analysis were employed to examine the relationships among the investigated traits (JMP, 2019).

Table 1. Climatic data of the region where the research was conducted*

Climate Factors		Month						Total
	Years	May	Jun	July	August	September	October	Average
Rain (mm)	2021	77.4	65.8	8.2	49.3	119.4	169.2	489.3
	Long Years	47.6	47.0	29.8	47.7	61.9	98.8	332.8
Average Temperature (°C)	2021	16.6	20.2	24.9	24.0	18.5	14.7	19.8
	Long Years	15.6	20.2	22.9	23.0	19.6	15.6	19.5
Relative Humidity (%)	2021	71.2	74.5	70.1	74.0	76.2	81.7	
	Long Years	79.42	75.85	74.22	75.36	77.33	79.97	

* Samsun Meteorology Station Climate data (2021-Long years/1981).

3. Results and Discussion

In this study, investigating the yield and yield parameters of certain maize genotypes, the mean values and TUKEY grouping of plant height, main stem thickness, and leaf count are provided in Table 2. The examined parameters of plant height, main stem thickness, and leaf count were found to have statistically significant ($P<0.001$) effects (Table 2).

When examining the maize genotypes in terms of plant height, the tallest plant height of 207.00 cm was observed in the P3 population, while the shortest plant height of 128.33 cm was recorded in the TBCM-19 line. The average plant height of the maize populations was determined to be 193.33 cm, while the average plant height of the lines and cultivars was 163.64 cm and 160.33 cm, respectively. The average plant height of the populations was significantly higher than that of the lines and cultivars (Table 2). Plant height is a parameter that varies depending on environmental factors and genetic characteristics (Sezer and Yanbeyi, 1997; İdikut et al., 2020). It has been noted that the plant height of popcorn is generally shorter compared to sweet corn and dent corn (Ülger, 1998). When examined for main stem diameter, the maize genotypes ranged from 11.91 mm to 20.20 mm. The P2 population had the highest main stem diameter of 20.20 mm, followed by TBCM-4 (20.00 mm) and TBCM-32 (19.97 mm), which were in the same statistical group. The lowest main stem diameter was observed in the R997 variety, measuring 11.91 mm. When genotypes were compared for average main stem diameter, the highest value of 18.53 mm was found in the lines, while the lowest value of 12.93 mm was observed among the cultivars (Table 2).

Table 2. The average values and variance analysis results for plant height, main stem diameter, and leaf number of the maize genotypes are presented

Genotypes		Plant height	Main stem diameter	Leaf number
P1		191.00 ± 2.61 b	13.47 ± 0.12 gh	9.67 ± 0.44 c-f
P2		191.00 ± 0.67 b	20.20 ± 0.21 a	12.33± 0.44 a
P3		207.00 ± 2.00 a	15.45 ± 0.14 e	12.00 ± 0.67 ab
P4		186.67 ±1.11 b	15.59 ± 0.25 e	11.33 ± 0.44 abc
P5		191.00 ± 1.33 b	15.80 ± 0.23 e	11.00 ± 0.67 a-d
Population Ave.		193.33± 5.69	16.10 ± 1.64	11.27 ± 0.95
TBCM-3		175.33 ± 1.11 c	18.56 ± 0.33 bc	10.33 ± 0.44 b-e
TBCM-4		164.67± 1.11 ef	20.00 ± 0.66 a	10.67 ± 0.44 a-d
TBCM-6		163.33 ±0.44 f	19.57 ± 0.11 ab	9.33 ± 0.44 def
TBCM-19		128.33 ±1.11 ı	18.31 ± 0.30 cd	9.33 ± 0.44 def
TBCM-23		188.33 ± 1.11b	15.28 ± 0.08 ef	9.67 ± 0.44 c-f
TBCM-32		172.67 ± 3.78 cd	19.97 ± 0.14 a	9.67 ± 0.44 c-f
TBCM-41		154.67 ± 1.56 g	17.89 ± 0.26 cd	8.33 ± 0.44 f
TBCM-44		183.67 ±2.89 b	18.56 ± 0.19 bc	11.00 ± 0.01 a-d
TBCM-49		143.67 ±2.22 h	18.72 ± 0.29 bc	9.67 ± 0.44 c-f
TBCM-62		153.33 ±1.11 g	19.62 ± 0.19 ab	11.00 ± 0.01 a-d
TBCM-109		172.00 ±1.33 cde	17.32 ± 0.11 d	11.67 ± 0.44 ab
Line Ave.		163.64 ± 13.67	18.53 ± 1.04	10.06 ± 0.80
SH9201		165.00 ± 0.67 def	14.06 ± 0.11 fg	11.00 ± 0.67 a-d
BAHARCİN		164.00 ± 2.00 f	12.82 ± 0.36 hı	8.33± 0.44 f
R997		152.00 ± 1.33 g	11.91 ± 0.55 ı	8.67 ± 0.44 ef
Variety Ave.		160.33 ± 5.56	12.93 ± 0.84	9.33 ± 1.11
Total Average		170.93 ± 15.89	17.01 ± 2.28	10.26 ± 1.07
		Mean Squares	Mean Squares	Mean Squares
Genotypes	18	1152.69**	20.88**	4.32**
Block	2	3.70	0.18	0.37
Error	36	6.22	0.16	0.41
CV		1.46	2.35	6.24

**= There is no difference between the means shown with the same letters in the same column (P<0.01).

Upon examining the analysis of the variance table, it was determined that the variation among genotypes had a highly significant effect on the number of leaves per plant (Table 2). The highest number of leaves per plant, 12.33 piece (leaves/plant), was observed in the P2 population, which was in the same statistical group as several other genotypes. The lowest number of leaves, 8.33 piece (leaves/plant), was found in the TBCM-41 genotypes. When the average number of leaves per plant was considered, it was determined to be 11.27 piece in populations, 10.06 piece in lines, and 9.33 piece in cultivars (Table 2).

In this study investigating the yield and yield parameters of certain corn genotypes, the average values and TUKEY grouping of cob length, cob diameter, number of cob rows, and number of kernels per cob are presented in Table 3. It was determined that these examined parameters had statistically significant effects (P<0.001) (Table 3).

The average cob length of genotypes was determined as 16.66 cm, with a range of 11.60 cm (R997) to 20.33 cm (TBCM-3) (Table 3). When examined in terms of average cob length, genotypes were found to be 17.14 cm in populations, 16.64 cm in lines, and 15.92 cm in cultivars. Several studies have also demonstrated significant differences among genotypes in terms of cob length (Sezer and Yanbeyi, 1997; Kaya and Kuşaksız, 2004; Shafai et al., 2020; Şahin and Kara, 2021). In the conducted studies, it has been determined that cob length varies between 15.7-17.0 cm (Gökmen et al., 2001), 11.5-18.2 cm (Şahin and Kara, 2021), and 16.3-16.9 cm (Shafai et al., 2020), and this research yields similar results. The observed differences in cob length among the research findings can be attributed to variations in environmental conditions where the studies were conducted and the different genotypes used.

When examined for cob diameter, the highest cob diameter of 36.49 mm was observed in the TBCM-23 line, while the lowest cob diameter of 27.63 mm was recorded in the TBCM-49 line. The average highest cob diameter among corn populations was 32.08 mm in lines, while the lowest average cob diameter was 30.36 mm in populations (Table 3). Previous studies by Sade and Çalış (1993), Kahramanoğlu (2009), and Şahin and Kara (2021) reported cob diameter ranges of 24.3-35.5 mm, 29.9-37.6 mm, and 29.3-35.6 mm, respectively. Our study's cob diameter values align with the findings of these researchers.

Table 3. The average values and variance analysis results of cob length, cob diameter, number of rows per cob, and number of grains per cob for different popcorn genotypes are presented

Genotypes		Cob length	Cob diameter	Number of rows per cob	Number of grains per cob
P1		17.50 ± 0.67 bcd	29.80 ± 0.74 efg	13.67 ± 0.44 c-f	608.00 ± 5.33 cd
P2		19.03 ± 0.31 ab	29.49 ± 0.63 fg	14.33 ± 0.44 a-d	772.67 ± 1.11 a
P3		16.50 ± 0.67 c-f	33.50 ± 0.79 bc	15.33 ± 0.44 ab	551.67 ± 27.78 de
P4		17.33 ± 0.62 b-e	29.33 ± 0.25 fg	15.67 ± 0.44 a	684.33 ± 45.11 bc
P5		15.33 ± 0.44 efg	29.67 ± 0.44 fg	13.33 ± 0.44 d-g	516.67 ± 10.22 ef
Population Ave.		17.14 ± 1.16	30.36 ± 1.33	14.47 ± 0.90	626.67 ± 81.47
TBCM-3		20.33 ±0.56 a	30.68 ± 0.14 def	14.00 ± 0.67 b-e	667.67 ± 11.56 bc
TBCM-4		19.00 ± 0.33 ab	32.63 ± 1.08 bcd	14.00 ± 0.01 b-e	706.33 ± 5.11 ab
TBCM-6		17.67 ± 0.56 bc	33.01 ± 0.22 bcd	14.67 ± 0.44 abc	610.33 ± 33.56 cd
TBCM-19		14.87 ± 0.58 fgh	30.62 ± 0.47 def	14.33 ± 0.44 a-d	454.00 ± 5.33 fg
TBCM-23		17.60 ± 0.27 bc	36.49 ± 0.49 a	15.33 ± 0.44 ab	644.33 ± 29.56 bc
TBCM-32		17.93 ± 0.62 bc	31.01 ± 0.91 c-f	12.00 ± 0.01 g	516.00 ± 8.00 ef
TBCM-41		16.83 ± 0.22 c-f	29.35 ± 0.72 fg	12.67 ± 0.44 efg	443.00 ± 8.00 fg
TBCM-44		16.50 ± 0.33 c-f	33.82 ± 0.99 ab	15.00 ± 0.01 ab	498.33 ± 4.44 ef
TBCM-49		13.97 ± 0.38 gh	27.63 ± 0.20 g	12.33 ± 0.44 d-g	415.00 ± 9.33 g
TBCM-62		15.50 ± 0.33 d-g	34.87 ± 0.74 ab	14.00 ± 0.01 a-d	444.33 ± 16.22 fg
TBCM-109		12.87 ± 0.36 hı	32.70 ± 0.54 bcd	14.00 ± 0.02 a-d	444.67 ± 23.56 fg
Line Ave.		16.64 ± 1.76	32.08 ± 2.09	13.85 ± 0.88	531.27 ±91.56
SH9201		18.33 ± 0.44 abc	32.52 ± 0.54 b-e	14.00 ± 0.02 a-d	501.33 ± 7.56 ef
BAHARCİN		17.83 ± 0.78 bc	32.57 ± 0.56 b-e	14.00 ± 0.03 b-e	518.00 ± 9.33 ef
R997		11.60 ± 0.27 ı	29.19 ± 0.97 fg	14.67 ± 0.25 a-d	405.67 ± 9.56 g
Variety Ave.		15.92 ± 2.88	31.42± 1.49	14.22 ± 0.35	475.00 ± 46.22
Total Average		16.66 ± 1.76	31.52 ± 1.98	14.07 ± 0.76	547.49 ± 92.41
		Mean Squares	Mean Squares	Mean Squares	Mean Squares
Genotypes	18	14.52**	15.74**	2.91**	35334.87**
Block	2	0.11	0.56	0.91*	668.44
Error	36	0.46	0.82	0.21	652.55
CV		4.07	2.87	3.26	4.67

**= There is no difference between the means shown with the same letters in the same column (P<0.01).

In the study, the number of rows per cob varied between 12.00 and 15.67 piece, with an average of 14.07 piece rows per cob for the genotypes. The highest number of rows, 15.67 piece, was observed in the P4 population, which was in the same statistical group as several other genotypes. The lowest number of rows, 12.00 piece, was found in the TBCM-32 line. When examining the average number of grains per row, the populations had the highest value of 14.47 piece, followed by cultivars with 14.22 piece, while the lines had the lowest average of 13.85 piece rows per cob (Table 3). It has been reported that the number of rows per cob in the ear is influenced by climate, genetic traits, and cultivation techniques, with genetic factors being the most influential factor (Sezer and Yanbeyi, 1997; İdikut et al., 2020). In this study, significant variations in the number of rows per cob among the genotypes were observed, indicating that these differences are attributed to the genetic makeup of the genotypes and the effect of cob diameter (Kaya and Kuşaksız, 2004; Tekkanat and Soylu, 2005; Kahramanoğlu, 2019).

The number of grains per cob varied between 405.67 and 772.67 piece, averaging 547.49 piece. The lowest values were recorded in the R997 variety with 405.67 piece and the TBCM-49 line with 415.0 piece. It has been reported that the number of grains per cob varies significantly depending on genetic factors and is strongly influenced by ecological conditions (Abendroth et al., 2011; İdikut et al., 2021).

In this study investigating the yield and yield parameters of certain maize genotypes, the average values and TUKEY grouping of cob weight, grain weight per cob, and grain yield are presented in Table 4. It has been determined that these examined yield parameters have statistically significant effects (P<0.001) (Table4).

The cob weights of genotypes ranged from 46.95 g to 133.49 g, with an average cob weight of 94.45 g. The highest cob weight of 133.9 g was observed in the TBCM-3 genotype. The lowest cob weights were recorded in the P4, TBCM-19, TBCM-49, and R997 genotypes, measuring 52.37 g, 46.95 g, 54.05 g, and 57.35 g, respectively, and they were statistically grouped together (Table 4). It was determined that cob weight is influenced by genotype and ecological differences, and similar results were obtained in previous studies (Sezer and Yanbeyi, 1997; Özkaynak and Samancı, 2003; Özkan, 2007; Özsoy, 2017).

Table 4. The average values and variance analysis results for cob weight, grain weight per cob, and grain yield of maize genotypes are presented

Genotypes		Cob weight	Grain weight per cob	Grain yield
P1		85,83 ± 2.10 hı	67.62 ± 0.79 gh	320.99 ± 2.72 f
P2		103.81 ± 3.53 efg	81.58 ± 1.26 de	379.61 ± 13.69 de
P3		107.02 ± 2.00 def	79.79 ± 3.25 ef	298.42 ± 1.10 g
P4		52.37 ± 1.69 j	40.63 ± 1.26 ı	295.37 ± 5.76 g
P5		78.82 ± 2.96 ı	61.78 ± 1.12 h	326.49 ± 0.07 f
Population Ave.		85.57 ± 16.37	66.28 ± 12.06	324.18 ± 23.22
TBCM-3		133.49 ± 1.49 a	104.71 ± 2.34 a	395.59 ± 1.22 cd
TBCM-4		120.40 ± 3.70 bc	94.88 ± 0.71 b	329.53 ± 0.62 f
TBCM-6		112.96 ± 0.99 cde	92.48 ± 0.50 bc	482.43 ± 7.05 a
TBCM-19		46.95 ± 2.91 j	31.34 ± 0.73 j	228.94 ± 0.67 h
TBCM-23		128.55 ± 1.43 ab	103.33 ± 1.15 a	458.01 ± 5.66 b
TBCM-32		94.87 ± 3.16 gh	73.42 ± 2.24 fg	362.61 ± 3.59 e
TBCM-41		81.68 ± 2.35 ı	64.30 ± 1.37 h	232.08 ± 2.08 h
TBCM-44		108.47 ± 3.34 def	71.95 ± 1.87 g	319.97 ± 1.60 f
TBCM-49		54.05 ± 1.24 j	42.33 ± 0.61 ı	280.49 ± 1.23 g
TBCM-62		100.85 ± 0.26 fg	79.16 ± 1.60 ef	404.83 ± 4.41 c
TBCM-109		95.52 ± 2.92 gh	79.87 ± 0.63 e	320.31 ± 1.35 f
Line Ave.		97.98 ±21.46	76.16 ± 17.76	346.80 ± 67.18
SH9201		115.85 ± 1.40 cd	87.58 ± 1.15 cd	460.95 ± 7.41 b
BAHARCİN		115.70 ± 2.99 cd	84.00 ± 0.53 de	225.44 ± 5.16 h
R997		57.35 ± 1.97 j	42.80 ±1.61 ı	183.05 ± 0.63 ı
Variety Ave.		96.30 ± 25.97	74.46± 19.11	289.81 ±114.09
Total Average		94.45 ± 21.74	72.82 ± 16.96	331.85 ± 65.38
		Mean Squares	Mean Squares	Mean Squares
Genotypes	18	2102.42**	1364.93**	21232.52**
Block	2	10.48	0.45	38.79
Error	36	11.73	4.27	43.22
CV		3.59	2.84	2.11

**= There is no difference between the means shown with the same letters in the same column (P<0.01).

When cob grain weights were examined, the highest cob grain weight was observed in the TBCM-3 genotype (104.71 g) and TBCM-23 genotype (103.33 g), while the lowest cob grain weight of 31.34 g was recorded in the TBCM-19 population. The average cob grain weight of the genotypes was determined as 72.82 g (Table 4).

The average grain yield of the genotypes ranged from 183.05 to 482.43 kg/da, with an overall average of 331.85 kg/da. The highest grain yield of 482.43 kg/da was achieved in the TBCM-6 genotype, followed by SH9201 (460.95 kg/da) and TBCM-23 (458.01 kg/da). The lowest grain yield of 183.05 kg/da was obtained from the R997 genotype. The observed differences among the genotypes in this study can be attributed to variations in individual cob yield, cob grain count, plant population, and grain-to-cob ratio. Previous research has also observed wide variations in grain yield among genotypes, and various studies have reported different ranges of grain yield. Sade and Çalış (1993) reported a range of 198.0-435.0 kg/da, Belen (1999) reported a range of 239.0-642.0 kg/da, Özkaynak and Samancı (2003) reported a range of 141.0-464.0 kg/da, Özkan (2007) reported a range of 204.0-529.0 kg/da, Öztürk et al. (2020) reported a range of 412.0-629.0 kg/da, and Şahin and Kara (2021) reported a range of 317.6-504.0 kg/da (Table 4).

The relationships between the traits and genotypes as a whole are presented in Figure 1 through a biplot graph. In this study, Principal Component 1 accounted for 43.5% of the variation, while Principal Component 2 accounted for 16%, resulting in a total variation of 59.5%. The biplot analysis explains both the relationships between the traits and the relationships between the genotypes. The angle value (0° to 90°) between vectors representing two traits indicates a positive relationship when it becomes narrower and a negative relationship when it becomes wider (90° to 180°). Additionally, the positioning of the genotypes indicates which genotypes have higher values for specific traits. (Yan and Tinker, 2006). Among the examined traits, it is evident that there is a positive and strong relationship based on the angles being less than or equal to 90°. The genotypes P2, P3, TBCM-23, TBCM-44, TBCM-4, TBCM-62, TBCM-6, TBCM-3, and SH9201 exhibited a strong positive relationship among the examined traits in the study (Figure 1).

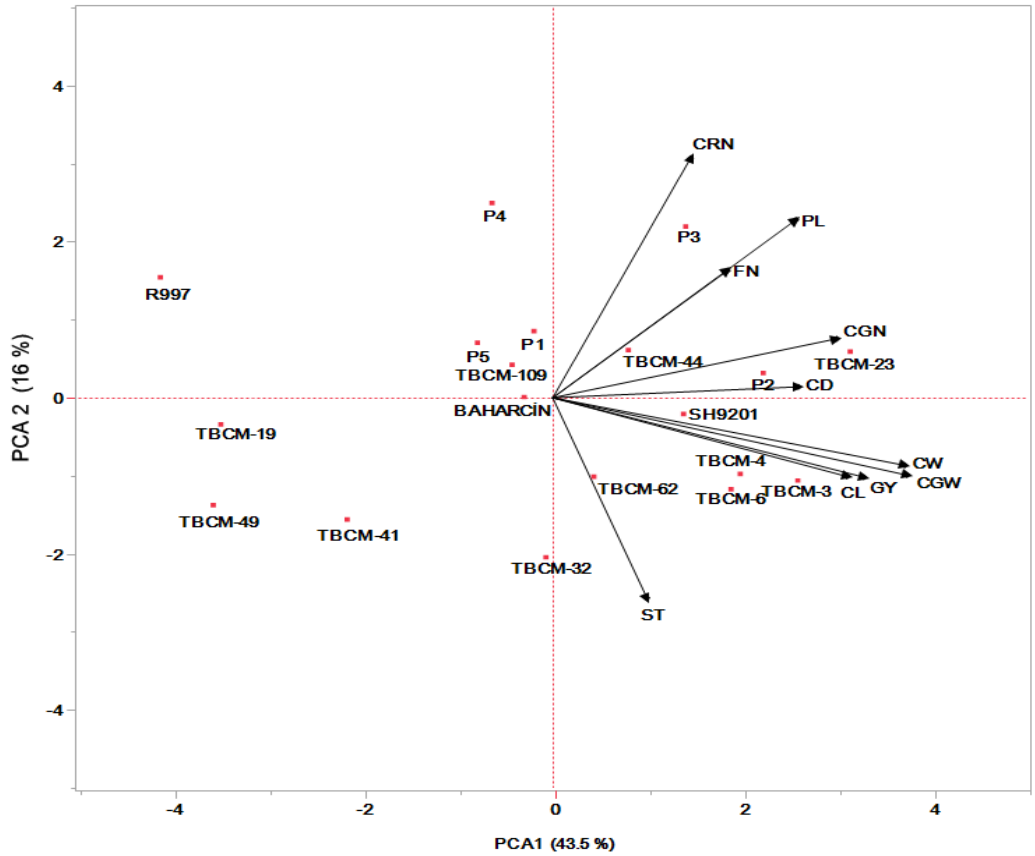

Figure 1. Biplot plot showing relationships between investigated traits and genotypes.

The variation between genotypes increases as the vectors move away from the origin, while the variation decreases as the vectors get closer to the origin. In the study, low variation was determined between P1, P5, TBCM-109 and Baharcin genotypes. Since it has the shortest vector, the number of leaves was determined as the least distinguishing feature (Mut et al., 2017). "Cob row number" parameter showed high variation with P3 genotype, while "plant height" and "leaf number" showed variation with TBCM-44 genotype. While " number of grains on the cob" and "cob diameter" traits were associated with P2 and TBCM-23 genotypes, SH9201 genotype showed significance in terms of "cob diameter", "cob weight", "grain weight per cob", "grain yield" and "cob length". TBCM-3, TBCM-4, TBCM-6 and TBCM-62 genotypes "main stem diameter", "cob weight", "grain weight per cob", "grain yield" and "cob length" " parameters (Mut et al., 2017).

4. Conclusion

The growth of the cinema and film industries has led to an increased demand for snacks, which in turn has prompted an increase in the production of the most popular snack, popcorn. As the consumption quantity has increased, our efforts to improve the quality characteristics and increase the yield of the product have gained momentum. This study was conducted to determine the yield and some yield components of popcorn genotypes grown under Samsun Bafra conditions, and significant results were obtained in terms of the examined parameters. In terms of grain yield, the TBCM-6 genotype had the highest yield of 482.43 kg/da. The biplot analysis revealed that the P2 and P3 populations, as well as the TBCM-3, TBCM-4, TBCM-6, TBCM-23, TBCM-44, and TBCM-62 lines, stood out in terms of yield and yield components.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	F.A.	E.Ö.	H.A.	İ.S.
C	25	25	25	25
D	25	25	25	25
S			50	50
DCP	50	25	25	
DAI	25	20	25	30
L	50	50		
W	25	25	25	25
CR			40	60
SR	50	50		
PM	40	20	20	20
FA		30	30	40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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SCREENING FOR SALT TOLERANCE OF 12 TURKISH TRITICALE CULTIVARS DURING GERMINATION AND EARLY SEEDLING STAGE

Mehmet ATAK[1], İbrahim ERTEKİN[1]*, İbrahim ATIŞ[1]

[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, 31060, Hatay, Türkiye*

Abstract: This investigation was carried out to determine the response of 12 Turkish cultivars to different salt stress levels during the germination and early seedling stages at Hatay Mustafa Kemal University in 2022. In present research, four different sodium chloride treatments (60, 120, 180 and 240 mM) and a control (0 mM) treatment were used. Germination ratio, germination index, mean germination time, root and shoot length, root and shoot fresh weight, root and shoot dry weight and biomass weight parameters were investigated to determine the salt tolerance of triticale cultivars. Cultivars, salt doses and their interactions had significant effects on all examined characteristics. In general, germination parameters and early seedling stage characteristics of the cultivars were adversely affected by increasing salt doses. Yet, it was determined that the responses to increasing salt doses were different among the cultivars. Although Mehmetbey cultivar was undesirably affected by increased salt doses, it was found to be more resistant than the other cultivars. On the other hand, Umranhanim was determined as the most sensitive cultivar to salt stress. After investigating the resistance of Mehmetbey cultivar to salt stress under field conditions, this cultivar can be included into breeding programs or cultivated in fields with salinity problems.

Keywords: Abiotic stress, Germination, Salt stress, Seedling stage, Triticale

***Corresponding author:** Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, 31060, Hatay, Türkiye
E mail: ibrahim.ertkn@hotmail.com (İ. ERTEKİN)
Mehmet ATAK https://orcid.org/0000-0003-1196-0413
İbrahim ERTEKİN https://orcid.org/0000-0003-1393-8084
İbrahim ATIŞ https://orcid.org/0000-0002-0510-9625

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1. Introduction

From germination to emergence stage, seeds are exposed to various abiotic stress factors such as drought, flooding, extreme heat or cold, salinity, mineral deficiency and toxicity (Khan et al., 2013). It is estimated that on-going global warming and climate change will even further aggravate the effects of abiotic stressors on plants. Climate change, naturally or caused by anthropogenic activities, is posing incredible challenges to agricultural activities around the world. Some estimates suggest that over 90 % of rural farmland was affected by abiotic stressors at some point during the growing season (Cramer et al., 2011). Therefore, it is essential to understand the stress responses of plants to identify resistant varieties that can tolerate abiotic stressors.

Salinity is a soil-based abiotic stress factor and generates important problems in agricultural production of arid and semi-arid regions. Data show that 933 million hectares, equivalent to 7% of arable land worldwide, are affected by salinity (Gangwar et al., 2020; Mora et al., 2020; Ahmadi et al., 2022). Salinity in agricultural lands significantly affects the germination, emergence, growth and crop formation of plants (Pakniyat et al., 2003; Gorji et al., 2017). Thus, it is very important to select species and/or cultivars suitable for such agricultural lands for production.

Triticale is primarily grown for feed grain and biomass production. In addition, the stems are used as straw, while the grains are used in human nutrition. Whatever the advantages of this plant, history of triticale has been shaped by humans. Nearly 100 years of research has been conducted on botanical and genetic properties of triticale species to turn them into a product (Haesaert and de Baets, 1996; Mergoum and Gomez-Macpherson, 2004). It has been found that triticale could tolerate some abiotic stress conditions better than the other cereal species (Roake et al., 2009). For instance, Motzo et al. (2013) reported that triticale was more drought tolerant than durum wheat. The USDA salinity laboratory has reported that the salinity tolerance of triticale was better than wheat and even similar to barley (Blum, 2014).

Triticale is usually seen as an alternative of other small grain cereals, primarily wheat and barley, for growing under adverse conditions or in low-input farming systems. The presence of appropriate genetic variation is a pre-requisite for the development of any character through selection and reproduction. It has been determined that there was an intraspecific variation in salt tolerance of triticale plants (Norlyn and Epstein, 1984). On the other hand, triticale has the potential to be

an important genetic resource for the transfer of resistance genes against biotic and abiotic stress conditions (Vaillancourt et al., 2008). Successful seed germination plays a critical role in ensuring the desired plant density and economic production. Saline soil conditions significantly limit seed germination (Almansouri et al., 2001; Atis, 2011). The first step in determining the genetic potential of this plant for salt tolerance is testing it at germination and early seedling stages.

Various techniques have been suggested to protect plants from environmental stresses. One of these techniques is the identification of salt-resistant species and/or cultivars. In this study, tolerance of some Turkish triticale cultivars to different salinity levels during germination and early seedling stages were investigated.

2. Materials and Methods

The research was conducted under laboratory conditions in Field Crops Department of Mustafa Kemal University, Faculty of Agriculture in 2022. Twelve triticale (X Triticosecale Wittmak) cultivars were used as plant material of the present study. Information about triticale cultivars are provided in Table 1.

Table 1. Turkish triticale cultivars used in the study and their registration institutions

Name of cultivars	Registration institutions
Alperbey	Bahri Dagdas International Agricultural Research Institute Directorate
Aysehanim	Eastern Mediterranean Transitional Zone Research Institute Directorate
Bera	Yonca Agriculture Products
Egeyildizi	Ege Agricultural Research Institute Directorate
Esin	Gap International Agricultural Research and Centre of Education Directorate
Karma 2000	Transitional Zone Agricultural Research Institute Directorate
Mehmetbey	Eastern Mediterranean Transitional Zone Research Institute Directorate
Mikham-2002	Bahri Dagdas International Agricultural Research Institute Directorate
Ozer	Bahri Dagdas International Agricultural Research Institute Directorate
Tatlicak 97	Bahri Dagdas International Agricultural Research Institute Directorate
Truva	Limagrain Turkiye
Umranhanim	East Anatolian Agricultural Research Institute Directorate

The experimental design was two-factorial arranged in a completely randomized design with four replications. Four salt doses (60, 120, 180 and 240 mM) and control (distilled water) were used for salt stress in germination media. All salt solutions were prepared with sterile distilled water. Triticale seeds were sterilized using sodium hypochlorite (rate of 1%). To sterilize the seeds, 60 g seeds were weighed and then were washed with 100 ml of 1% sodium hypochlorite by shaking for 10 minutes. Sterilized seeds were washed 3 times with sterilized distilled water to remove chemical residues over the seeds. Sterile disposable Petri dishes with 9 cm diameter were used to germinate the seeds under salt stress. Two-layer filter paper was placed in Petri dishes and 10 mL salt solution for salt stress and 10 mL sterilized distilled water for control was used in germination media. To sow the seeds into Petri dishes, a sterile cabin was used to ensure sterile media during the germination and early seedling stage. Seeds treated with control and salt doses were incubated in an incubator at 24±1 °C for 10 days. Germinated seeds were counted in every 24 hours during 7 days. With the use of number of germinated seeds, germination ratio (GR), germination index (GI) and mean germination time (MGT) were calculated. At the end of 10 days, all Petri dishes were opened and root and shoot length (RL and SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW) and shoot dry weight (SDW) characteristics were measured on seedlings. The RFW and SFW were weighed and the samples were dried in an oven at 65 °C for 24 hours. Weighing was made to determine the RDW and SDW characteristics of the dried samples (Equations 1-3).

$$GR\% = \frac{Total\ Germinated\ Seeds}{Total\ Seeds\ Used\ for\ Germination\ Test} \times 100 \quad (1)$$

$$GI = \sum G/T \quad (2)$$

where; G is the number of germinated seeds per day and T is the germination period.

$$MGT\ (day) = \sum (n \times d)/N \quad (3)$$

where; n is the number of seeds germinated on each day, d is the number of days from the beginning of the test and N is the total number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981).

All data obtained from the present experiments were subjected to two-way ANOVA and biplot analysis with the use of SAS JMP 13 statistical software. The Tukey pair-wise test was applied to compare significant means ($P<0.05$) (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Effects of Cultivars on Germination Parameters and Seedling Properties

Effects of cultivars on germination and seedling parameters are provided in Table 2. All characteristics were significantly (P<0.0001) affected by cultivars. The data for GR, GI, MGT, RL and SL are given Table 3. GR values of the cultivars ranged from 36.50% to 87.25% with the highest value from Mehmetbey cultivar and the lowest value from Esin cultivar. Egeyildizi and Aysehanim cultivars gave similar results with Mehmetbey cultivar. Esin cultivar with lowest GR was placed into the same group with Bera and Karma 2000 cultivars. The GI values of the cultivars varied between 3.05 and 8.16 with the highest value from Mehmetbey cultivar and the lowest value from Bera cultivar. However, Bera, Esin and Karma 2000 cultivars were placed into the same group. MGT values ranged from 2.55 days to 6.47 days with the highest value from Umranhanim cultivar and the lowest value from Ozer cultivar. In other words, Ozer cultivar achieved the fastest germination under different salt doses. RL values of the cultivars varied between 1.99 - 9.67 cm with the highest value from Aysehanim cultivar and the lowest value from Umranhanim cultivar. SL values of the cultivars changed between 4.56 and 9.97 cm.

The mean values for the parameters of RFW, SFW, RDW, SDW and BW are given in Table 4. RFW values of the cultivars varied between 21.07 - 82.50 mg plant^{-1} with the highest value from Mehmetbey cultivar and the lowest value from Umranhanim cultivar. SFW values of the cultivars ranged between 38.81 and 85.95 mg plant^{-1} with the highest value from Karma 2000 cultivar and the lowest value from Umranhanim cultivar. RDW values of the cultivars varied between 4.00 and 10.85 mg plant^{-1} with the highest value from Mikham-2002 cultivar and the lowest value from Umranhanim cultivar. SDW values of the cultivars changed between 5.44 and 10.45 mg plant^{-1} with the highest value from Mikham-2002 cultivar and the lowest value from Umranhanim cultivar. BW values of the cultivars varied between 994.4 and 3019.2 mg Petri^{-1} with the highest value from Mehmetbey cultivar and the lowest value from Umranhanim cultivar.

3.2. Effects of Salt Doses on Germination Parameters and Seedling Properties

Salt doses had significant effects on germination parameters and seedling properties of triticale cultivars (Table 2). Mean values for GR, GI, MGT, RL and SL at different salt doses are given Table 5. Decreasing GR values were seen with increasing salt doses. As compared to control treatment, 240 mM salt dose yielded 38.29% reduction in GR. The GI values also decreased with increasing salt doses.

Table 2. Effects of cultivars, salt doses and their interactions on investigated parameters

Items	Cultivars (C)	Salt doses (SD)	C×SD
Germination ratio, GR, (%)	<.0001	<.0001	<.0001
Germination index, GI	<.0001	<.0001	<.0001
Mean germination time, MGT, (day)	<.0001	<.0001	<.0001
Root length, RL, (cm)	<.0001	<.0001	0.0002
Shoot length, SL, (cm)	<.0001	<.0001	0.0050
Root fresh weight, RFW, (mg/plant)	<.0001	<.0001	0.0002
Shoot fresh weight, SFW, (mg/plant)	<.0001	<.0001	0.0037
Root dry weight, RDW, (mg/plant)	<.0001	<.0001	0.0016
Shoot dry weight, SDW, (mg/plant)	<.0001	<.0001	0.0055
Biomass weight, BW, (mg/Petri)	<.0001	<.0001	<.0001

Table 3. Mean values and Tukey pair-wise test results for GR, GI, MGT, RL and SL of the cultivars

Cultivars	Investigated parameters				
	GR (%)	GI	MGT (day)	RL (cm)	SL (cm)
Alperbey	71.00±5.21 ^{bcd}	6.01±0.45 ^{cd}	4.19±0.20 ^{bc}	2.38±0.68 ^{f}	5.37±0.90 ^{de}
Aysehanim	81.25±4.59 ^{ab}	7.07±0.49 ^{b}	3.38±0.19 ^{cde}	9.67±1.46 ^{a}	7.78±1.18 ^{bc}
Bera	38.75±5.39 ^{g}	3.05±0.42 ^{f}	4.14±0.23 ^{bc}	3.32±0.40 ^{ef}	6.42±0.79 ^{cd}
Egeyildizi	78.75±4.78 ^{ab}	5.80±0.39 ^{cd}	4.84±0.21 ^{b}	5.30±0.77 ^{cde}	7.27±1.03 ^{bc}
Esin	36.50±5.80 ^{g}	3.21±0.48 ^{f}	4.58±0.37 ^{b}	6.81±1.38 ^{bc}	7.83±1.07 ^{bc}
Karma 2000	38.00±3.86 ^{g}	3.45±0.28 ^{f}	3.10±0.26 ^{de}	5.78±0.82 $^{b-e}$	8.33±1.06 ^{ab}
Mehmetbey	87.25±3.49 ^{a}	8.16±0.27 ^{a}	3.21±0.12 ^{de}	8.42±1.13 ^{ab}	7.35±1.19 ^{bc}
Mikham-2002	60.75±4.52 ^{def}	4.79±0.39 ^{e}	4.88±0.21 ^{b}	4.50±0.48 $^{c-f}$	7.66±1.03 ^{bc}
Ozer	60.50±5.06 ^{ef}	5.71±0.50 ^{d}	2.55±0.11 ^{e}	6.59±0.97 ^{bcd}	8.51±1.10 ^{ab}
Tatlicak 97	67.50±6.00 ^{cde}	5.61±0.51 ^{de}	4.61±0.24 ^{b}	4.12±0.63 ^{def}	5.21±0.92 ^{de}
Truva	74.75±5.97 ^{bc}	6.61±0.51 ^{bc}	3.71± 0.17^{cd}	5.45±0.75 ^{cde}	9.97±1.20 ^{a}
Umranhanim	54.75±5.97 ^{f}	4.83±0.47 ^{e}	6.47±0.45 ^{a}	1.99±0.19 ^{f}	4.56±0.88 ^{e}

GR= germination ratio, GI= germination index, MGT= mean germination time, RL= root length, SL= shoot length.

Increasing MGT values were seen with increasing salt doses. The salt doses of 120, 180 and 240 mM yielded similar MGT values with 4.47, 4.51 and 4.37 days, respectively. RL values varied between 1.99 - 8.76 cm. Increasing salt doses significantly reduced RL values. As compared to control treatment, 240 mM salt dose reduced RL values by 22.72%. Similar findings were also observed for SL values. As compared to RL values, SL results were more affected by increasing salt doses.

The mean values for RFW, SFW, RDW, SDW and BW at different salt doses are given Table 6. RFW values ranged from 32.88 to 74.49 mg plant^{-1} with the highest value from the control treatment and the lowest value from 240 mM salt dose. Decreasing RFW values were seen with increasing salt doses. The salt doses of 180 mM and 240 mM yielded similar results. SFW values varied between 21.11 - 120.70 mg plant^{-1}. Significant decreases were seen in SFW values with increasing salt doses. RDW values varied between 6.51 - 8.90 mg plant^{-1} with the highest value from 60 mM salt dose and the lowest value from 240 mM salt dose. The 180 mM and 240 mM salt doses yielded similar results. In addition, control, 60 mM and 120 mM treatments yielded similar results. SDW values of control, 60 mM, 120 mM, 180 mM and 240 mM treatments were respectively recorded as 11.86, 10.95, 9.62, 7.20 and 4.18 mg plant^{-1}. BW values changed between 434.2 and 3599.9 mg Petri^{-1}. Decreasing BW values were seen with increasing salt doses.

Table 4. Mean values and Tukey pair-wise test results for RFW, SFW, RDW, SDW and BW of the cultivars

Cultivars	Investigated parameters				
	RFW (mg plant^{-1})	SFW (mg plant^{-1})	RDW (mg plant^{-1})	SDW (mg plant^{-1}	BW (mg Petri^{-1})
Alperbey	38.61±4.61 ^{def}	63.63±7.59 ^{bc}	5.78±0.53 ^{de}	8.14±0.78 ^{ab}	1057.9±231.7 ^{d}
Aysehanim	70.12±8.50 ^{ab}	75.80±10.04 ^{ab}	8.87±0.53 ^{ab}	8.75±0.83 ^{ab}	2635.9±394.5 ^{ab}
Bera	35.86±5.70 ^{ef}	68.59±6.69 ^{abc}	6.25±0.74 ^{cd}	9.16±0.79 ^{ab}	965.8±180.1 ^{d}
Egeyildizi	55.75±8.06 $^{b-e}$	68.56±9.11 ^{abc}	6.54±0.47 ^{cd}	9.30±0.76 ^{ab}	2180.9±350.1 ^{bc}
Esin	62.63±7.59 ^{abc}	73.37±10.60 ^{ab}	7.88±0.43 ^{bc}	9.81±0.83 ^{a}	2352.6±387.3 ^{b}
Karma 2000	57.46±4.84 ^{bcd}	85.95±11.64 ^{a}	6.59±0.60 ^{cd}	8.60±0.63 ^{ab}	1325.9±219.0 ^{d}
Mehmetbey	82.50±6.42 ^{a}	72.43±12.57 ^{ab}	10.49±0.34 ^{a}	8.54±1.04 ^{ab}	3019.2±379.7 ^{a}
Mikham-2002	70.17±5.86 ^{ab}	74.22±9.63 ^{ab}	10.85±0.57 ^{a}	10.45±0.96 ^{a}	2112.0±321.0 ^{bc}
Ozer	74.57±5.93 ^{ab}	80.04±10.58 ^{ab}	10.35±0.45 ^{a}	9.74±0.86 ^{a}	2111.5±345.8 ^{bc}
Tatlicak 97	43.21±4.64 ^{cde}	49.84±9.04 ^{cd}	6.76±0.41 ^{cd}	7.35±0.85 ^{bc}	1571.9±281.2 ^{cd}
Truva	57.16±7.10 ^{bcd}	81.40±9.85 ^{ab}	6.87±0.59 ^{bcd}	9.86±0.83 ^{a}	2452.4±364.2 ^{ab}
Umranhanim	21.07±1.98 ^{f}	38.81±6.10 ^{d}	4.00±0.36 ^{e}	5.44±0.65 ^{c}	994.4±153.4 ^{d}

RFW= root fresh weight, SFW= shoot fresh weight, RDW= root dry weight, SDW= shoot dry weight, BW= biomass weight.

Table 5. Mean values and Tukey pair-wise test results for GR, GI, MGT, RL and SL values at different salt doses

Salt doses (mM)	Investigated parameters				
	GR (%)	GI	MGT (day)	RL (cm)	SL (cm)
Control	88.96±1.74 ^{a}	7.77±0.22 ^{a}	3.48±0.13 ^{b}	8.76±0.75 ^{a}	12.35±0.33 ^{a}
60	76.15±3.35 ^{b}	6.53±0.28 ^{b}	3.87±0.15 ^{b}	8.05±0.74 ^{a}	11.04±0.39 ^{b}
120	62.50±3.59 ^{c}	5.21±0.31 ^{c}	4.47±0.23 ^{a}	5.11±0.40 ^{b}	7.57±0.42 ^{c}
180	50.73±3.25 ^{d}	4.19±0.25 ^{d}	4.51±0.23 ^{a}	2.90±0.20 ^{c}	3.63±0.27 ^{d}
240	34.06±2.49 ^{e}	3.09±0.22 ^{e}	4.37±0.26 ^{a}	1.99±0.12 ^{c}	1.34±0.13 ^{e}

GR= germination ratio, GI= germination index, MGT= mean germination time, RL= root length, SL= shoot length.

Table 6. Mean values and Tukey pair-wise test results for RFW, SFW, RDW, SDW and BW at different salt doses

Salt doses (mM)	Investigated parameters				
	RFW (mg plant^{-1})	SFW (mg plant^{-1})	RDW (mg plant^{-1})	SDW (mg plant^{-1})	BW (mg Petri^{-1})
Control	74.49±4.50 ^{a}	120.70±4.02 ^{a}	8.20±0.33 ^{a}	11.86±0.31 ^{a}	3599.9±176.5 ^{a}
60	73.52±4.92 ^{a}	96.78±3.83 ^{b}	8.90±0.43 ^{a}	10.95±0.48 ^{a}	2801.4±188.6 ^{b}
120	58.50±4.08 ^{b}	66.96±3.28 ^{c}	7.86±0.45 ^{a}	9.62±0.38 ^{b}	1772.5±152.4 ^{c}
180	39.40±2.98 ^{c}	41.39±4.60 ^{d}	6.52±0.39 ^{b}	7.20±0.38 ^{c}	883.8±68.4 ^{d}
240	32.88±2.82 ^{c}	21.11±1.97 ^{e}	6.51±0.47 ^{b}	4.18±0.35 ^{d}	434.2±41.8 ^{e}

RFW= root fresh weight, SFW= shoot fresh weight, RDW= root dry weight, SDW= shoot dry weight, BW= biomass weight.

3.3. Effects of Interactions (Cultivar × Salt Dose) on Germination Parameters and Seedling Properties

Cultivar × salt dose interactions had significant effects germination parameters and early seedling characteristics. Interaction bar-charts of all investigated properties are provided in Figure 1 - 10. The GR values of

all cultivars decreased with increasing salt doses (Figure 1). In terms of GR, Mehmetbey cultivar was identified as the most resistant at 240 mM salt dose. The GI values of all Triticale cultivars also decreased with increasing salt dose (Figure 2). The highest GI at 240 mM salt dose was obtained from Mehmetbey cultivar. MGT values did change significantly with changing salt doses (Figure 3). However, as compared to other cultivars, MGT values of Umranhanim cultivar increased with increasing salt doses. RL values of Triticale cultivars substantially decreased with increasing salt doses (Figure 4). Similar results were also found for SL (Figure 5). These results indicated that seedling growth was adversely affected by increasing salt doses. The RFW values of the cultivars decreased with increasing salt doses (Figure 6). The lowest decrease in RFW was seen in Ozer cultivar. SFW values of all cultivars significantly decreased with increasing salt doses (Figure 7). In terms of SFW, Mehmetbey cultivar was identified as the most adversely affected from salt stress. The RDW and SDW values significantly decreased with increasing salt doses (Figure 8 and 9). All cultivars practically yielded identical results for RDW under 240 mM salt stress as compared to the control. The lowest SDW value was seen in 240 mM salt dose of Mehmetbey cultivar. The BW values of triticale cultivars decreased as the salt dose increased (Figure 10).

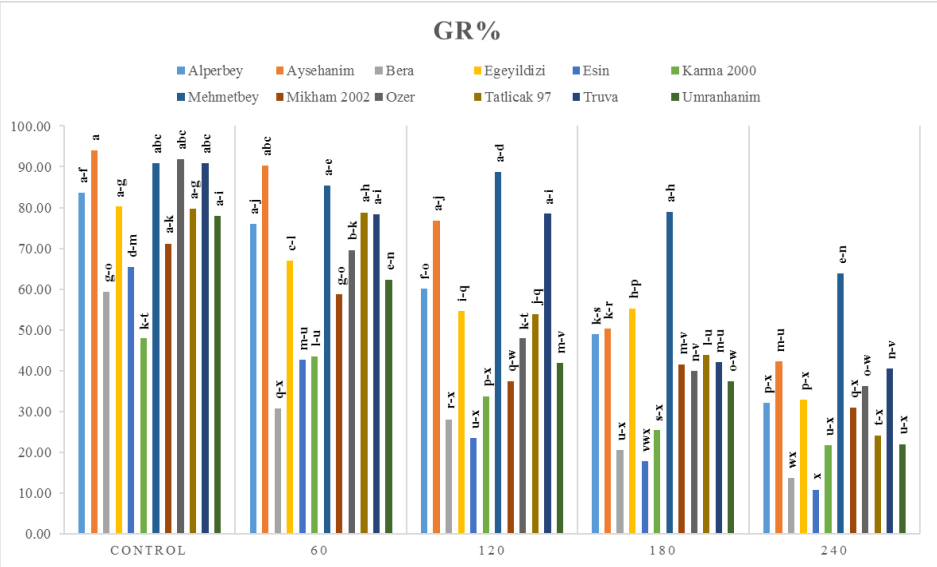

Figure 1. Effects of interactions on germination ratio (GR) of some Turkish triticale cultivars.

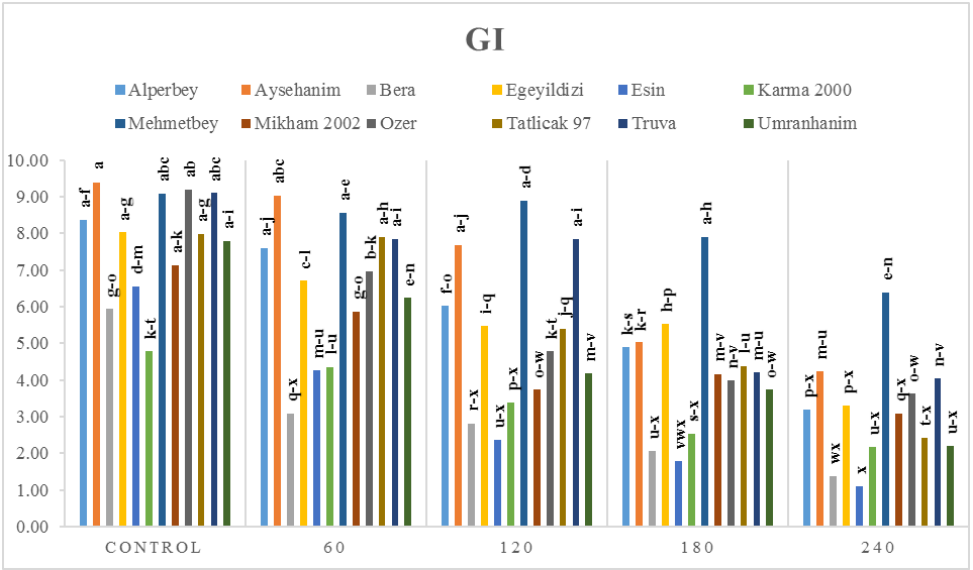

Figure 2. Effects of interactions on germination index (GI) of some Turkish triticale cultivars.

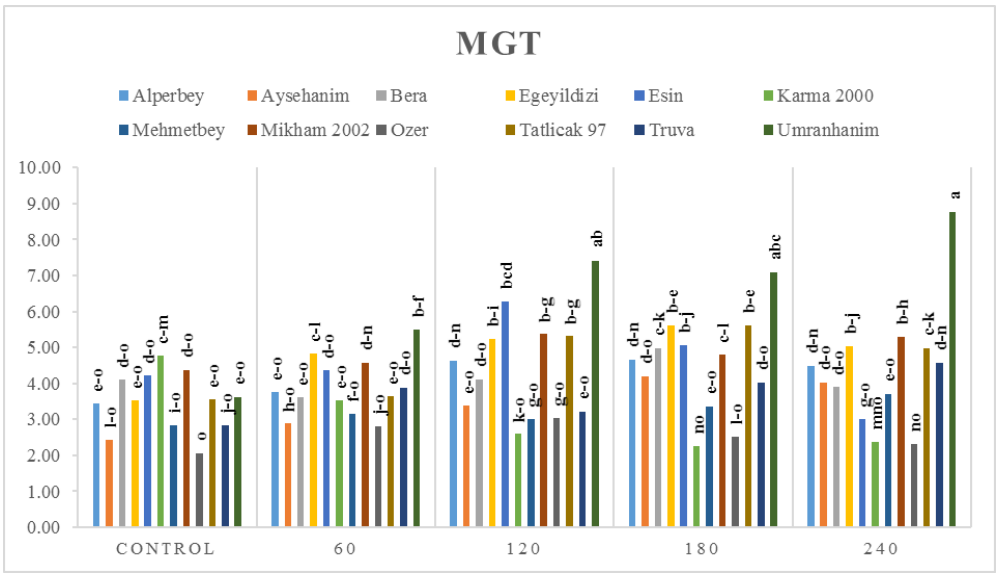

Figure 3. Effects of interactions on mean germination time (MGT) of some Turkish triticale cultivars.

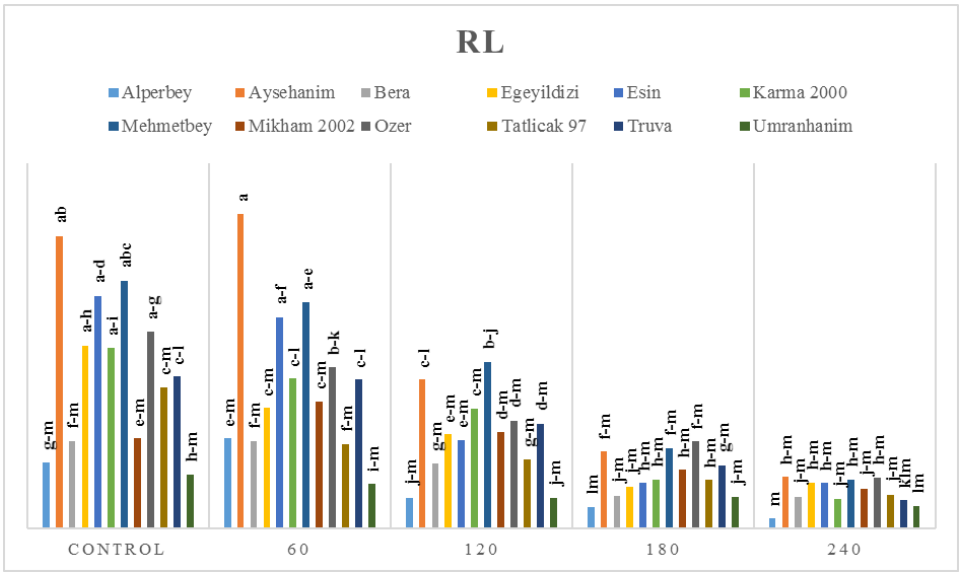

Figure 4. Effects of interactions on root length (RL) of some Turkish triticale cultivars.

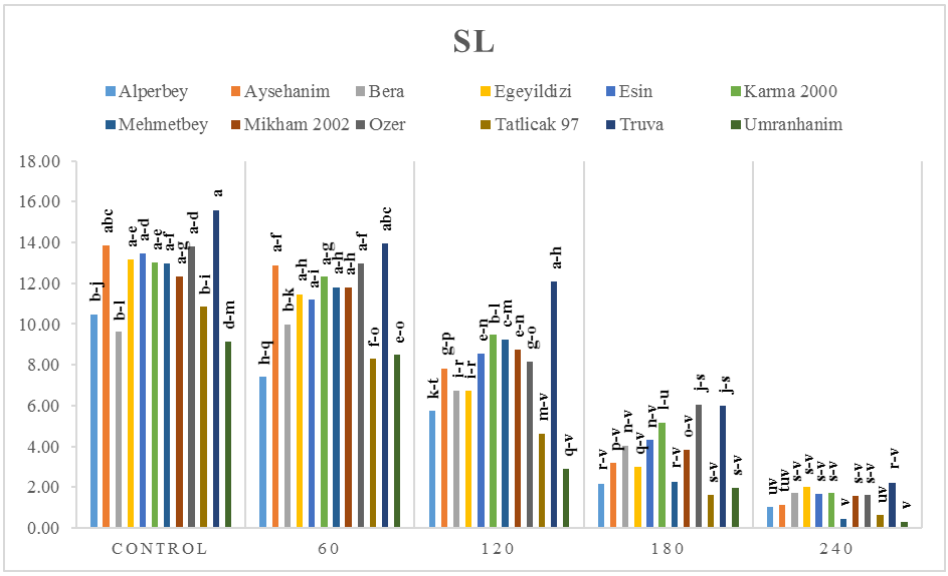

Figure 5. Effects of interactions on shoot length (SL) of some Turkish triticale cultivars.

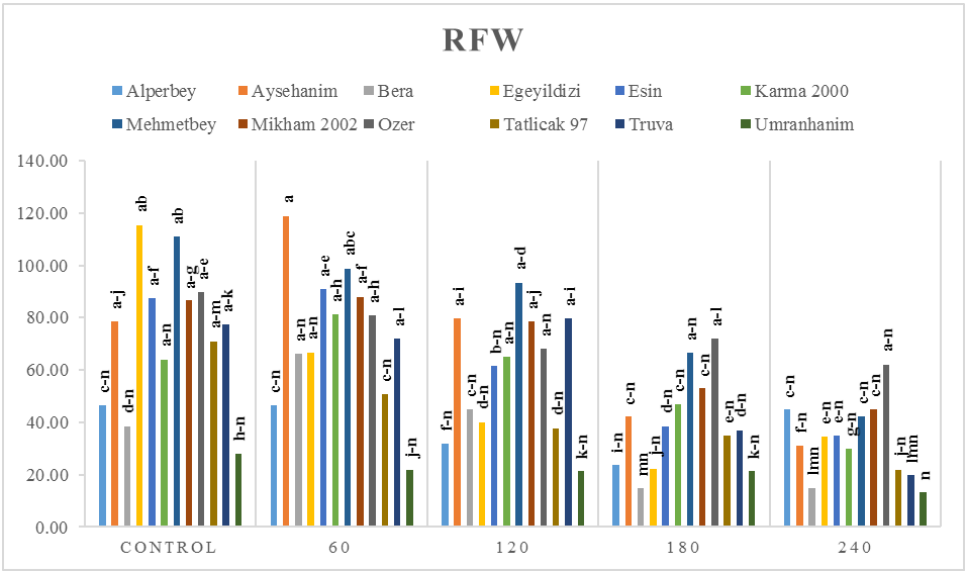

Figure 6. Effects of interactions on root fresh weight (RFW) of some Turkish triticale cultivars.

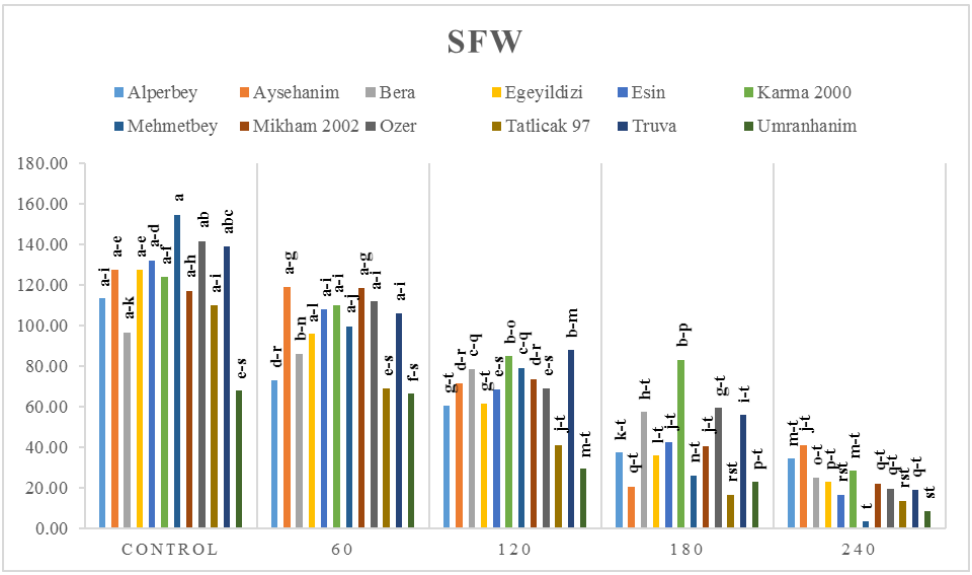

Figure 7. Effects of interactions on shoot fresh weight (SFW) of some Turkish triticale cultivars.

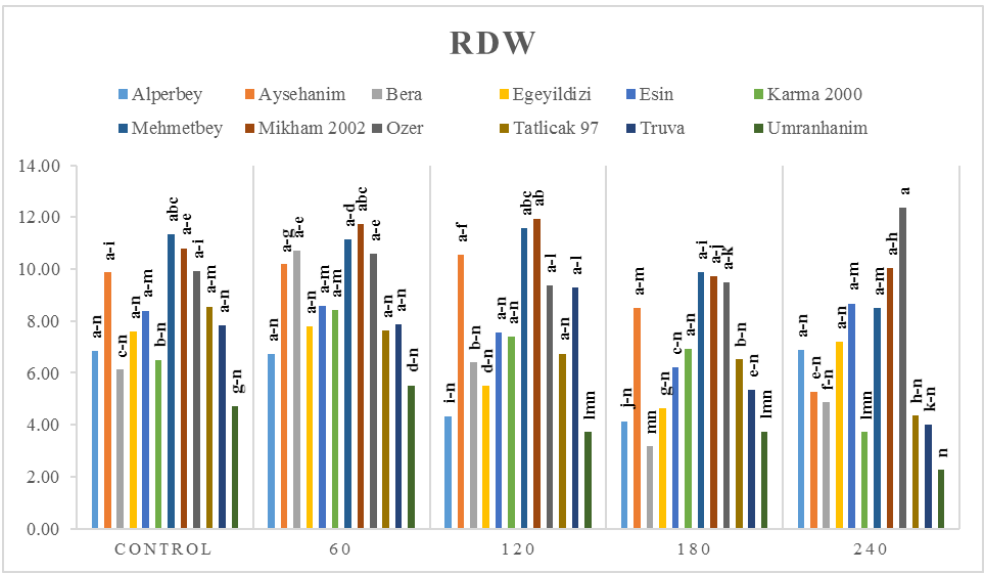

Figure 8. Effects of interactions on root dry weight (RDW) of some Turkish triticale cultivars.

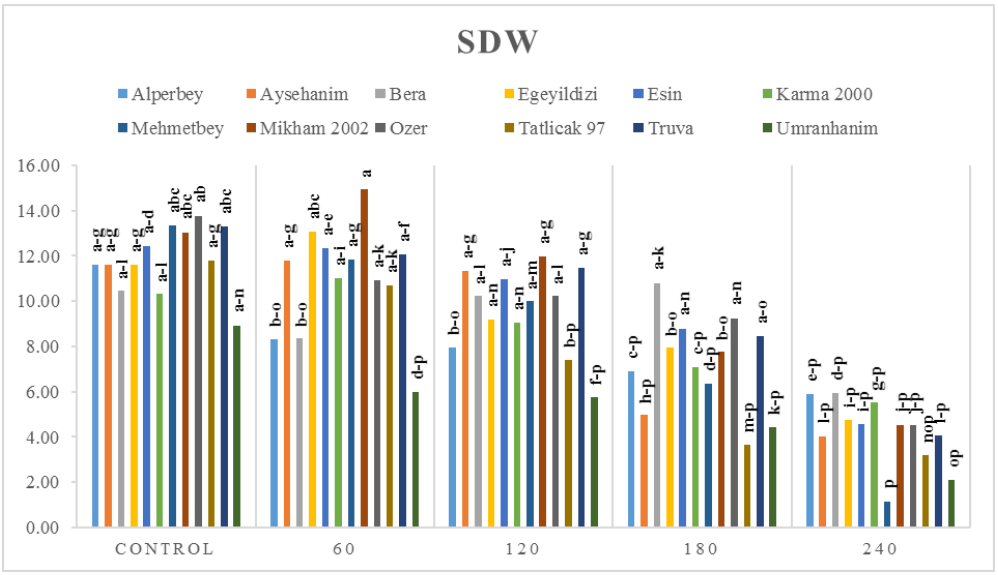

Figure 9. Effects of interactions on shoot dry weight (SDW) of some Turkish triticale cultivars.

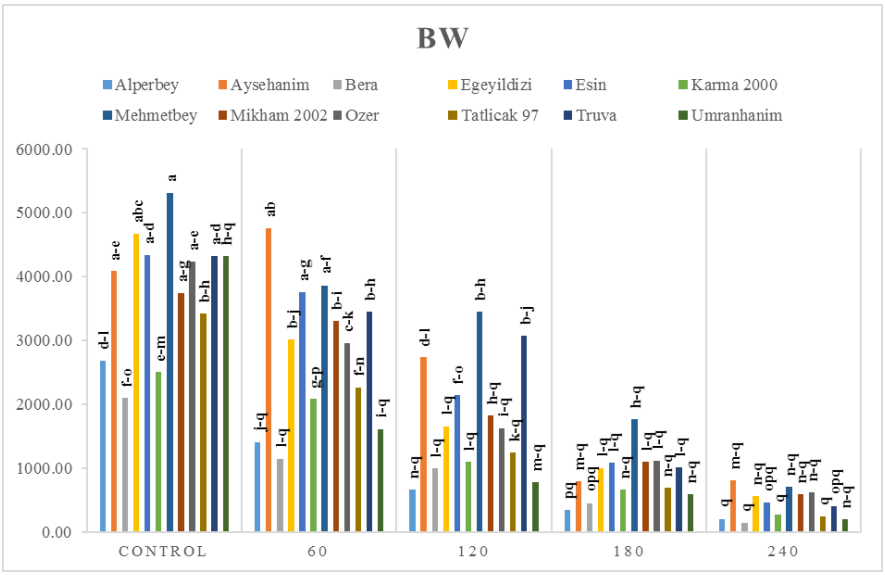

Figure 10. Effects of interactions on biomass weight (BW) of some Turkish triticale cultivars.

The GR values of the cultivars generally decreased with increasing salt doses (Figure 1). Germination ratio is a highly significant parameter for successful production of field crops particularly in salt-affected soils (Farooq et al., 2015). Similar to present germination ratios, it has been generally reported that germination of plants was adversely affected by increasing salt stress levels during the germination stage (Ashraf and Foolad, 2005; Atak et al., 2006; Farsiani and Ghobadi, 2009; Atış, 2011; Konuşkan et al., 2017). The GI values of the cultivars also decreased significantly with increasing salt doses (Figure 2). However, each triticale cultivar yielded different germination index results under different salt stress levels (Table 2). It has been reported in many studies that the germination index of plants decreased significantly with increasing salt doses (Khayatnezhad and Gholamin, 2011; Ologundudu et al., 2014; Ertekin et al., 2017; Süren and Kızılşimşek, 2020). On the other hand, similar to present findings, it was determined that different responses to increased salt stress levels occurred even among the cultivars of different plants (Bybordi and Tabatabaei, 2009; Khayatnezhad and Gholamin, 2011; Ologundudu et al., 2014). Salt stress caused an increase in mean germination time (MGT) characteristic of the cultivars (Figure 3). It has been reported by various research that mean germination time of plants was prolonged due to increasing salt stress doses (Nizam, 2011; Elouaer and Hannachi, 2012; Cokkizgin, 2012). Root and shoot lengths (RL and SL) of triticale cultivars decreased significantly with increasing salt doses. In terms of RL and SL characteristics, there were significant differences among cultivars (Table 2). It has been reported by various studies that increasing salt doses inhibit shoot and root growth of field crops (Rios-Gonzalez et al., 2002; Szalai and Janda, 2009; El Sayed and El Sayed, 2011; Qu et al., 2012). Root and shoot fresh

weights (RFW and SFW) decreased as the salt dose increased (Figure 6 and Figure 7). It has been reported that root and shoot growth were significantly reduced in canola due to increasing doses (Bybordi and Tabatabsei, 2009). Although there was a growth regression in the roots at increasing salt stress levels, it was determined that the shoots were more adversely affected than the roots (Munns, 2012). In this study, in terms of root dry weight (RDW), each cultivar yielded different responses to different salt doses (Figure 8). Indeed, it has been reported that some cultivars have increased ion uptake and dry weight due to the salt in the germination medium, while some have no ion uptake and their root dry weights have decreased (Khayatnezhad and Gholamin, 2011; Nizam, 2011; Elouaer and Hannachi, 2012). Shoot dry weight (SDW) property of triticale cultivars was also adversely affected by increasing salt doses (Figure 9). It has been reported by various studies that shoot growth in plants was significantly restricted due to increasing salt doses (Atış, 2011; Ertekin et al., 2017; Ertekin et al., 2018; Ertekin et al., 2022). Biomass weight (BW) of triticale cultivars decreased as the salt dose increased (Figure 10). It has been reported by various studies that growth was restricted and biomass weight decreased in plants under various abiotic stress conditions (Ertekin et al., 2020; Ertekin and Bilgen, 2021; Aygün et al., 2022).

3.4. Evaluation of Cultivars by Principal Component Analysis

Correlation biplots of principle components 1 and 2 of the PCA results obtained from data of germination and early seedling properties of twelve triticale cultivars subjected different NaCl doses are illustrated in Figure 11. Principle component 1 (F1) described 59.46% of the original information and principle component 2 (F2) described 22.25% (81.1% by two principle components). PCA analysis allowed easy visualization of complex data on germination parameters and early seedling parameters of twelve triticale cultivars. To examine the contributors to the principle components, the germination and early seedling characteristics in F1 and F2 were compared. It was clear that GR, GI, RL, RFW, RDW and BW were grouped with positive axis on the upper right side of the biplot, suggesting that these parameters had a positive correlation among themselves. The cultivars clustered in the upper right of the biplot graph were Mehmetbey and Ayşehanim cultivar and thus, it can be said that these cultivars were superior in terms of the characteristics in the upper right. MGT values were clustered on the upper left side of the biplot, while SL, SFW and SDW were found on lower right portion of the biplot. Bera, Esin and Karma 2000 cultivars were further away from GR and GI parameters. Ümranhanim cultivar was found further away from many of the studies traits.

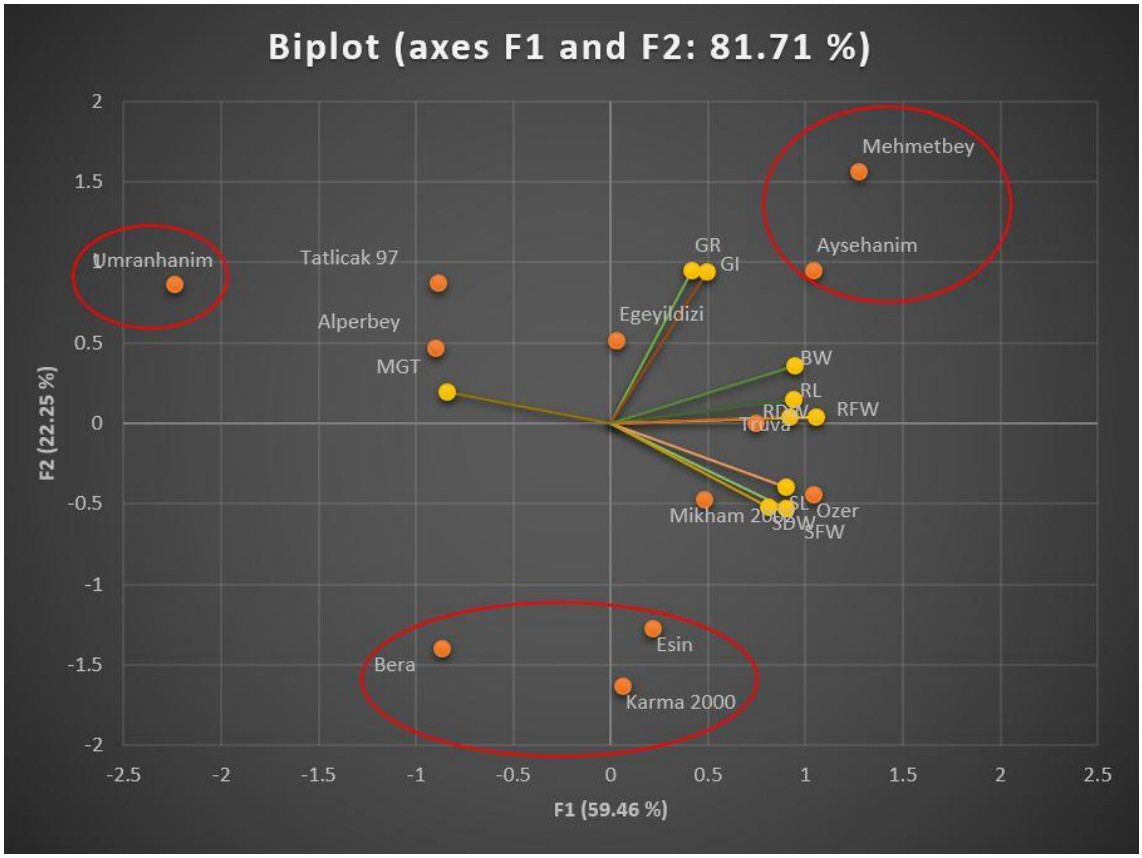

Figure 11. Correlation biplots of principle components 1 and 2 of the PCA results obtained from data on germination parameters and early seedling properties of twelve triticale cultivars subjected to different NaCl doses.

4. Conclusion

In this study, germination parameters and early seedling properties of Turkish triticale cultivars were investigated under different salt stress levels. In general, germination parameters and early seedling stage characteristics of the cultivars were adversely affected by increasing salt doses. Yet, it was determined that the response to increasing salt doses were different among the triticale cultivars. Although Mehmetbey cultivar was adversely affected by increased salt doses, it was found to be more resistant to saline conditions than the other cultivars. On the other hand, Umranhanim cultivar was identified as the most sensitive cultivar to salt stress. After investigating the resistance of Mehmetbey triticale cultivar to salt stress under field conditions, this cultivar can be included into breeding programs or cultivated in areas with salinity problems.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.A.	İ.E.	İ.A.
C	30	40	40
D	50	40	10
S	50		50
DCP	25	50	25
DAI	10	70	20
L	25	50	25
W	30	40	30
CR	40	20	40
SR	30	50	20
PM	60	20	20
FA	30	40	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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Research Article
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ESTIMATION OF SOYBEAN SEEDS WEIGHT USING IMAGE PROCESSING

Hayrettin KARADÖL[1]*, Hamza KUZU[1], Mualla KETEN GÖKKUŞ[2]

[1]*Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Biosystems Engineering, 46100, Kahramanmaras, Türkiye*
[2]*Nevşehir Hacı Bektaş Veli University, Engineering-Architecture Faculty, Biosystems Engineering, 50300, Nevsehir, Türkiye*

Abstract: Today, image processing techniques are frequently used in irrigation, fertilization and spraying applications in order to increase agricultural input efficiency and product quality. In this study, the relationship between the image and weight of soybeans was investigated. For this purpose, some image processing applications were carried out on the images of soybeans grown with the deficit irrigation (100%, 75, 50 and 25) method. In the study, the relationship between the weight of soybeans and the number of pixels occupied on the images was 88.78%. The weights belonging to the displayed soybean grains decreased from 100% watered to 50% watered, in the 25% irrigated area, it increased again. The 25% irrigated case created significant stress for soybeans. However, as in some plants, this situation caused an increase in grain weight in soybeans.

Keywords: Image processing, HSV color space, Soybean

***Corresponding author:** Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Biosystems Engineering, 46100, Kahramanmaras, Türkiye
E mail: hayrettinkaradol@gmail.com (H. KARADÖL)
Hayrettin KARADÖL https://orcid.org/0000-0002-5062-0887
Hamza KUZU https://orcid.org/0000-0001-8585-4467
Mualla KETEN GÖKKUŞ https://orcid.org/0000-0001-7741-922X

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1. Introduction

Soybean (*Glycine max*. L.), a one-year warm climate plant belonging to the legume family, is used in human and animal nutrition and has a high nutritional value (Sahar, 2017). Its seeds contain an average of 36-40% protein, 18-24% fat, 26% carbohydrates and 18% mineral matter, and about 20% of the world's vegetable oil production is met by soybeans (Arioglu, 2007). Soybean protein has the closest protein to animal protein and has a high biological value (Seckin Dinler and Tasci, 2020). Soybean can be grown for grain, dry hay and silage, can be used as a cover crop for erosion control and as a green fertilizer that adds nitrogen to the soil (Acikgoz et al., 2013). After itself, soybean, which is included in the crop rotation, has an important potential for sustainable agriculture due to its increased yield in the products to be sown and its fertilizer savings, both economically and ecologically, providing positive contributions to the soil (Agin and Malasli, 2016; Ozel and Acar, 2020). Water stress is an effective factor that limits the production of this important plant in semi-arid and semi humid regions of the world. In these regions, the frequency and amount of precipitation during the growing season is usually quite variable. Under non-irrigated conditions in humid areas, the variability in seasonal rainfall leads to variability in water and nutrient uptake, as well as growth, development, and yield from year to year (Scott et al., 1987; Sincik et al., 2008).

Growing soybean is economically feasible in places where there is water and low irrigation costs, however, water stress caused by limited irrigation in places where water is not available significantly reduces seed yield and components of soybean (Scott et al., 1987; James, 1988; Karam et al., 2005; Sincik et al., 2008; Candogan, 2009; Turgut, 2021). Water stress has different effects on plants depending on the period. If water stress is applied at the beginning of flowering period in soybean, it can reduce the number of pods in the plant, while in the flowering period it can cause a decrease in the number and size of pods. If water stress is applied during the pod filling period, it can negatively affect the size of the seeds (Oya et al., 2004; Candogan, 2009; Turgut, 2021).

Thanks to the advances in computer technology, one of the fields of application that has a very wide scope is image processing (Balci et al., 2016; Demir et al., 2016). The advantages of image processing such as providing more accurate and reliable results than traditional methods, being fast and economical at the same time, have enabled its widespread use (Balkir et al., 2019). In agricultural production, different image processing applications are carried out from the sowing process to the obtaining of the products and then the determination of their quality, size, weight, etc. characteristics. The processing of any image data generally involves (1) reading the images by an image processing program (Image Acquisition), (2) performing some preprocessing (changing image size, color space transformations, normalization, etc.) to eliminate distortions and noise in the images (Preprocessing), (3) extracting information such as color and shape from the image (Feature

Extraction), (4) classifying the extracted information from the image to recognize objects (Classification) (Pedreschi et al., 2004; Rzanny et al., 2017). Image processing is widely used in agricultural applications such as color analysis of agricultural products, quality control, classification, detection of weeds, spraying and automation processes in agriculture. Many studies have been conducted in this field (Kilic et al., 2007; Chen et al., 2010; Sabanci et al., 2012; Cai et al., 2013; Sofu et al., 2013; Paap, 2014; Sabanci and Aydin, 2014; Rahman et al., 2015; Demir et al., 2016; Yilmaz, 2016; Karadol, 2017; Sert, 2018; Sharif et al., 2018; Solak and Altinisik, 2018; Turkoglu et al., 2020).

In this study, the aim was to estimate the weight of the pods based on the area they occupy on the image with the limited irrigation method for soybean seeds. The results obtained were compared with the real weight and the success rate of the system was calculated and an effective method was presented for the soybean quality value.

2. Materials and Methods

In the study, soybeans produced in a total of 12 parcels with three repetitions of irrigation, 100% irrigation, 75% irrigation with 25% deficit of 100% irrigation, 50% irrigation with 50% deficit of 100% irrigation, and 25% irrigation with 75% deficit of 100% irrigation, were used to meet the plant's needs.

The soybeans collected from the parcels were divided into groups and 80 soybeans were selected for each group. The 12 bean groups were then randomly placed on an A4 paper. Afterwards, images of the soybeans were obtained from a CMOS sensor type cell phone camera with a resolution of 64 MP at a distance of 40 cm. The images obtained in RGB format were then processed in the MATLAB environment using the following steps.

1. The region of interest (ROI) was determined and cropped out from the RGB image.
2. The resulting new image was converted from the RGB color space to the HSV color space.
3. The "saturation" channel in the HSV color space was converted to a binary image using a static threshold value due to its ability to separate the soybeans more clearly from the gray level value.
4. The noises on the binary image were cleaned using a small-valued structural element and the total area values (total pixel count) of the regions (soybeans) with white color values on the image were recorded. Images related to these processes are shown in Figure 1.

3. Results

The number of pixel counts and weights of soybean groups on the image are shown in Table 1 and the change states of these two values are shown in Figure 2. When the irrigation rate was reduced from 100% to 50%, the average pixel count and weight of the bean groups decreased. At 25% irrigation rate, both the weight and total pixel count increased. As in some plants, it is understood that soybean also causes grain growth and weight gain as a result of the plant's exposure to water stress. The relationship between total pixel counts and weights belonging to images of soybean seed groups is given in Figure 3. The actual weights of the 12 manually separated soybean seeds were determined with 88% accuracy across all irrigation method using the total number of pixels they occupied on the image. For production purposes only to obtain seed weight without taking into account quality and nutritional content, 25% irrigation can be recommended. However, to make a definite conclusion on this, the number of experiments on this subject needs to be extended.

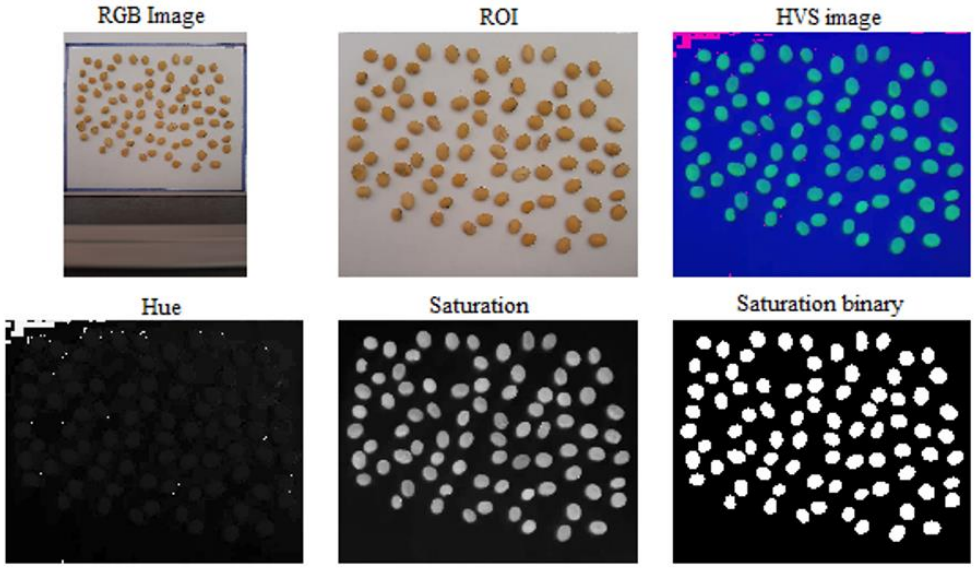

Figure 1. Image transformation processes.

Table 1. Total pixel counts and weights belonging to images of soybean groups

Group No	Irrigation method (%)	Number of pixels (Pcs)	Mean	Weight	Mean
1		1773989		10.4566	
2	100	181422	184621	11.8426	11.6711
3		198452		12.7141	
4		190834		10.8710	
5	75	144092	174757	6.6646	9.5187
6		189345		11.0204	
7		175557		9.6025	
8	50	162188	165019	8.8541	8.8513
9		157311		8.0952	
10		183987		11.0756	
11	25	179750	183628	11.5957	11.5026
12		187147		11.8365	

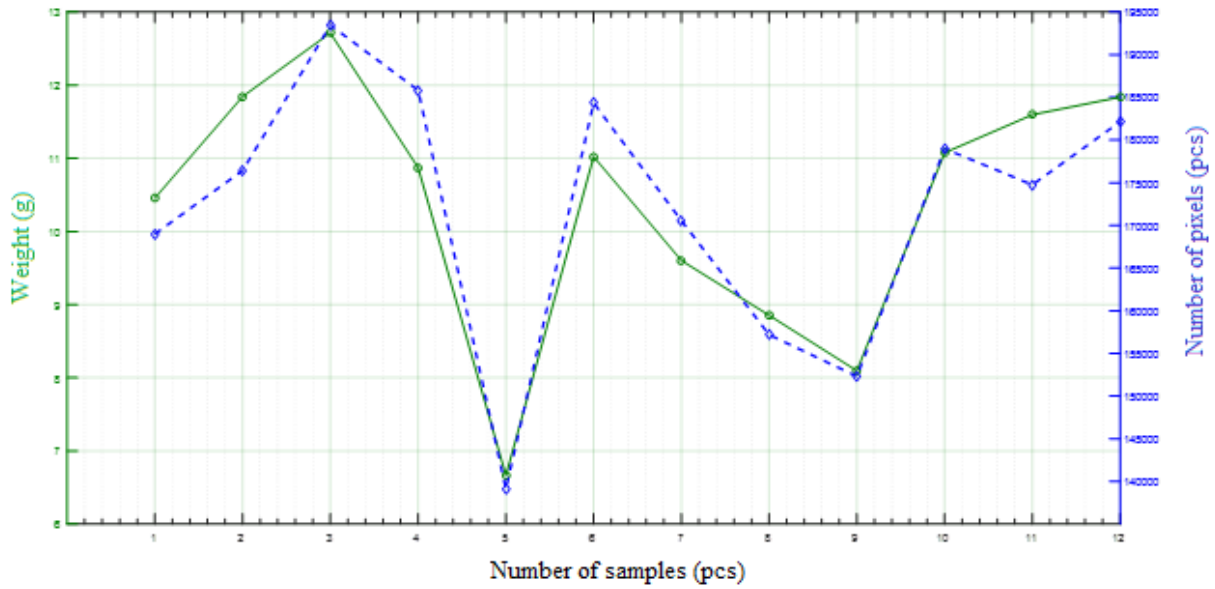

Figure 2. Graph of total pixel counts and weights belonging to images of soybean groups.

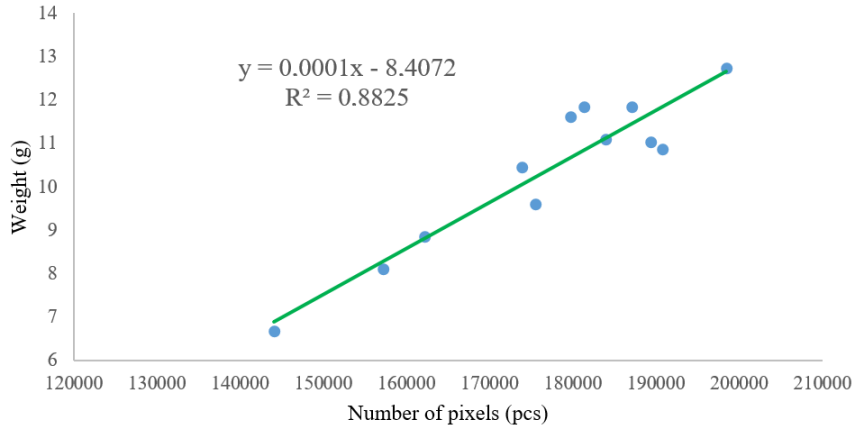

Figure 3. Relationship between total pixel counts and weights belonging to images of soybean deed groups.

4. Discussion

In many studies, image processing techniques have been used to determine product quality by analyzing the shape, size and weight of agricultural products. Sharma et al. (2021) performed an image processing based investigation to classify wheat physical properties such as size, shape, color, and texture. They examined the relationship between the weight and volume of four wheat speices. A linear relationship (R^2 in the range of 0.841–0.920) was found between individual kernel weight and projected area estimated using image processing methodology. Sabanci et al. (2016) were

performed weight estimation of bread wheat (Average success rate, 98.29) and durum wheat (Average success rate, 97.54) in diffirent amounts was performed by using image processing techniques. In this study, this value was obtained as 88.25% for soybean. This difference was thought to be due to the fact that the shape and structural properties of soybean, which is an oil seed plant, are different from cereals.

Many studies have shown that when water-stressed, grain crops such as soybeans have decreased in grain weight and yield value as water stress increases (Shaw and Laing, 1966; Huck et al., 1983; Foroud et al., 1993; Lopez et al., 1996a; Lopez et al., 1996b;). In this study, while the results of the experiments up to 25% irrigation were similar, there was a difference in the results of 25% irrigation. This indicates that soybean was subjected to significant stress due to 25% irrigation. Taiz and Zeiger (2008) have mentioned that some plants can manage water efficiently by physiologically storing it in the soil in lands where water stress is severe and can use it for the remaining period of its life for the development of certain regions. It is assumed that 75% water limitation applied to soybean in this study has improved the plant seeds. Therefore, due to excessive stress, the seed weights and sizes have increased and consequently, the total pixel count has also increased in the 25% irrigation condition.

5. Conclusion

In this study, the plant seed weight and pixel size decreased from 100% irrigation rate to 50% irrigation rate and increased at 25% irrigation rate. The increase in plant seed weight and pixel size at 25% irrigation rate is an important indication of the response of plants to stress. In conclusion, the actual weights of soybean seeds for all irrigation rates were determined with 88% accuracy using the total number of pixels occupied on the image. Investigating the effects of moisture content and species differences on weight estimation could lead to more accurate results.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.Ka.	H.Ku.	M.K.G.
C	40	30	30
D	30	40	30
S	40	30	30
DCP	30	30	40
DAI	40	30	30
L	20	50	30
W	60	20	20
CR	60	20	20
SR	70	30	
PM	30	40	30
FA	40	30	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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EVALUATION OF THE EFFECTIVENESS OF DIFFERENT TRAP TYPES AND PHEROMONES AGAINST *Halyomorpha halys* (HEMIPTERA: PENTATOMIDAE)

Mansur ULUCA[1]*, Kibar AK[1]

[1]*Black Sea Agricultural Research Institute, Department of Plant Healthy, 55300, Samsun, Türkiye*

Abstract: Brown Marmorated Stink bug (BMSB) (*Halyomorpha halys*, Hemiptera: Pentatomidae) was first detected in Türkiye in 2017 and is an important invasive insect species worldwide. About 300 hosts have been identified for this pest until recently. Among the hosts of the pest in the Black Sea region, mainly hazelnuts, fruits and vegetables are included. This study was carried out to evaluate the effectiveness of different traps in 2020 and 2021 in a mandarin garden in the Kemalpaşa district of Artvin, one of the places where the pest was first transmitted to Türkiye. Small Funnel Trap (SFT), McPhail Funnel Trap (MPT), Multi-Funnel Trap (MFT), and Big Funnel Trap (BFT) and two different pheromones SMC and TRC were tested against *H. halys*. Each trap was tested with two different pheromones, and the trapping performances of different trap types were followed from the beginning of July to the end of October. The adults were counted every week in the traps. In the traps, it was determined that the most insect-attractive pheromone and trap type combination was TRC+BFT and that the least effective insect trapping type was the SMC+MPT combination. In general, it was determined that TRC, which is the most effective pheromone, also works effectively with BFT and MPT. It was determined that SMC pheromone formed a more effective combination with SFT. In addition, it is predicted that larger-scale designs of MPT will be more effective together with TRC, and SFT with SMC. In this study, it was determined that the performances of different types of traps and pheromones that can be used in the control and monitoring of BMSB vary up to 29 times, and the importance of the combination of pheromone and trap was revealed.

Keywords: Attract-kill, Brown marmorated stink bug, Monitoring, Pheromone trap

***Corresponding author:** Black Sea Agricultural Research Institute, Department of Plant Healthy, 55300, Samsun, Türkiye
E mail: mansur.uluca@tarimorman.gov.tr (M. ULUCA)
Mansur ULUCA https://orcid.org/0000-0001-9805-6464
Kibar AK https://orcid.org/0000-0002-8004-2686

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1. Introduction

Brown Marmorated Stink Bug (BMSB), *H. halys* is an invasive pest that continues to spread and damage in ecological areas suitable for its biology, as in all countries where it spreads. BMSB was first identified outside of its homeland in 1996 in the USA (Hoebeke and Carter, 2003). First report of this pest was in Türkiye in 2017 and it has since spread to many countries in North America, South America, Europe, and Africa (Çerçi and Koçak, 2017; Ak et al., 2019a; Ak et al., 2023; Anonymous, 2023).

The large host range of BMSB includes many cultivated plants such as peach, apple, pear, hazelnut, almond, soybean, corn, pepper, tomato, as well as ornamental and forest plants (Nielsen and Hamilton, 2009b; Haye et al., 2015; Bariselli et al., 2016; Musolin et al., 2017; Hamilton et al., 2018; Leskey and Nielsen, 2018; Ak et al., 2019b; Dumbadze et al., 2019).

The nymphs and adults of the pest cause damage by feeding on the parts such as fruits, bud and leaves of the plants they host (Haye and Weber, 2017). For example; late season feeding by adults induced the highest proportion of injured apples and early season adult feeding resulted in the highest percentage of wounded peaches. As a consequence, various necroses that deepen from the fruit's outer surface to the inside cause significant economic losses (Acebes-Doria et al., 2016).

Since the beginning of the epidemic in many countries, it has been determined that this pest has caused significant economic damage to these plants. (Nielsen and Hamilton, 2009a; Hedstrom et al., 2014; Bariselli and 2016; Bosco et al., 2018; Dumbadze et al., 2019). The population monitoring and control of the BMSB gains importance as it spreads to the level of damaging agricultural areas in a short time.

Because of its high flight capacity of up to 117 km in season, this invasive pest needs to be monitored dynamically, and the time of whatever control must be correctly predicted (Lee and Leskey, 2015). As the population of this insect, which has a high reproductive and spreading force, increases and the damage intensifies, studies on the correct detection and monitoring of the population of the pest in agricultural areas have increased. In this respect, the aggregation

pheromones of the pest, which play a very important role in the intraspecific communication of the pest in determining the annual population change, are combined with different types of traps and used for different purposes in the USA, Europe, Georgia, and Türkiye (Joseph et al., 2013; Aigner and Kuhar 2014; Sargent et al., 2014; Murvanidze et al., 2018; Rice et al., 2018; Ak et al., 2019b). In previous research on the subject, the most important attractants were the two component aggregation pheromones (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (Khrimian et al., 2014). Furthermore, with this combination's synergist, methyl (2E, 4E, 6Z)-decatrienoate (MDT), the impact of the attractant is increased (Weber et al., 2014).

Using these pheromones, it is killed by insecticide nets or collected in funnel traps and destroyed by mechanical means, which can be evaluated within the scope of the attract-kill method (Kuhar et al., 2017; Ak et al., 2019a). However, the common use of pheromone traps against this insect is by using sticky trays, pyramid traps, McPhail traps, and funnel traps for population monitoring throughout the season (Hamilton et al., 2018). Besides these, when the pheromone traps of BMSB are evaluated with population monitoring, meteorological data of the region, and biological information of the pest, the spreading parameters of the pest can also be explained (Wallner et al. 2014; Nielsen et al., 2013). Using black pyramid traps, researchers have followed the seasonal abundance and phenology of BMSB populations across the United States, while in Türkiye they use sticky pheromone traps for monitoring (Leskey et al. 2015a; Hadden et al., 2022; Ak et al., 2023). The catching BMSB data were compared to the pyramid and sticky traps, which revealed equivalent population fluctuations for a lot of areas. As a result, throughout the season, the number of *H. halys* adults and nymphs trapped in pyramid traps was much higher compared to sticky traps. However, both may be trusted to monitor this pest in different regions (Acebes-Doria et al, 2020).

In this research, aggregation pheromones of BMSB were used in four different trap types and their performance was compared. Because it was unclear whether this different trap type would function effectively in the Eastern Black Sea Region's ecological conditions. The possibility of using aggregation pheromones, which are an effective component in population monitoring, with these traps for various reasons has been investigated.

2. Materials and Methods

2.1. Materials

We compared trap performances with *H. halys* adults and nymphs, four different trap types, and two distinct aggregation pheromones. These pheromones are TRC (Pherocon®, Trécé Inc., Adair, OK, USA) containing 5 mg aggregation pheromone+50 mg MDT synergist and SMC® [100 mg murgantiol/dispenser and 80 mg methyl (E,E,Z)-2,4,6-decatrienoate (MDT)]. In the research, the following traps were used:

a) Small-funnel Trap (SFT): It includes of an umbrella-type funnel cover at the top and a pheromone holder, a yellow funnel, a transparent bucket (3 l) at the bottom, and a hanging wire (Figure 1 a).
b) McPhail Trap (MPT): There is a transparent plastic cover and a pheromone holder with a diameter of 15 cm at the top and a length of 20 cm, and an inverted yellow funnel base with a 3 cm inlet hole at the bottom. Insects attracted by the pheromone fixed at the top inside the transparent cover enter the trap through the funnel mouth and gather in the chamber (Figure 1 b).
c) Multi-funnel Trap (MFT): This trap consists of a chamber (1 liter) at the bottom, 3 black plastic funnels (diameter 18 cm, height 10 cm), and a funnel cover. Insects that enter the funnel entrance with the pheromone placed under the cover are caught by falling into the chamber (Figure 1 c).
d) Big-funnel Trap (BFT): Funnel traps are made by cutting the top 15 cm of a 19 lt plastic carboy (bottom diameter: 28 cm, height: 58 cm, mouth diameter: 5 cm) and mounting it on the rest of the carboy. By hanging the pheromone at the entrance of the trap's open part, insects are attracted from this point and collected in the lower chamber (6 liter) (Figure 1 d).

Figure 1. Different traps types: (a) Small funnel trap; (b) McPhail trap; (c) Multi funnel trap; (d) Big funnel trap.

2.2. Method

Traps placed in the mandarin orchard in Kemalpaşa (Artvin) between July and October were checked for 21 weeks in 2020 and 19 weeks in 2021, and the captured adults were recorded. In each trap type, 2 different pheromones were tested with 3 replications. The traps were hung at a height of 1–1.5 m on the mandarin trees at the yield age at 10 m intervals. The performances of the traps were determined according to the trap types, pheromones, and months with the insects caught.

3. Results

3.1. Efficacy of Pheromones

Adults of BMSB was captured in small numbers by both pheromones in July and August, and it was determined that the number of insects caught increased starting in September. As a result, the temporal attraction frequency of the two pheromones during the season is equivalent (Figure 2). The pheromones caught significantly more adults at a rate of 94.1%–1.9%; that is, a total of 1225 adults and 62 nymphs (Table 1). In this respect, the overall trap performance of all traps is represented by total adults and nymphs. A one-way ANOVA was used to compare total weekly adult and nymph trap captures from July to September 2020 and 2021.

BMSB captures in SMC pheromone was correlated with all trap types. SMC+SFT was demonstrated to be 2.6 times more effective than TRC+SFT, and the performance of TRC in the other 3 traps was higher. In this study, most insects were caught in the TRC+BFT combination, and the TRC+MPT combination captured significantly more BMSB compared with all SMC+trap types. Although the TRC pheromone has the highest total capture percentage, catching frequencies are similar for both pheromones in July and October (Table 2). Accordingly, capture rates of SMC and TRC pheromones in July and August are very low; levels of insects peak in September and decrease in October.

3.2. Efficacy of Traps

The average weekly performance of the SFT and BFT with SMC pheromone is 1.3 and 1 insect per trap in 2020 and 2021, respectively. Therefore, the performances of these traps are equivalent in SMC pheromone. The two-year seasonal performance of the traps is shown in Figures 3 and 4.

In the time-dependent performance of traps, an increasing number of insects were caught from the end of August, until overwintering. Using this temporal definition as a baseline, captures of BMSB were considerably higher in SMC+SFT, TRC+BFT, and TRC+MPT combinations than in other traps at the relative population density in September. Furthermore, these pheromone-trap combinations caught BMSB almost constantly during the experiment.

The highest capture is detected in the BFT trap in TRC, which performs up to 10 times better compared to other traps. The number of insects caught in the MPT trap was determined to be between 2-4 times, resulting in the closest performance to the BFT (Figure 5 and 6).

In general, SMC and TRC pheromones were determined more effective at catching insects in September. In addition, BMSB abundance peaked in the BFT trap, which is the most effective trap, in September, as indicated by peak captures (Table 2).

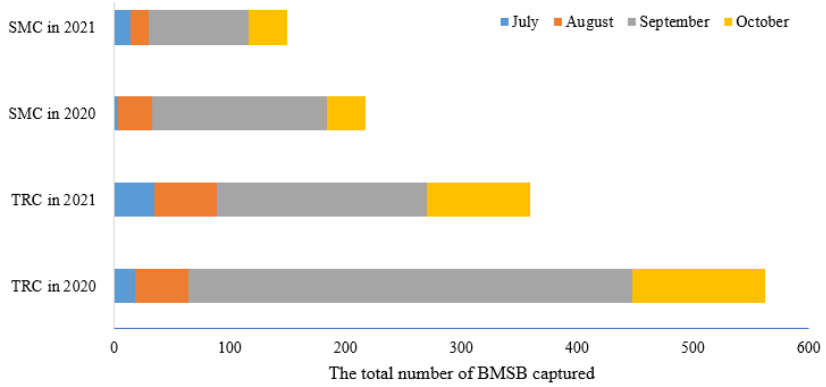

Figure 2. Performance of SMC and TRC pheromones by month in 2020 and 2021.

Table 1. Capture of BMSB adults and nymphs with four different traps and two pheromones in 2020 and 2021

		Adult Mean ± SE	Nymph Mean ± SE
SMC	SFT	68.00±8.00 ^{b}	4.00±3.00 ^{a}
	MPT	10.50±3.50 ^{a}	0.50±0.50 ^{a}
	MFT	29.00±5.00 ^{a}	1.00±0.00 ^{a}
	BFT	69.50±14.50 ^{b}	0.50±0.50 ^{a}
TRC	SFT	25.00±8.00 ^{a}	3.00±2.00 ^{a}
	MPT	76.00±4.00 ^{a}	14.5±10.50 ^{a}
	MFT	45.00±4.00 ^{a}	2.00±1.00 ^{a}
	BFT	289.50±92.50 ^{b}	5.50±1.50 ^{a}

Significant changes are indicated by rows with different letters (Duncan's HSD, α = 0.05).

Table 2. Capture of BMSB in four trap types and TRC and SMC pheromones in 2020 and 2021

Traps	Mounts	SMC		TRC	
		2020 Mean ± SE	2021 Mean ± SE	2020 Mean ± SE	2021 Mean ± SE
SFT	July	0.00±0.00 a	0.40±0.19 a	0.11±0.11 a	0.20±0.14 a
		0.00±0.00 A	0.40±0.19 A	0.13±0.13 A	0.20±0.14 A
	August	1.00±0.26 b	0.46±0.19 b	0.50±0.17 a	0.27±0.15 a
		1.00±0.26 B	1.11±0.32 AB	0.5±0.17 AB	0.39±0.14 A
	September	3.44±0.54 b	2.73±0.47 b	1.05±0.27 a	0.73±0.25 a
		3.44±0.54 C	1.89±1.96 C	1.05±0.27 C	0.61±0.22 A
	October	0.17±0.12 a	0.47±0.24 a	0.22±0.13 a	0.27±0.12 a
		0.20±0.14 AB	0.17±0.40 A	0.27±0.15 A	0.17±0.17 A
MPT	July	0.11±0.76 a	0.13±0.09 a	0.83±0.26 b	0.73±0.21 a
		0.07±0.07 A	0.13±0.35 A	0.67±0.27 A	0.73±0.21 AB
	August	0.11±0.76 a	0.00±0.00 a	1.28±0.31 b	0.80±0.30 ab
		0.17±0.9 A	0.22±0.17 A	1.56±0.29 AB	1.78±0.46 BC
	September	0.61±0.18 a	0.33±0.21 a	2.39±0.43 a	3.4±0.61 b
		0.61±0.18 B	0.06±0.06 A	2.39±0.43 C	2.28±0.54 C
	October	0.00±0.00 a	0.00±0.00 a	0.89±0.32 a	0.67±0.27 a
		0.00±0.00 A	0.00±0.00 A	1.07±0.37 A	0.00±0.00 A
MFT	July	0.56±0.56 a	0.27±0.15 a	0.56±0.56 a	0.87±0.29 a
		0.00±0.00 A	0.27±0.15 AB	0.00±0.00 A	0.87±0.30 AB
	August	0.33±0.14 a	0.47±0.22 b	0.39±0.12 a	1.60±0.55 b
		0.39±0.14 A	0.67±0.21 C	0.44±0.12 A	1.83 ±0.47 B
	September	1.22±0.30 a	0.67±0.21 a	1.61±0.40 a	0.93±0.32 a
		1.22±0.30 B	0.50±0.17 AB	1.61±0.40 B	0.33±0.14 A
	October	0.33±0.14 a	0.27±0.15 a	0.28±0.14 a	0.67±0.67 a
		0.40±0.16 A	0.00±0.00 A	0.33±0.16 A	0.00±0.00 A
BFT	July	0.00±0.00 a	0.13±0.09 a	0.00±0.00 a	0.53±0.22 a
		0.00±0.00 A	1.33±0.09 A	0.00±0.00 A	0.53±0.22 A
	August	0.22±0.13 a	0.13±0.09 ab	0.39±0.20 a	0.93±0.36 ab
		0.22±0.13 A	1.22±0.43 AB	0.39±0.20 A	3.00 ±0.93 A
	September	3.11±0.42 b	2.00±0.48 b	16.22±1.87 b	7.00±1.32 c
		3.11±0.42 C	1.50±0.33 C	16.22±1.87 C	6.94±1.05 B
	October	1.33±0.46 b	1.47±0.32 b	5.00±1.24 b	4.93±0.96 b
		1.60±0.53 B	0.83±0.30 AB	6.00±1.35 B	2.33±1.11 A

"A-B-C" characters are used for grouping by months and "a-b-c" characters are used for grouping by performance traps. Significant changes are indicated by rows with different letters (Duncan's HSD, α = 0.05).

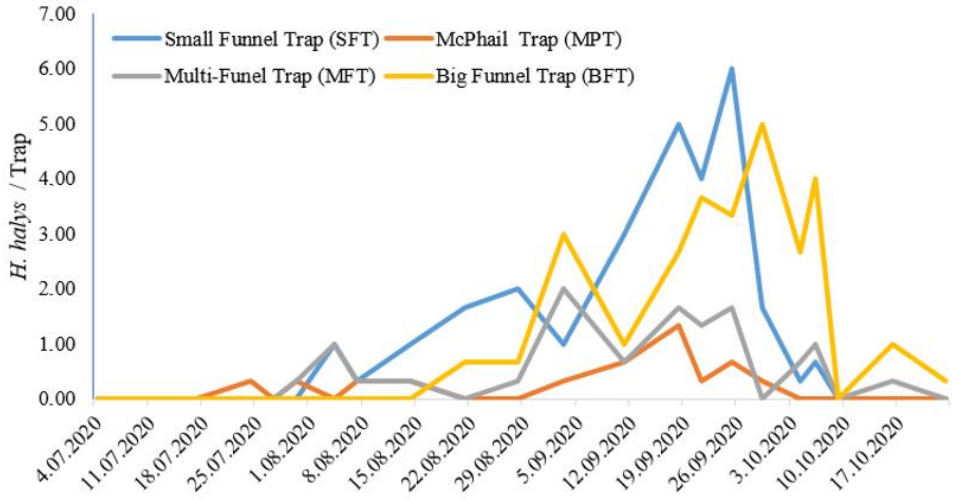

Figure 3. Performance of all traps in SMC pheromone in 2020.

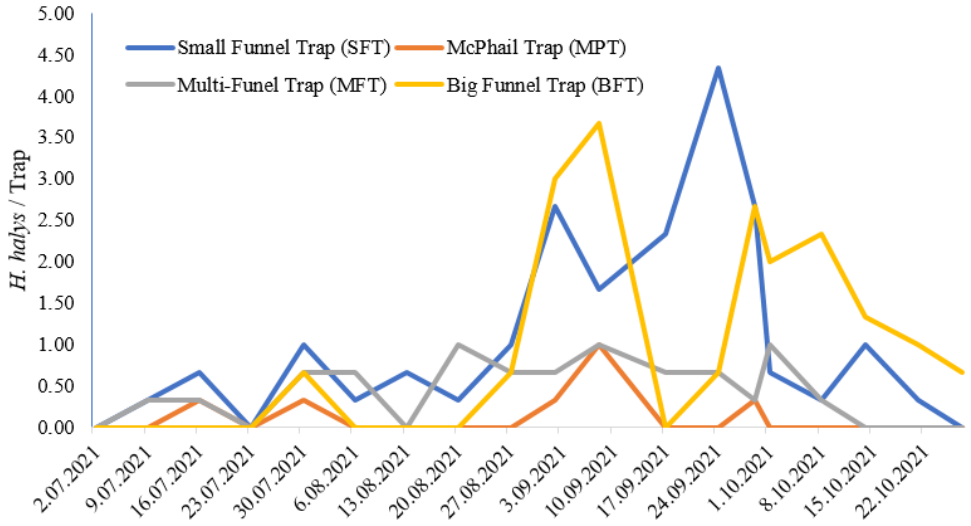

Figure 4. Performance of all traps in SMC pheromone in 2021.

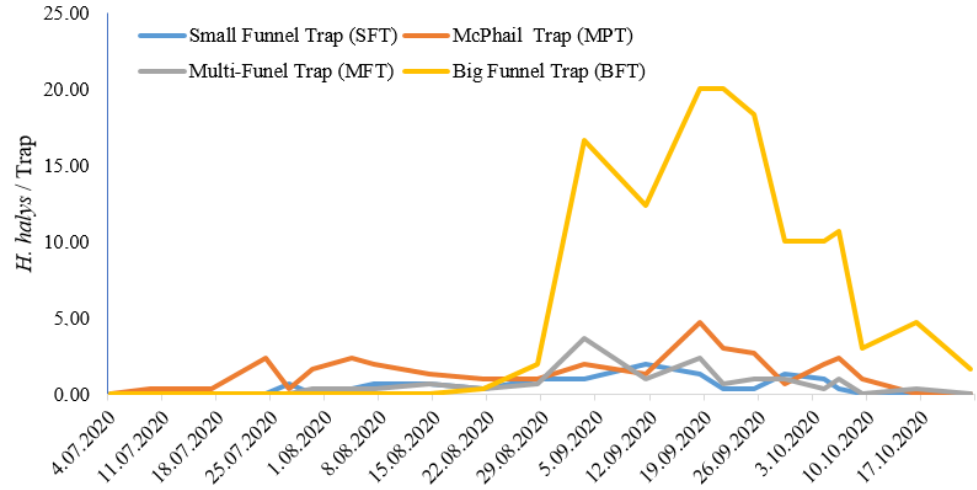

Figure 5. Performance of all traps in TRC pheromone in 2020.

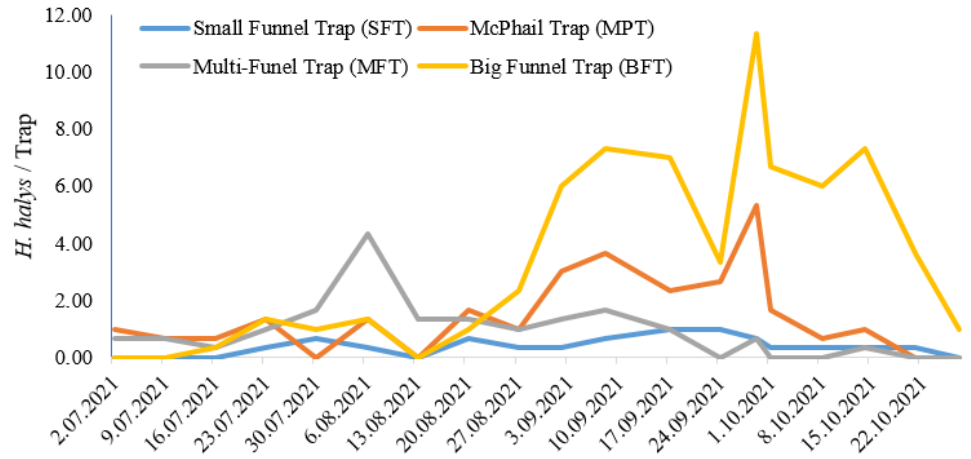

Figure 6. Performance of all traps in TRC pheromone in 2021.

4. Discussion and Conclusion

According to the results of many studies, the use of various traps utilized to control BMSB aims at monitoring, attract-kill, and live capture (Sargent et al., 2014; Leskey et al., 2015b; Rice et al., 2018; Suckling et al., 2019). These traps are generally used together with aggregation pheromones as an attractant and methyl (E,E,Z)-2,4,6-decatrienoate (MDT) lure as a synergist (Weber et al., 2014; Rice et al., 2017; Chase et al.,2018; Ak et al., 2019b; Acebes-Doria et al., 2020).

In this study, TRC, the most effective pheromone, was determined to combine successfully with BFT and MPT,

whereas the SMC pheromone combined more effectively with SFT. As the study was evaluated in general, the TRC+BFT combination was determined to have 2-29 times more performance than other traps. In this case, using the BFT trap only for attract-kill in terms of its size, cost, and time-dependent performance is more appropriate. Especially the 6-liter capacity chamber of the trap, which is very effective at capturing a large number of insects. Moreover, the catching rate increases rapidly in September and October, while a small number of insects are caught in BFT until the end of August. This type of trap captured 23 times more BMSB than the sticky trap (Pherecon®) used for monitoring with the same pheromone, and these sticky traps are also used for monitoring purposes in the same region (Ak et al., 2019a; Ak et al., 2019b). As a result, while it's important to capture many insects in a brief period of time with attract-kill traps, it is critical for the monitoring traps to catch insects for a long time.

The attract-kill method should be not preferred for agricultural parcels of small sizes because may cause a bad effect. For example, damage from using attract-kill traps to catch BMSB in *Solanum lycopersicum* small gardens is greater than damage without traps (Sargent et al., 2014). Monitoring traps may provide longer-term data on relative population densities, whereas attract-kill traps provide data on high populations in a short period of time. Against a large number of BMSB adults and nymphs, insecticide applications were made to the killing blocks, and the use of attract-kill blocks resulted in 2–7 times less damage compared with standard areas. Overall, attract-kill proved successful in controlling low to moderate *H. halys* populations in agricultural lands (Morrison et al., 2018). The killing blocks edges of orchards may be good assist-solution in the fight against BMSB throughout the season (Rice et al., 2018). For growers with organic agriculture and beekeeping in the Eastern Black Sea Region, the attract-kill method may not be feasible because of the pesticide residue.

In this context, synthetic pheromones and LED combined with one trap can be evaluated as alternative methods. (Rondoni et al., 2022). The Aluminum Foil Pan Trap type can catch up to 144 insects per week over the overwintering period (Aigner and Kuhar 2014). In addition, the combined use of synthetic pheromone and LED at the same pyramid trap showed a synergistic effect on *H. halys* positive phototaxis. This multiple trap combination attracted up to 8 times more BMSB, increasing performance (Rondoni et al., 2022). However, more research should be conducted to accurately characterize BMSB behavior utilizing light-based attractants alone or in combination with specific pheromone monitoring methods (Leskey et al., 2015b).

In order to improve the design of BMSB traps, efforts were initially focused on identifying disadvantages and increasing efficiency. For example, although large pyramid traps are effective, they are unsuitable for agricultural use because they are expensive, cumbersome, and difficult to establish (Rice et al., 2018). In our study, the accumulation of rainwater in the reservoir of the BFT was a drawback. Because insect cadavers block the base drainage holes during rain, the reservoir may fill with water from time to time. Furthermore, during periodic controls, thin plastic material deforms quickly, which may cause insects to escape. Horizontal funnel traps designed to capture BMSB live provide an alternative to these drawbacks (Suckling et. al., 2019). In addition, trap designs should be cheaper, lightweight, durable, and uncomplicated (Morrison et al., 2015). In another study, modified pyramid traps were more successful than standard pyramid traps and small pyramid traps, and different designs of the same trap type also affected performance considerably. The researchers determined that adults were caught more frequently in modified pyramid traps, and more nymphs were caught in all other traps (Rice et al., 2018). However, considering that the rate of catching adults is higher in all traps in our study, it is clear that the trap type is very effective on the adult-nymph ratio. Consequently, lower nymphal captures may indicate relative populations, although in a smaller overall than adults.

Transparent sticky traps combined with BMSB pheromone and synergist are stated to be basic and simple to use for monitoring purposes (Acebes-Doria et al., 2018). SMC+SFT and TRC+MPT can be used because of their small size and durable materials for long-term monitoring programs in early July–early October. Interestingly, using the SMC pheromone, the seasonal peak of BMSB captures in the SFT trap paralleled those captured by BFT that most efficacy combination. Although the reservoir of SFT was quite small compared to that of BFT. In this research, SMC+BFT has been deemed to be the best trap with regard to performance and usability. Additional studies must be conducted to examine alternative trap designs with additional trap types in varying populations in order to shift to simpler designs. Therefore, the evaluation criteria of BMSB traps vary according to the intended use and performance of the traps. At this point, it is important to make the right choice. The application of insecticides, in apple orchards has decreased by 40% due to the use of pheromone traps in making decisions about insecticide treatments (Short et al., 2016). Using pheromonal monitoring can help determine susceptible crops to invasion and make proactive applications.

Recently, using semiochemicals and semi-physical attractants together as synergists (viotraps) and technological applications (BugMap, image analysis system, sensor, smart trap) have been researched for more sustainable IPM strategies and sophisticated approaches (Malek et al., 2019; Čirjak et al., 2022; Zapponi et al., 2023).

In general, the availability of various types of traps and attractants that can be used against BMSB expands research possibilities. This study showed that trap design

and pheromone composition may influence trap effectiveness significantly. According to the results of the study, the potential of using the four traps tested in our study for the indirect and direct control of BMSB should be evaluated with new research.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.U.	K.A.
C	80	20
D	50	50
S	60	40
DCP	50	50
DAI	60	40
L	70	30
W	60	40
CR	50	50
SR	70	30
PM	20	80
FA		100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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TECHNICAL AND ECONOMIC EXAMINATION OF THE MECHANICAL HAZELNUT COLLECTING MACHINE MANUFACTURING

Hüseyin SAUK[1]*, Mehmet Arif BEYHAN[1], Kübra Meriç UĞURLUTEPE[1]

[1]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies 55139, Samsun, Türkiye

Abstract: In this study, taking into account the conditions of Türkiye, it will be possible to harvest hazelnuts grown on flat and nearly flat lands, in line with ergonomic principles, with an approach that consider operator comfort and work safety, with a mechanically effective sweeping unit with high work success, which removes the kernel+husk hazelnut from the ground, some performance values of the hazelnut harvesting machine such as collecting efficiency, labor requirement, fieldwork success, product work success, and foreign material separation efficiency, which cleans the mixed hazelnuts in the separation unit at a level that does not affect the performance of the husk peeling machine and stores the cleaned grain+husk hazelnut mixture, were determined and obtained. In light of the data obtained, it is contribution to the economy is discussed. The collecting efficiency of the mechanical hazelnut harvesting machine was 93.26%, the labor requirement was 47.85 h/ha, the fieldwork success was 0.134 ha/h, the product work success was 295.92 kg/h, and the foreign material separation efficiency was 96.85%. Considering the performance values obtained, very successful results have obtained from the field trials performed with the mechanical hazelnut harvesting machine. As a result, the production of the hazelnut harvesting machine and its implementation will make hazelnut production sustainable by significantly reducing production costs, and the import of machinery that is not suitable for the conditions of Türkiye will be no longer needed.

Keywords: Hazelnut harvester, Mechanical harvesting, Hazelnut, Mechanization

***Corresponding author:** Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies 55139, Samsun, Türkiye
E mail: hsauk@omu.edu.tr (H. SAUK)
Hüseyin SAUK https://orcid.org/0000-0001-5622-6170
Mehmet Arif BEYHAN https://orcid.org/0000-0002-4536-0865
Kübra Meriç UĞURLUTEPE https://orcid.org/0000-0001-7184-3388

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1. Introduction

Türkiye has 74.50% of the world's hazelnut planting areas, with approximately 700 thousand hectares. The amount of production as in-shell hazelnut is about 684 thousand tons, meeting 76% of the world hazelnut production. Hazelnut export is approximately 500 thousand tons, realizing 75% of the world hazelnut export (TÜİK, 2023).

In Türkiye, hazelnut harvesting is still done by hand-picking from the branch or the ground, based on human labor. Afterward, the collected hazelnuts are separated from their husks in the husker. Hand harvesting of hazelnuts in Türkiye requires 306 BIGh/ha. This figure constitutes 71% of the total working time for production and 55% of the production cost (İlkyaz, 1986). This situation significantly increases the hazelnut production cost and causes a labor-based labor requirement during harvest. Reducing the high labor requirement and the price is possible by mechanizing the harvesting process (Beyhan and Yıldız, 1996).

In the paper of the General Secretariat of the Black Sea Exporters' Association on the subject, In Azerbaijan and Georgia, which have meager production costs, hazelnut production is increasing every year due to profitability; international companies that are important hazelnut users have started to realize hazelnut plantations in countries in the southern hemisphere such as Chile, Argentina, and Australia to meet their own needs. These countries have encouraged their farmers to produce hazelnuts. It is reported that efforts to promote it in various ways have started to pose a clear threat to our country in the medium and long term. In addition, in the article, it is stated that the high hazelnut production costs in our country always pose an obstacle to our competitiveness as a handicap, even though the application of state support purchases was abandoned with the new hazelnut strategy and the application was started to create the prices within the free market rules and the basis was prepared for the formation of competitive prices with rival countries is expressed. In particular, it is underlined that the expectation of selling prices above world prices, caused by the high cost of harvesting expenses, is a chronic problem of the sector (KİB, 2023).

In Italy, Spain, and the USA, which produce hazelnuts economically, mechanical harvesting has become widespread as much as planting techniques and land topography allow. For this purpose, machines with

pneumatic (vacuum), pneumatic+mechanical, and mechanically effective total units are used. Working with these machines requires good ground preparation (leveling and compaction) and spread product should be lined with lateral sweepers. The fact that the hazelnuts grown in these countries have a short husk as a variety feature causes the hazelnuts to fall out as grains during the harvest period. For this reason, husk peeling machines are not used. Due to the identifiable geometric shape of the hazelnut kernels, it is possible to clean it with known separation methods effectively. Elements such as stones, soil, etc. that cannot be cleaned are separated in water pools, and the cleaned hazelnuts are dried in dryers.

The fact that Turkish hazelnut varieties have a long husk and tightly wrap the fruit, and do not have an identifiable geometric shape makes the separation systems of these machines ineffective. In addition, the efficiency of the sweeper units decreases due to the differences in planting techniques and the characteristics of the garden floor. Also, the large size of the machines creates a problem depending on the sewing technique (Beyhan, 1992). Although Italian agricultural machinery manufacturers have tried to enter the Turkish market for years, they have not succeeded due to the machines' unsuitability.

This study was carried out by taking into account the conditions of Türkiye our country, which can harvest the hazelnuts grown on flat and nearly flat lands, in line with ergonomic principles, with an approach that consider operator comfort and work safety, with a mechanically effective sweeping unit with high work success, which removes the kernel + husk hazelnut from the ground, It covers the design and prototype manufacturing of the hazelnut harvester, which cleans the mixed hazelnuts in the separation unit without affecting the performance of the husk peeling machine and stores the cleaned grain + husk hazelnut mixture. In addition, some performance values (picking efficiency, labor requirement, fieldwork success, product business success, and foreign material separation efficiency) of the self-propelled hazelnut harvester, whose prototype was manufactured, were determined, and its contribution to the economy was discussed.

2. Prototype Manufacturing and Technical Specifications of Hazelnut Harvesting Machine

In the field of agricultural machinery engineering, the biological factor is essential in terms of engineering applications. Notably, in designs for garden mechanization, factors such as ecology, topography, cultivation techniques, variety characteristics, etc., are the essential elements to be considered. Agricultural Machinery Chair of Berlin Technical University, Faculty of Machinery and Agriculture, Prof. Dr. Eng. Kurt Marks, in the summer of 1958 at I.T.U. At a conference attended by the Faculty of Machinery as a guest, "the knowledge and tools that can be taken into consideration for calculating agricultural machines are very lacking. For this reason, it can be said that the creation of a device becomes a work of personal ability fed by experience and general knowledge rather than calculating, just like in architectural work. In the construction of agricultural machinery, especially in some harvesting machines, the function is so crucial that besides, issues such as strength and drawbar force are not in question. However, the issues of strength and pulling force come into account very quickly." Considering these explanations, designing a hazelnut harvester with adequate agrotechnical functionality requires acquiring research-based primary data and relevant experience in evaluating the obtained data.

Prof. Dr. Mehmet Arif Beyhan, whose majority of his academic studies are on obtaining basic data on hazelnut harvesting mechanization, has designed many experimental purpose hazelnut harvesters based on different principles and tested them in practice. In these trials, he revealed the problems encountered and possible solution methods (Beyhan, 1992; Yıldız, 2000; Keskin, 2004).

Considering the conditions in Türkiye, the self-propelled hazelnut harvester with a mechanically effective sweeping system for the hazelnut harvest, which is grown on flat and nearly flat lands, consists of 4 central units: the collection unit, the separation unit, the unloading unit, the power supply, and the walking system. The collecting unit is a mechanically effective unit consisting of metal chain fingers. Based on a concept design that will sweep the ground and weed bottoms, considering the soil characteristics, the system has been developed according to the results of the experimental studies carried out during the harvest periods, and the manufacturing dimensions have been determined. The separation system consists of a straw walker sieve with longitudinal bars and cutting star wheels placed in the sieve channels. In addition, metal combs have been added to the system to separate grass, leaves, and twigs. Experimental methods have optimized the dimensions subject to manufacturing. The unloading unit uses an endless helix in the unloading team, and the hazelnuts with grain + husk are conveyed to the sack tied to the outlet. 12 HP diesel engine is used in the power supply and travel system. Power transmission systems to the machine (clutch system, safety system, gearboxes, shafts, etc.) are designed using known methods. The walking system is a hydrostatic system. The system consists of variable-flow hydraulic pumps, motors, and a mechanical transmission system.

In the existing hazelnut orchards, the basic dimensions of the machine were determined depending on the factors such as the distance between the rows, the height of the branches, the angle of the components, and the systems in question were placed in the chassis suitable for these dimensions. The moving parts (shafts, axles, gearboxes,

rolling bearing housings, couplings, etc.) that make up the units in question were assembled by machining. The machine is 3200 mm long, 1600 mm high, and 1200 wide, with three wheels. The device is driven by a gasoline engine of 1800 min^{-1}, a cylinder volume of 420 cc, and an engine power of 12 HP.

3. Results on the Performance Values of the Hazelnut Harvesting Machine

After the prototype production of the hazelnut harvester was made, it was subjected to garden trials (Figure 1). Harvest experiments were carried out according to the randomized plots trial design, assuming that the hazelnut yield per decare in Türkiye is 106 kg (FAO, 2020) and at a garden yield of 110 kg/da as milled hazelnuts with 10% moisture (w.b.) content, each parcel containing 30 quarries was carried out in triplicate. The average of the performance values of the machine obtained as a result of the trials is given in Table 1.

Figure 1. General view of the hazelnut harvester during gardening.

Table 1. Performance values of the hazelnut harvester

Working width, (mm)	1200
Working velocity, (m/s)	1.6
Collection efficiency, (%)	93.26
Labor requipment, (h/ha)	47.85
Area business success, (ha/h)	0.134
Product business success (kg/h)	295.92
Foreign material separation efficiency, (%)	96.85
Fuel consumption(L/h)	2.4

In working with the machine, a product loss of 6.74% occurred. Nuts poured into the quarries account for 2.54% of these losses. The remaining 4.20% loss is due to hazelnuts that the machine cannot collect. It was observed that the hazelnuts that could not be collected were found in the soil crevices. The amount of foreign material coming into the sacks, together with the kernels + husk hazelnuts, is 3.15%. Most of the foreign materials are soil. Most of the soil was collected from the grass-free area. Most grass, twigs, leaves, and coarse dust are materials accumulated in previous years. The amount of other material remaining outside the soil will decrease if the harvest is carried out continuously by machine.

According to the performance values obtained, the designed hazelnut harvester can be calculated at 110 kg/da orchard yield, the fieldwork success is 13.4 da/10h, and the product work success is 2.95 t/10h. Since the hazelnut harvest season lasts about 30 days, a machine can collect approximately 90 tons of shelled hazelnuts-kernel during the harvest season. In today's conditions, in the ideal conditions (well-cleaned garden ground and hand-picking from the ground in situations where the entire product is spilled on the floor), the cost of harvesting 1 ton of milled, dried hazelnuts vary between 9-12 thousand ₺ and requires approximately 30 İİG/day. These data reveal the technical, social, and economic necessity of mechanization of hazelnut harvest.

4. Conclusion

Italy is the essential machine manufacturer country for the harvest mechanization of hard-shelled fruits. Turkish major hazelnut producers go to Italy and say that they are examining these machines and they are not suitable for the conditions of our country. In addition, in 1996, the Italian manufacturer brought a pneumatically effective (vacuum) machine to the Ordu region and conducted a demonstration study. However, they were not successful. Despite this, another Italian producer company brought a device to the Çarşamba region in the 2015 harvest season and conducted a demonstration study. According to the information obtained from the manufacturers, this company was not successful either. However, recently, it has been heard that the companies in question are trying to develop hazelnut harvesters suitable for the conditions of Türkiye.

Although it changes yearly, approximately 684 thousand tons of shelled milled hazelnuts are produced in Türkiye. Considering that more than half of the production areas are on flat or near-flat and productive areas, mechanical harvesting of approximately 300 thousand tons of hazelnut seems possible. Considering the fieldwork success of the hazelnut harvester, about 3300 hazelnut harvesters are needed. In addition, hazelnut production areas are constantly expanding in Azerbaijan and Georgia, which produce varieties similar to hazelnut varieties in our country. According to FAO 2020 data, Azerbaijan has 34 thousand tons of milled, dried hazelnuts on approximately 30 thousand hectares.

Otherwise, Georgia produces 30 thousand tons of dried hazelnuts in roughly 16 thousand hectares. If the harvest of half of the hazelnut production amounts of both countries is mechanized, they will need about 350 machines.

According to data, it can be said that there will be a significant demand for machinery both in the domestic and foreign markets. This will add considerable added value to the country's economy.

As a result, if the projected concept design is realized, a new product with high business success, harvest efficiency, and energy use efficiency will emerge. The manufacture of such a hazelnut harvesting machine will make hazelnut production enjoyable by ergonomically improving the working conditions of the producers, will make the hazelnut production sustainable by significantly reducing the production costs, will reduce the use of child labor intensively used in the hazelnut harvest, and will help academics, designers and agricultural machinery manufacturers working on the subject. It will contribute to making new designs that take this concept as a reference. Thus, an important step will be taken to mechanize hazelnut, which is one of the most critical problems in Türkiye with the production of a machine suitable for our garden structure and hazelnut varieties.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.S.	M.A.B.	K.M.U.
C	40	30	30
D	100		
S	100		
DCP	70	30	
DAI	100		
L	40	30	30
W	40	30	30
CR	40	30	30
SR	40	30	30
PM	40	30	30
FA	40	30	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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THE EFFECT OF DONORS USED IN *IN-VIVO* MATERNAL HAPLOID TECHNIQUE ON HAPLOID INDUCTION RATE

Rahime CENGİZ[1]*

[1]*Sakarya University of Applied Sciences, Faculty of Agricultural, Department of Field Crops, 54580, Sakarya, Türkiye*

Abstract: Maize is an important plant grown to obtain grain and silage, and is used in human and animal nutrition. In conventional maize breeding studies, inbred line development studies are carried out for at least 7 years if a single generation is obtained in a year, while it is possible to develop 100% homozygous lines in a short period of 2 years with the *in vivo* maternal haploid technique. The *in vivo* maternal haploid technique is widely used in advanced maize breeding programs. The choice of donor or source material to be used for haploid induction depends on the purpose of the breeding program. Generally, breeders use F_1 or F_2 populations as source material for haploid induction. In this study; 30 F_1 genotypes and their F_2s were crossed with the inducer line. The putative haploid seed was identified by considering the *R1-nj* color marker, and the haploid induction rate was determined. The effect of the generations of the donor genotypes on the haploid induction rate was compared by performing an independent sample test, and the haploid induction rate obtained from the F_1 donors was found to be higher than the haploid induction rate of the F_2 donors. It was determined that there was a change in the haploid induction rate as the genotypes changed within the F_1 and F_2 donor groups.

Keywords: Maize, Breeding, Haploid technique, Inducer line, Source material

***Corresponding author:** Sakarya University of Applied Sciences, Faculty of Agricultural, Department of Field Crops, 54580, Sakarya, Türkiye
E mail: rahimecengiz@subu.edu.tr (R. CENGİZ)
Rahime CENGİZ https://orcid.org/0000-0001-6355-7496

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1. Introduction

Maize is an important plant that is grown for grain and silage, and is used in human and animal nutrition. Due to the support of maize production in Türkiye in recent years, there has been a remarkable increase in maize cultivation area and production. In the last 10-15 years, there has been a great increase in maize cultivation areas, especially in our Central Anatolia and Southeastern Anatolia regions. The increase in the profitability of maize production, the development of high-yielding varieties in the cultivation technique, the effective use of water and fertilizer, and ease of mechanization and marketing are the most important reasons for the increase in cultivation area and production. Today, maize is cultivated in approximately 65-70 provinces (Soylu, 2022).

The development of high-yielding and quality hybrids in maize requires the continuous development of new inbred lines. A very long time is needed to obtain the lines that are settled with conventional methods. The haploid technique provides significant advantages in shortening this period. The potential of doubled haploids in maize breeding has long been demonstrated (Chase, 1969).

Today, doubled haploids are widely used in many areas of maize research and in conventional hybrid maize breeding around the world. In research, doubled haploid genotypes have been a valuable tool in structural and functional genomics, proteomics, metabolomics, marker-assisted studies, molecular cytogenetics, genetic engineering, and other fields. In breeding, doubled haploid lines allow the efficiency of selection to increase, shorten the breeding period, and save time and effort (Geiger, 2009).

Haploids are developed from an unfertilized female egg (gynogenesis) or a male cell (androgenesis). The *in vitro* androgenesis method is performed by anther culture. There are two known methods of obtaining haploids from the embryo of the maize. These are maternal haploids and paternal haploids. In this method, some native genotypes are used, called 'inducer lines', with which maternal haploids can be obtained. The method in which inducer lines are used as pollinators is defined as *in vivo* gynogenesis. The rate of obtaining haploid from modern inducer lines is between 5-8% (Geiger, 2009).

When maize plants are crossed with specific genotypes called inducers, maize kernels with haploid and normal diploid embryos show a clear distinction. This is called *in vivo* haploid induction. Generally, kernels with haploid embryos have normal triploid endosperm. Therefore, these grains show the same germination rate and germination strength as diploid embryonic grains (Coe and Sarkar, 1964).

The inducer genotype is used as a pollinator for the production of maternal haploids. Both the cytoplasm and the chromosomes carried by the resulting haploids come

from the donor plant. Different inducer genotypes are used in paternal and maternal haploid induction methods. Both methods of *in vivo* haploid induction are much less dependent on the donor genotype structure than the *in vitro* technique (Röber et al., 2005; Spitko et al., 2006).

In haploid inducer lines commonly used today, the *R1-nj* allele has been combined with other genes required for anthocyanin biosynthesis. Most maize germplasms used in breeding programs do not have the anthocyanin biosynthesizing genes or the *R1-nj* allele, which imparts the red-violet color in grain or plant tissue. When the source material that does not contain the anthocyanin color gene and the inducer lines used as the male are crossed, the *R1-nj* gene is dominant to the colorless *r1* allele, so the emergence of the Navajo phenotype in the embryo and endosperm is expected in all the seeds obtained. However, differential expression of the *R1-nj* allele allows the differentiation of maternal haploids from diploids. When inducer lines with a high haploid induction rate are used for induction cross, maternal haploids usually occur between 6-10% (Chaikam and Boddupalli, 2012).

The choice of donor or source material to be used for haploid induction depends on the purpose of the breeding program. Generally, breeders use F_1 or F_2 populations as source material for haploid induction. Prigge et al. (2011) used single hybrids, village populations, and open-fertilized varieties as source material for *in vivo* maternal induction. In addition, haploid induction rate (HIR) and false haploid selection rates were compared when these materials were used as source material according to kernel type (dent and flint maize). Significant variations were detected among the source materials used in terms of response to haploid induction. Higher HIR was determined in single hybrids compared to other source materials.

Significant differences in induction rate were determined between donor genotypes (eg, dent, flint maize, local variety) (Roux, 1995; Eder and Chalyk, 2002; Röber et al., 2005). However, the range of variation determined for these differences was small compared to the response to anther and microspore culture. Environmental conditions also affect the success of the *in vivo* haploid technique.

Hu (2014) used six inducer lines and 10 different F_1 as donors in his study to determine the induction rates of different inducer lines under the same conditions. The haploid induction rate ranged from 2.17 to 5.33%, and the induction rate of the haploid inducers was ranked from low to high as KMS-3<WY-1<PR-2<YP-13<KMS-2<KMS-1. Considering the haploid seeds obtained from different donors, the average haploid induction rate differed significantly between donors and ranged from 1.26% to 10.27%.

In vivo haploid technique has been widely used in maize breeding studies in recent years as a method that shortens the breeding period and increases its efficiency. The success of obtaining haploid seeds with the *in vivo* technique using haploid inducers varies according to the characteristics of the inducer line and the genotypes used as the donor.

In our country, the use of *in vivo* maternal haploid method in hybrid maize breeding has become increasingly widespread. This work was planned to determine the effect of donor genotypes used in *in vivo* maternal haploid technique on the haploid induction rate (HIR). This study determined the effect of 30 F_1 donors and their F_2s on HIR. The study's results aimed to facilitate breeders using this technique in choosing donor generations.

2. Materials and Methods

This study was carried out in the Sakarya location in 2020-21. MHI (Moldovian Haploid Inducer) available from Maize Genetics and Genomics Database (Maize GDB) was used as the inducer in the *in vivo* maternal haploid technique. The MHI inducer line has *R1-nj*, *B1*, and *Pl1* alleles. It creates anthocyanin in the seed and the rootlets during the germination period. According to Chalyk (1994), it has an average haploid induction rate of 4.5%. As source material for *in vivo* maternal haploid technique, 30 numbers of F_1 (single hybrid) and 30 numbers of F_2 (30 of F_2 derived from F_1 were used) were counted and packaged, and sprayed against subsoil pests and fungal diseases. Hybrid maize varieties used as donors are planted which were for grain and silage purposes, in the dent kernel type, early, mid-late, and late maturity groups. F_2s of donor F_1s were obtained by the bulk selfing method in 2020. Each of the F_1 and F_2 donors was planted in 2 rows, 70 cm row spacing, and 18 cm in row spacing, in 5 m long plots with a hand sowing machine on two times in 2021.

After the experimental area was prepared with suitable tillage tools before planting, a composite fertilizer containing 10 kg of pure nitrogen (N) and 10 kg of pure phosphorus (P_2O) was applied per decare. The second fertilizer of nitrogen (12 kg N da-1) was given when the plants were 40-50 cm. Cultural struggle (hand hoe) was made with weeds. Plant tags are attached before flowering.

When the flowering period of the F_1 and F_2 genotypes came, all tassels were cut and removed. Ears are closed with shoot bags without flowering. The hybridization process was carried out according to Russell and Eberhart (1975). Plants of the inducer genotype were covered with tassel bags when the anthers started pollinating 50% in the main axis after the tassel emergence. When the silk was 3-5 cm in the ears covered with shoot bags in the source material, the isolation bags at the tassel of the inducer line were collected and put together and poured onto the silks of the donors. The process continued until the hybridization of all source material was complete. At least 5 ears were crossed in each donor. Crossed ears were kept in isolation papers until harvest. At harvest, the ears of each donor were

taken into separate mesh bags and labels were attached. When the grain moisture decreased to 14%, grains of each genotype were separated from the cob, and the total number of seeds was determined. Haploid seeds were selected considering the *R1-nj* color marker carried by the inducer line (Chaikam and Boddupalli, 2012).

Four different types of kernel samples are obtained from induction cross; i) normal diploid or hybrid seeds (F_1) have purple-colored endosperm and embryo, ii) seeds considered haploid (H) have purple endosperm and colorless embryo, iii) seeds with diploid endosperm (DE) have no color in the endosperm but have color in the embryo, iv) considered non-hybrid (MD) are seeds without coloration in the embryo and endosperm (Figure 1).

The haploid induction rate for each genotype was determined according to the formula below.

HIR = (Haploid seed number/Total number of seeds) x 100

The t-Test analysis of the data obtained from the study was made using the MSTAT-C package program.

3. Results

30 different F_1s used as trial material was planted in 2020, and their F_2s were obtained. In 2021, 30 F_1 and 30 F_2 source materials were planted according to the method. Induction cross was performed using the *in vivo* maternal haploid technique. The images of the cobs obtained at the harvest are given in Figure 2. In the seeds taken from each source material, haploid seed selection was made according to the method (Figure 3).

Figure 1. Four different categories of seeds from induction crossing.

Figure 2. Ear samples from induction crossing.

Figure 3. Identification of haploid seeds according to the *R1-nj* color marker.

Although the *R1-nj* marker system is an effective way of distinguishing haploids, the expression of the *R1-nj* allele is highly influenced by the genetic background of the source material. Navajo crown coloration ranged from a small dot (at the point where the silk attaches to the grain) to coloration that spanned the entire endosperm. In addition, the darkness of color in the endosperm and embryo also differed from very light to dark and deeper (Figure 4).

HIRs of each genotype were calculated according to the method. HIR of F_1 genotypes varied between 3.2-7.1%. HIR of F_2 genotypes was found to be the lowest at 2.8% and the highest at 6.5%. Two groups were compared using a t-test in terms of HIRs of 30 F_1s and their F_2s used as donors. According to the results of the analysis, F_1 donors were superior to F_2 donors and took place in group a (Table 1).

HIR obtained from induction crossing of F_1 genotypes used as donor source material was found to be statistically significant at the 5% level compared to HIR values of F_2s derived from the same F_1s (Table 2). These results obtained from the study by Prigge et al. (2011) are in line with the findings. At the same time, it was determined that there was a change in the induction rate as the genotypes changed within the F_1 and F_2 donor groups. The haploid induction rate was not affected by whether the cultivars were early or late, grain or silage. Since all of the genotypes used in the study are in dent kernel type, they do not carry an inhibitor gene that will prevent the emergence of the *R1-nj* allele. The expression of the *R1-nj* allele in F_1 and F_2 donor genotypes supports this situation.

Figure 4. The expression of the *R1-nj* allele in the kernel from different donors.

Table 1. t-Test group analysis of F_1 and F_2 donor source materials

	Groups	Number of Donors	Mean	Std. Deviation	Std. Error Mean
F_1 and F_2 Donors	1.00 F_1	30	5.3500 [a]	1.09379	0.19970
	2.00 F_2	30	4.7267 [b]	1.00032	0.18263

Table 2. Independent sample test for HIR between F_1 and F_2 donor source materials (t-Test).

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
F_1 and F_2 Donors	Equal variances assumed	0.189	0.666	2.30	58	0.025*	0.62333	0.27062	0.0816	1.16503
	Equal variances not assumed			2.30	57.5	0.025	0.62333	0.27062	0.0815	1.16513

* Significant at 5%.

4. Discussion and Conclusion

It has been stated in other studies that the effect of donor genotypes on the success of obtaining haploids in the *in vivo* maternal haploid technique is less compared to the *in vitro* haploid technique. Although the success rates of obtaining haploid plants are not very different between the two techniques, they depend on the genotype in both methods (Beckert, 1994). In our study, it was determined that there was a change in the haploid induction rate as the donor genotypes changed.

The study was conducted under greenhouse conditions using 12 different local maize landraces and ADAIL-I inducer line to develop doubled haploid lines with different oil and zein content. Haploid induction rates of donor materials ranged from 6.08% to 11.71%, and the mean HIR value of the ADAIL-I inducer line was determined as 8.20% (Kahriman et al, 2022).

The nature of the source material to be used as a donor in the development of doubled haploid lines can change the breeding scheme. Hybrids created for special purposes and populations obtained from them by inbreeding can be used in induction hybridization as donors by choosing appropriate for the purpose. However, a strong selection is required in terms of the characteristics of the source material. The development of homozygous maize lines has an important place in hybrid maize breeding. Breeders use different source materials to develop

genetically different maize lines. It is quite common to use different F_1 (single hybrid) source materials in breeding studies for this purpose. Considering the haploid induction rate in studies of obtaining doubled haploid maize lines using the *in vivo* maternal haploid technique, the use of F_1s comes to the fore.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	R.C.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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BIOACTIVE CONTENTS AND FRUIT TRAITS OF SOME APPLE CULTIVARS GROWN IN KAYSERI

Umut ATEŞ[1]*, Burhan ÖZTÜRK[1], Serkan UZUN[2]

[1]*Ordu University, Faculty of Agriculture, Department of Horticulture, 52200, Ordu, Türkiye*
[2]*Düzce University, Çilimli Vocational School, Department of Plant and Animal Production, 81750, Çilimli, Düzce, Türkiye*

Abstract: This study was carried out to determine the physical properties, biochemical and bioactive compounds of different apple cultivars grown in Yahyalı district of Kayseri province. The plant material of the research consisted of apple cvs. 'Scarlet Spur', 'Starking Delicious', 'Starkrimson Delicious', 'Golden Delicious', and 'Super Chief'. In the study fruit weight, length, width, firmness, soluble solid contents (SSC), titratable acidity (TA), vitamin C, total phenolics, total flavonoids, and antioxidant activities (DPPH and FRAP assays) were investigated. In the findings, significant differences were determined between the fruit characteristics of the cultivars. The highest fruit weight (289.96 g) and length (32.61 mm) were measured from 'Scarlet Spur', while the highest fruit width (34.86 mm) was measured from 'Scarlet Spur' and 'Starkrimson Delicious'. The SSC was determined between 11.65 ('Super Chief')-14.30% ('Starkrimson Delicious'), and TA between 0.47% ('Super Chief')-1.13% ('Golden Delicious'). The highest vitamin C content was measured in 'Starking Delicious' cultivar (66 mg100 g^{-1}) compared to the others. In terms of total phenolic content, 'Scarlet Spur' (38.2 g GAE L^{-1}) and 'Super Chief' (36.5 g GAE L^{-1}) cultivars had the highest values, while 'Super Chief' had the highest values in terms of total flavonoids (23.5 g QE L^{-1}), and antioxidant activity (FRAP, 490.7 mmol TE L^{-1}; DPPH, 1145.9 mmol TE L^{-1}). As a result, it was revealed the differences between the physical, biochemical, and bioactive contents of apple cultivars grown in Yahyalı district of Kayseri province.

Keywords: Antioxidant, Firmness, *Malus domestica*, Total phenolics, Vitamin C

***Corresponding author:** Ordu University, Faculty of Agriculture, Department of Horticulture, 52200, Ordu, Türkiye
E mail: umutates.es@gmail.com (U. ATEŞ)
Umut ATEŞ https://orcid.org/0000-0002-8050-0616
Burhan ÖZTÜRK https://orcid.org/0000-0002-0867-3942
Serkan UZUN https://orcid.org/0000-0002-3857-6561

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1. Introduction

The Apple is one of the fruit species with high economic value and consumption preference, which is widely grown in our country and worldwide (Çorumlu, 2010; Uzun et al., 2016). The spread of cultivation in large areas in Türkiye is seen as the favorable ecological conditions (Öztürk and Öztürk, 2016; Şensoy and Bostan, 2019). For this reason, Türkiye is an important apple production center and its apple products are valuable both in the domestic market and in exports. So, determining the physical and biochemical properties of different cultivars in regional cultivation is essential in obtaining fruits with high market value for producers and consumers.

Apples can be stored longer than many other types of fruit (Öztürk et al., 2018). This ensures the quickly and cheaply availability of fruit for people almost throughout the year. Apple exhibits an increasing consumption trend due to its rich nutritional contents including bioactive ingredients for health. Apple contains many biochemical components, such as phenolic compounds, antioxidants, and vitamin C, which are important for human health and effectively prevent many diseases (Teshome et al., 2023). In particular, its antioxidant activity helps protect cells from oxidative damage (Deuchande et al., 2023). Consequently it provide resistance against cancer and cardiovascular diseases, obesity, and other chronic diseases (Millán-Laleona et al., 2023). However, known that cultivars have a significant effect on the biochemical content.

The physical properties of fruits play an important role in consumption preference. In addition, it determines the suitability for industrial use (Özçağıran et al., 2005). However, it has been stated that ecological factors may cause some changes in the physical properties of fruits (Öztürk and Öztürk 2016). For this reason, it is necessary to determine the cultivars physical characteristics and select the appropriate cultivars for the region (Turan and Karlıdağ, 2022). This way, a higher quality product is obtained, a higher economic profit is provided based on the producer, and a contribution to the country's economy is provided. In addition, easier marketing of the quality products obtained is of particular importance for producers (Kaya et al., 2022).

This study was carried out to determine the physical properties, biochemical and bioactive compounds of different apple cultivars grown in Kayseri province. Within the scope of the study, it aims to help the producers who grow apples or plan to grow in the region in selection of ideal apple cultivars.

2. Materials and Methods

2.1. Plant Materials

This research was conducted in 2022, in the Yahyalı district of Kayseri province. The research material was obtained from the orchard of a person who produces commercially production in this area. The area where the research was conducted is a very flat area with an average of 1-2% slope. The terrestrial climate prevails in the district. The average temperature is 20 °C in summer and -18 °C in winter. The plant material of the research consists of 'Scarlet Spur', 'Super Chief', 'Golden Delicious', 'Starkrimson Delicious', and 'Starking Delicious' cultivars grafted on MM106 rootstock at seven years old. The orchard was established north-south, and the soil structure is sandy loam. All cultural processes in the orchard were performed at a similar level and in a way for each cultivar.

2.2. Methods

The study was designed as three replications with five plants in each repetition. Approximately 10 kg of fruit from trees of each cultivar was collected by hand to represent the tree. Harvesting was carried out when the fruits reached commercial maturity (based on days after full blooming for each cultivars). The harvested fruit were placed in the crates in a single row and transferred to the postharvest physiology laboratory (24±2 °C and 80±5% relative humidity) of the department of horticulture at Ordu University without wasting time.

2.3. Observations

2.3.1. Fruit weight, length, width, and firmness

Measurements were carried out on 30 fruits at each replications for each cultivar, with three replications for a total of 90 fruit. Fruit weight was measured using a digital balance with an accuracy of 0.01 g (Radwag PS/C/1 model, Poland). Dimensional properties were measured with a 0.01 mm precision digital caliper (Mitutoyo CD-6CSX, Tokyo, Japan). The firmness expressed in N after being measured with a hand penetrometer (FT-327, Effegi, Italy) with an 11.1 mm tip (Ozturk et al., 2018).

2.3.2. Soluble solid contents (SSC), titratable acidity (TA) and vitamin C

A total number of 45 fruits (15 for each test) were separated for SSC, TA and vitamin C analysis. The separated fruit were crushed with a blender (Model: Promix HR2653 Philips). The SSC was determined using a digital refractometer (Atago, PAL-1, USA). For TA measurement, 15 mL of extract was mixed with 15 mL of distilled water and the amount of 0.1 mol L^{-1} NaOH (sodium hydroxide) required to titrate the solution to pH 8.1 was recorded as titratable acidity (mg malic acid 100 mL^{-1}). To determine the vitamin C (mg 100 g^{-1}) of the samples, approximately 0.5 mL of the extract was mixed with 0.5% oxalic acid to a final volume of 5 mL. Vitamin C test strips (Catalog no: 116981, Merck, Germany) were used for the measurement (Ozturk et al., 2019).

2.3.3. Total phenolics, total flavonoids, antioxidant activity

During the measurement, 10 fruit were taken from each replication, and washed with distilled water. The fruit were then homogenized in a blender for 5 min. Approximately 30-40 mL of fruit pulp was placed into a 50 mL tube. The prepared tubes were kept at -20°C until the day of analysis. Before the analysis, the frozen samples were thawed for approximately 5 h at room temperature (21 °C). Pulp and juice were separated from each other by centrifugation at 12,000 × g at 4°C for 30 min. The filtrate, which completely separated from the pulp, was used to determine the content of total phenolics, total flavonoids, DPPH, and FRAP antioxidant activities. Spectrophotometric measurements for total phenolics, total flavonoids, and antioxidant activity were performed using a UV-Vis spectrophotometer (Shimadzu, 1280, Kyoto, Japan). Total phenolics were determined according to the method described by Singleton and Rossi (1965), total flavonoids were determined according to the method described by Chang et al. (2002), and antioxidant activity was determined using two different procedures: 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay (Aglar et al., 2017) and ferric ions (Fe^{+3}) reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). The results were expressed as follows: total phenolics as g GAE (gallic acid equivalent) L^{-1} fresh weight (fw), total flavonoids as g QE (quercetin equivalent) L^{-1} fw, and DPPH-FRAP as mmol Trolox equivalent (TE) L^{-1} fw.

2.4. Statistical Analysis

The normality of the data was tested using the Kolmogorov-Smirnov Test, while Levene's test was utilized for variances homogeneity. Subsequently, an analysis of variance (ANOVA) was conducted to evaluate the data, and Tukey's multiple-comparison test was employed to detect significant differences among the cultivars. All statistical analyses were performed with the aid of SAS software (version 9.1, USA).

3. Results and Discussion

Fruit weight, length, width, and firmness values of the cultivars examined in the study were presented in Table 1. In apples, fruit weight, shape, size, and firmness are important criteria that affect consumer preferences (Drkenda et al., 2021). On the other hand, it is important to know the physical properties of fruit so that the producer can obtain more yielding, higher quality and marketable products (Cătălina et al., 2015). Because it is known that environmental conditions affect these features as well as genetic factors. In this study, differences between cultivars were significant regarding fruit weight, length, and width ($P<0.05$). The lowest fruit weight was determined in 'Starking Delicious' (165.93 g), while the highest was determined in the Scarlet Spur (289.96 g). Similarly, fruit length and width were the lowest in 'Starking Delicious' (26.79 mm and 27.99 mm) and the highest in 'Scarlet Spur' (32.61 mm and 34.86

mm). On the contrary, the lowest firmness was determined in the 'Scarlet Spur' (62.8 N) and the highest in 'Starking Delicious' (75.3 N). The difference between the cultivars in terms of firmness (62.8-75.3) was statistically insignificant (P<0.05). In previous studies carried out in different ecological conditions, Jemrić et al. (2003) were determined fruit weight 173.59-213.78 g, and firmness 64.13-67.66 N in 'Golden Delicious' in Croatia ecology; Karşı and Aslantaş (2016) were determined fruit weight 159.67 g, fruit width 73.8 mm, and fruit length 67.6 mm in 'Golden Delicious' in Erzurum ecology; Turan and Karlıdağ (2022) were determined fruit weight 139.31-139.87 g, fruit length 54.09-60.13 mm and firmness 44.12-49.03 N in 'Golden Delicious' in Malatya ecology; Özongun et al. (2014) were determined fruit weight 249 g, fruit width 82 mm, fruit length 73 mm, and firmness 68.82 N in 'Scarlet Spur' in Isparta ecology; Bolat et al. (2019) were determined fruit weight 157.5 g, fruit width 68.7 mm, fruit length 67.5 mm, and firmness 50.01 N in 'Scarlet Spur' in Antalya ecology; Uzun et al. (2022) were determined fruit weight 251.40 g, fruit width 78.37 mm, and fruit length 72.97 mm in 'Scarlet Spur' in Ordu ecology. Accordingly, it is seen that the findings obtained from this study have similarities with the results in the literatures. It is thought that ecological conditions and cultural practices such as genetic factors (Ateş and Öztürk, 2022), rootstocks (Ercişli et al., 2000; Yaman et al., 2022), pruning (Ates et al., 2022), fertilization (Bai et al., 2019), application of bio-stimulants (Sîrbu et al., 2023), and irrigation (Mpelasoka et al., 2000) can affect the physical properties of fruits.

The SSC, TA and vitamin C values of the cultivars examined in the study are presented in Table 2. Regarding these characteristics, the differences between the cultivars were statistically significant (P<0.05). While these features are effective on the maturity status of the fruits and the determination of the harvest date, they are also important in terms of taste and flavor formation. It is reported that the maturity status of fruits is related to ecological conditions (Güneyli and Onursal, 2014) and genetic factors (Bolat et al., 2019). In this study, the differences between the cultivars regarding biochemical properties were statistically significant (P<0.05). The lowest SSC and TA content were found in 'Super Chief' (11.65% and 0.47%), the highest SSC in 'Starkrimson Delicious' (14.3%), and the highest TA in 'Golden Delicious' (1.13%). Vitamin C was the lowest in 'Golden Delicious' (48 mg 100 g^{-1}) and the highest in 'Starking Delicious' (66 mg 100 g^{-1}). In previous studies carried out in different ecological conditions, Jemrić et al. (2003) were determined SSC between 12.11-13.38%, and between TA 0.39-0.41% in 'Golden Delicious' in Croatia ecology; Ghafir (2009) was determined SSC of 14,75%, and TA of 0.20% in 'Starkrimson' in Libya ecology; Maqsood et al., (2013) were determined vitamin C of 11.63-12.3 mg 100 g^{-1}, and TA of 0.31% in 'Starkrimson' and 'Starking Delicious' cultivars in Pakistan ecology.

Phenolic compounds and antioxidant activity, which positively affect human health, are secondary metabolites (Chen et al., 2013) found in fruits (Ozturk et al., 2016) or other parts of plants (Prakash et al., 2007). These contents can vary according to the species and depending on the variety and environmental factors. Total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activities (DPPH and FRAP assays) of the cultivars examined in the study were presented in Table 3. Regarding these characteristics, the differences between the cultivars were statistically significant (p<0.05). In the study, the highest TPC were obtained from 'Scarlet Spur' (38.2 g GAE L^1) and 'Super Chief' (36.5 g GAE L^{-1}) cultivars, while the lowest was found in 'Golden Delicious' (17.7 g GAE L^{-1}). TFC were highest in 'Super Chief' (23.5 g QE L^{-1}) and lowest in 'Starking Delicious' (14.5 g QE L^{-1}). According to DPPH and FRAP assays, the antioxidant activity was the highest in 'Super Chief' and the lowest in 'Golden Delicious'. Antioxidant activity of the 'Super Chief' cultivar was determined as 490.7 mmol TE L^{-1} according to the DPPH test and 1145.9 mmol TE L^{-1} according to the FRAP test. Similar to our study, TPC (Jindal et al., 2002; Maqsood et al., 2013; Turan and Karlıdağ, 2022), TFC (Awad et al., 2000), and antioxidant activities (Petkovsek et al., 2007; Vieira et al., 2011; Bahukhandi et al., 2019) show significant variation among cultivars. Plants contain essential ingredients both as a source of health and nutrients. Ingredients like bioactive compounds found in fruits are inexpensive and easily accessible. The amount of these bioactive contents varies depending on species-cultivars (Mertoğlu and Evrenosoğlu, 2019; Balta et al., 2022), rootstock (Kviklys et al., 2015), fertilization (Çakır et al., 2021), and ecological factors (Öztürk and Öztürk, 2016).

Table 1. Fruit weight, firmness and fruit dimensional characteristics of different apple cultivars grown in Kayseri

Cultivars	Quality characteristics			
	Fruit weight (g)	Length (mm)	Width (mm)	Firmness (N)
Scarlet Spur	289.96 ^{a}	32.61 ^{a}	34.86 ^{a}	62.8
Super Chief	222.30 ^{b}	31.51 ^{ab}	30.38 ^{b}	67.3
Golden Delicious	226.66 ^{b}	30.09 ^{abc}	30.80 ^{b}	72.8
Starking Delicious	165.93 ^{c}	26.79 ^{d}	27.99 ^{b}	75.3
Starkrimson Delicious	239.18 ^{b}	28.91 ^{cd}	34.86 ^{a}	68.3

* Means in the same column with different letters are significantly different according to Tukey's test at P<0.05.

Table 2. Soluble solid contents (SSC), titratable acidity (TA) and vitamin C contents of different apple cultivars grown in Kayseri

Cultivars	Biochemical characteristics		
	SSC (%)	TA (% malic acid)	Vitamin C (mg 100 g^{-1})
Scarlet Spur	12.85 ^{c}	0.64 ^{c}	60 ^{b}
Super Chief	11.65 ^{e}	0.47 ^{e}	59 ^{b}
Golden Delicious	13.65 ^{b}	1.13 ^{a}	48 ^{d}
Starking Delicious	12.35 ^{d}	0.59 ^{d}	66 ^{a}
Starkrimson Delicious	14.30 ^{a}	0.75 ^{b}	53 ^{c}

* Means in the same column with different letters are significantly different according to Tukey's test at P<0.05.

Table 3. Total phenolics (TPC), total flavonoids (TFC) and antioxidant activity (DPPH and FRAP assays) of different apple cultivars grown in Kayseri

Cultivars	Bioactive compounds			
	TPC (g GAE L^{-1})	TFC (g QE L^{-1})	DPPH (mmol TE L^{-1})	FRAP (mmol TE L^{-1})
Scarlet Spur	38.2 ^{a}	16.9 ^{ab}	346.6 ^{bc}	881.6 ^{b}
Super Chief	36.5 ^{a}	23.5 ^{a}	490.7 ^{a}	1145.9 ^{a}
Golden Delicious	17.7 ^{b}	17.7 ^{ab}	37.4 ^{e}	551.6 ^{c}
Starking Delicious	18.3 ^{b}	14.5 ^{b}	240.3 ^{c}	705.9 ^{bc}
Starkrimson Delicious	18.5 ^{b}	19.4 ^{ab}	75.6 ^{d}	936.8 ^{ab}

* Means in the same column with different letters are significantly different according to Tukey's test at P<0.05.

4. Conclusion

As a result, significant differences were determined regarding the physical, biochemical, and bioactive contents of apple cultivars are grown in Yahyalı district of Kayseri province. According to the analysis, 'Scarlet Spur' cultivar stood out regarding physical properties and 'Super Chief' cultivar in case of bioactive contents. In this context, preferring 'Scarlet Spur' and 'Super Chief' cultivars for growing in Yahyalı ecology can be recommended.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	U.A.	B.Ö.	S.U.
C	20	60	20
D	20	80	
S	25	50	25
DCP	60	10	30
DAI	80	10	10
L	60	20	20
W	60	20	20
CR	10	20	70
SR	40	30	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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INTEGRATED CONTROL OF *Echinochloa oryzoides*, *Echinochloa crus-galli* AND *Cyperus difformis* BASED ON PRE-SOWING HERBICIDE AND SOME HERBICIDE COMBINATIONS IN RICE

Emine KAYA ALTOP[1]*, Hüsrev MENNAN[1]

[1]*Ondokuzmayıs University, Faculty of Agriculture, Department of Plant Protection, 55139, Samsun, Türkiye*

Abstract: Cosmopolitan weeds are even constant elements where rice cultivation started just a century ago. There are few but important weed species with high adaptation in rice, which requires monoculture production systems and aquatic environment. Rotation difficulty resulted in the proliferation of highly competitive weed species strongly adapted to the aquatic environment. Constant use of herbicides with the same mechanisms of action leads to an evaluation of herbicide-resistant weed population and an increased number of herbicide-resistant populations in Türkiye. Chemical weed control and alternative integrated weed management strategies in rice production systems have significantly evolved throughout the years as well as rice herbicide traits and weed spectrum. The study was carried out in rice fields with resistance problems in two different locations, in the Black Sea and the Marmara Regions. The clamozone 480 EC, oxadiazon 200 CS, glyphosate potassium 441 g/l, glyphosate IPA 360 g/lt +carfentrazone-ethyl 5 g/l, cyhalofop-butyl 200 EC, penoxsulam 25.2 OD +bentazon-sodium 480 SL active ingredients and combinations' efficacy were investigated on ALS and ACCase inhibitor herbicides resistant *Cyperus difformis*, *Echinochloa oryzoides* and *Echinochloa crus-galli* populations. At the end of the study, it was determined that including pre-sowing herbicides such as clomazone and oxadiazon in the weed control program would help control the ALS and ACCase-resistant this species. Implementing integrated weed management strategies for managing existing herbicide-resistant weeds and reducing future development of herbicide resistance is one of our most influential and economical long-term strategies.

Keywords: Herbicide resistance, Pre-sowing herbicides, Rice, Weed

***Corresponding author:** Ondokuzmayıs University, Faculty of Agriculture, Department of Plant Protection, 55139, Samsun, Türkiye
E mail: kayae@omu.edu.tr (E. KAYA ALTOP)
Emine KAYA ALTOP https://orcid.org/0000-0002-0987-9352
Hüsrev MENNAN https://orcid.org/0000-0002-1410-8114

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1. Introduction

Weeds are very difficult to control in rice cultivation areas, and if not controlled, they cause more than 40% product loss (Busconi et al., 2012; Chauhan and Abugho, 2013). Because the rice production system is completely in an aquatic environment, we see that few but important weed species are adapted to this system. When we look at the weed species that are a problem in Asian, American and European countries where rice cultivation areas are dense, it is seen that the species of *Echinochloa, Cyperus* and *Alisma* genus cause significant problems and are difficult to control (Holm et al., 1977; Ruiz-Santaella et al., 2006; Talbert and Burgos, 2007; Mennan et al., 2012).

Weed competition is the primary pest for rice production, with yield reductions of more than 50% (Ziska et al., 2015). *Echinochloa crus-galli* (L.) P. Beauv. can cause more than 55% reduction in grain yield (Zhang et al., 2017), while competition with weedy rice can result in up to 72% reduction in the number of full grains (Martin and Tanzo, 2015). In addition to yield loss, rice weeds can reduce the land value (Ottis and Talbert, 2007), increase the soil seed bank (Bagavathiannan et al., 2011), and lead to price increases due to contaminated rice seeds. Rice growers worldwide rely heavily on herbicides for weed management (Rouse et al., 2018; Barber et al., 2022). However, chemical weed control and alternative integrated weed management strategies in rice production systems have evolved significantly over the years, along with rice herbicide properties and weed spectrum.

As in many developed countries, weed control in rice is carried out completely dependent on herbicides in Türkiye. It is seen that mainly ALS and ACCase inhibitor herbicides are used to control *Echinochloa* spp., *Cyperus difformis* L. and *Alisma plantago-aquatica* L. However, as a result of the use of this group of inhibitors in rice cultivation areas for a long time, resistance problems have emerged in different weed species belonging to these genera, and it has become impossible to control weeds at present (Itah et al., 1999; Park et al., 1999; Fischer et al., 2000). Mennan et al. (2012) investigated the populations of *Echinochloa oryzoides* L. and *E. crus-galli* resistant to ALS and ACCase inhibitor herbicides in

the Marmara and Black Sea Regions rice cultivation areas. In recent years, the abandonment of some areas in rice cultivation due to herbicide resistance and leaving them after planting because they cannot be combated have reached dimensions that threaten agricultural production. In addition, studies have shown that the majority of resistant populations are metabolic; therefore, farmers try to dissolve the existing herbicides at 3-4 times the dose or by making many mixtures. As a result of the applications made, it is partially successful, but it also brings many problems with product safety.

Pre-emergence herbicide application is effective during the dry period for early weed emergence just before or after rice emergence (Singh et al., 2016; Mahajan and Chauhan, 2015), but a post-emergence herbicide application is required for the second spraying of weeds during the flood period (Jordan et al., 1998). A narrow time window (0 to 3 DAS (day after sowing)) requires highly effective pre-emergence herbicides (Mahajan and Chauhan, 2015) to provide season-long weed control (Helms et al., 1995).

The resistance to herbicides in weeds changes the spraying programs. In this case, to control populations resistant to ALS and ACCase inhibitor herbicides, developing and practising local integrated control techniques in our country and the rest of the world is necessary. From this point of view, it is essential to use herbicides effectively within the scope of integrated control, which is one of the issues that must be solved in order to prevent product loss in rice production. In Türkiye, two herbicides with active ingredients, clomazone and oxadiazon, are licensed as pre-sowing and early post-emergence. Although these two active ingredients have different properties, clomazone is more effective against *E. crus-galli* and oxadiazon is effective against *C. difformis*. However, it is not known what effect the combination of these two will have on *E. oryzoides*, which is one of the important problems. There is also apply of total herbicides before planting to break the resistance. In order to clarify this situation and to see a practical result, if any, considering the known ecological characteristics of these weeds, it is planned to develop an integrated control system with the application of glyphosate or glyphosate + carfentrazone at different times and in combination with other herbicides after emergence.

In this study, it was aimed to determine the chemical integrated control possibilities of *C. difformis*, *E. oryzoides* and *E. crus-galli* populations resistant to ALS and ACCase inhibitor herbicides, which were previously detected in rice cultivation areas of Marmara and Black Sea Regions, based on pre-sowing herbicide applications and some herbicide combinations.

2. Materials and Methods

The study was carried out in the rice fields with resistance problems in two different locations, in Samsun, representing the Black Sea Region, and in the center of Edirne, representing the Marmara Region, in the third week of May 2015 and 2016. Experiments were set up in a randomized block design with four replications, and the parcel sizes were set to 50 m^2 (10m x 5m). A safety strip of at least 2 m is left between the parcel and the blocks. The spray volume was 200 L ha^{-1} and applied using a compressed CO_2 sprayer system that had 8004 flat-fan nozzles with a pressure of three bars. A control parcel was also created. In addition to the control parcel, it was created in separate parcels where the producer's routine operations (3-4 herbicide applications) against the resistance problem will also occur. Thus, all applications had the chance to be compared.

Observations were made on different dates during the experiment depending on the application time. These were made 7 days after the first observation and 20 days after the second observation. In addition, checks were made every week to observe whether there were new exits.

The applications given below were carried out.

2.1. Clomazone and Oxadiazon Application

1. Clomazone 480 EC formulation was applied at 720-960 g a.iha^{-1} as a pre-sowing preparation dose, and then water was added to the pans by sowing within 1-2 days.
2. Oxadiazon 200 SC formulation was applied at 300-400 g a.iha^{-1} as a pre-sowing preparation dose, and then water was added to the pans by sowing within 1-2 days.
3. Clomazone 480 CS formulation and oxadiazon 200 SC were applied in a dose of 720+300 g a.i. ha^{-1} as a mixture before planting, and then water was added to the pans by sowing within 1-2 days.

2.2. Glyphosate (Glyphosate Potassium (441 g/l)) Application

4. 7 days before sowing (DBS), the pans were filled with water and kept in the pans, and the germination of weed seeds was encouraged. The water in the pans was drained, and a dose of 2205 g a.i. ha^{-1} glyphosate was applied as a preparation dose within 1-2 days.
5. 15 DBS, the pans were filled with water and kept in the pans, and the germination of weed seeds was encouraged. Afterwards, the water in the pans was drained, and then a dose of 2205 g a.i. ha^{-1} glyphosate was applied as a preparation dose within 1-2 days.
6. 21 DBS, the pans were filled with water and kept in the pans, and weed seeds germination was encouraged. Afterwards, the water in the pans was drained, and a dose of 2205 g a.i. ha^{-1} glyphosate was applied as a preparation dose within 1-2 days.

2.3. Glyphosate+Carfentrazone (Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l) Application

7. 7 DBS, the pans were filled with water and kept in the pans, and the germination of weed seeds was encouraged. Afterwards, the water in the pans was

drained, and a 720+10 g a.i. ha^{-1} dose of glyphosate+carfentrazon was applied as a preparation dose within 1-2 days.

8. 15 DBS, the pans were filled with water and kept in the pans, and weed seeds germination was encouraged. Afterwards, the water in the pans was drained, and a 720+10 g a.i. ha^{-1} dose of glyphosate+carfentrazon was applied as a preparation dose within 1-2 days.
9. 21 DBS, the pans were filled with water and kept in the pans, and weed seeds germination was encouraged. Afterwards, the water in the pans was drained, and a 720+10 g a.i. ha^{-1} dose of glyphosate+carfentrazon was applied as a preparation dose within 1-2 days.

The characters, doses and application periods used in the trials are given in Table 1.

Table 1. The characters, doses and application periods of herbicides used in the integrated control of *Echinochloa oryzoides, Echinochloa crus-galli* and *Cyperus difformis*.

No	Active ingredients and formulations	Dose (g a.i. ha^{-1})	Application periods (BBCH-scale)
1	Clomazone 480 EC	720	B 00
2	Clomazone 480 EC	960	B 00
3	Oxadiazon 200 CS	300	B 00
4	Oxadiazon 200 CS	400	B 00
5	Clomazone 480 EC+Oxadiazon 200 CS	720+300	B 00
6	Glyphosate potassium 441 g/l (7 DAT water apply)	2205	B 00
7	Glyphosate potassium 441 g/l (14 DAT water apply)	2205	B 00
8	Glyphosate potassium 441 g/l (21 DAT water apply)	2205	B 00
9	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (7 DAT water apply)	720+10	B 00
10	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (14 DAT water apply)	720+10	B 00
11	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (21 DAT water apply)	720+10	B 00
12	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC	720+300+700	B00 +B 12-14
13	Clomazone 480 EC+Oxadiazon 200 CS+Penoxsulam 25.2 OD	720+300+40,3	B 00 +B 12-14
14	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD	720+300+126	B 00 +B 12-14
15	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD +Bentazon-sodium 480 SL	720+300+126+720	B 00+B 12-14+ B 21-22
16	Control	-	

2.4. Statistical Analysis

The 0-100% scale specified by EWRS and WSSA was used to measure the activity levels of herbicides. Accordingly, 0% means no weed control, and 100% of weeds are completely controlled (Zandstra et al., 2003; Mennan et al., 2006). Arcsine transformation was applied to the obtained weed control percentage values to improve homogeneity before analysis of variance (ANOVA). The averages obtained in the experiment were separated by LSD ($P<0.05$) by applying Fisher's protected test, and then the results were grouped according to the Tukey test.

3. Results

Regarding herbicide applications in both regions ($P<0.05$), the interactions between regional and year were found to be insignificant, so the averages of both regions were combined. Considering the efficacy values obtained on the 7th day after the application, 1500+1500 cc ha^{-1} dose of the clamzone+oxadiazon mixture was found to be effective at an acceptable level against *E. oryzoides*, *E. crus-galli* and *C. difformis* (Table 2). When the efficacy values obtained on the 14th day from Table 3 are examined, results similar to the counts made on the 7th day were obtained. The clomazone + oxadiazon mixture gave excellent results in the control of three species. It is observed that there is a decrease in the

effectiveness of glyphosate potassium application with quenching on the 7th day. Efficiency has decreased in these parcels with new exits. When we look at the glyphosate potassium application, no change was observed in the effectiveness of all three species as the number of days after the application increased (Table 2-3). Similar results were obtained in the application of glyphosate IPA + carfentrazone-ethyl, but the level of effectiveness was found to be higher. Clomazone + oxadiazon + cyhalofop-butyl (applied later) application showed an 85% efficiency as in the 5th application. Similar results were obtained in the applications numbered 13, 14 and 15 following this application (Table 1).

Considering the efficacy values obtained on the 14th day from Table 3, both doses of clomazone were successful, with an effect of over 90% on *E. oryzoides* and *E. crus-galli*. Similarly, both doses of oxadiazon were found to be effective against both *E. crus-galli* and *C. difformis*. No effect of this active substance on *E. oryzoides* was found. The clomazone + oxadiazon mixture gave an excellent result against all three species. In the application of glyphosate potassium, it is seen that there is a decrease in the effectiveness with quenching on the 7th day after the application. Efficiency has decreased in these parcels with new exits. On the other hand, the efficiency was found to be around 75% in the parcels where water was not given. Although similar results were obtained in the application of glyphosate IPA + carfentrazone-ethyl, the efficacy levels were found to be higher than glyphosate potassium (Table 3). Since the post-emergence applications to be made following clomazone + oxadiazon application have yet to be performed, the efficacy values obtained on the 14th day were found to be above 90%, as previously stated.

Considering the evaluations made on the 28th day, another counting period, from Table 4, the effectiveness of clomazone and oxadiazon applications was over 90%, as in the previous count. It is understood that very successful results were obtained in the parcels where these two active ingredients were applied together. Glyphosate potassium and glyphosate IPA + carfentrazone-ethyl applications decreased with quenching. Studies have shown us that these applications can be successful in the first weed emergence, but there is a need for post-emergence applications in the future. Post-emergence applications were also carried out in this counting period following the clomazone + oxadiazon application. However, these efficiencies above 90% come from pre-sowing applications. As it can be understood from here, the persistence and effectiveness of these active ingredients applied before planting continue on the 28th day.

A decrease in the efficacy of both doses of clomazone and oxadiazon started in the counts made on the 56th day after sowing. The activities decreased by 30-40% (Table 5). The effectiveness of glyphosate potassium and glyphosate IPA + carfentrazone-ethyl applications disappeared during these counting periods. Following the administration of clomazone + oxadiazon, the 3500 cc ha^{-1} dose of cyhalofop-butyl showed an efficiency of 75%. As can be seen from this, the dose of cyhalofop-butyl only contributed 10% in efficacy.

Table 2. Statistical grouping of herbicides used in the integrated control of *E. oryzoides*, *E. crus-galli* and *C. difformis* populations according to efficacy percentages on the 7th day after application in the biological efficacy trial of herbicides.

No	Active ingredients and formulations	Dose (g a.i. ha^{-1})	*E. oryzoides*	*E. crus-galli*	*C. difformis*
1	Clomazone 480 EC	720	85 ^{a}	85 ^{a}	0 ^{c}
2	Clomazone 480 EC	960	90 ^{a}	90 ^{a}	0 ^{c}
3	Oxadiazon 200 CS	300	20 ^{d}	65 ^{b}	95 ^{a}
4	Oxadiazon 200 CS	400	20 ^{d}	70 ^{b}	95 ^{a}
5	Clomazone 480 EC+Oxadiazon 200 CS	720+300	90 ^{a}	95 ^{a}	75 ^{b}
6	Glyphosate potassium 441 g/l (7 DAT water apply)	2205	50 ^{b}	55 ^{c}	65 ^{b}
7	Glyphosate potassium 441 g/l (14 DAT water apply)	2205	30 ^{cd}	30 ^{d}	60 ^{b}
8	Glyphosate potassium 441 g/l (21 DAT water apply)	2205	25 ^{cd}	30 ^{d}	60 ^{b}
9	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (7 DAT water apply)	720+10	55 ^{b}	55 ^{c}	70 ^{b}
10	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (14 DAT water apply)	720+10	50 ^{b}	50 ^{c}	70 ^{b}
11	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (21 DAT water apply)	720+10	40 ^{c}	50 ^{c}	75 ^{b}
12	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC	720+300+700	90 ^{a}	90 ^{a}	95 ^{a}
13	Clomazone 480 EC+Oxadiazon 200 CS+Penoxsulam 25.2 OD	720+300+40,3	90 ^{a}	95 ^{a}	95 ^{a}
14	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD	720+300+1126	90 ^{a}	100 ^{a}	95 ^{a}
15	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD +Bentazon-sodium 480 SL	720+300+1126+720	90 ^{a}	95 ^{a}	100 ^{a}
16	Control		0 ^{e}	0 ^{e}	0 ^{c}
	LSD (P<0.05)		7.88	9.56	8.53

*There is no difference at the level of (P<0.05) according to the Tukey test between the applications shown with the same letter in the columns.

Table 3. Statistical grouping of herbicides used in the integrated control of *E. oryzoides*, *E. crus-galli* and *C. differmis* populations according to efficacy percentages on the 14t[h] day after application in the biological efficacy trial of herbicides.

No	Active ingredients and formulations	Dose (g a.i. ha^{-1})	*E. oryzoides*	*E. crus-galli*	*C. differmis*
1	Clomazone 480 EC	720	90 ^{a}	95 ^{a}	0 ^{d}
2	Clomazone 480 EC	960	95 ^{a}	100 ^{a}	0 ^{d}
3	Oxadiazon 200 CS	300	10 ^{e}	85 ^{ab}	95 ^{a}
4	Oxadiazon 200 CS	400	15 ^{e}	95 ^{a}	100 ^{a}
5	Clomazone 480 EC+Oxadiazon 200 CS	720+300	95 ^{a}	100 ^{a}	100 ^{a}
6	Glyphosate potassium 441 g/l (7 DAT water apply)	2205	40 ^{d}	50 ^{c}	45 ^{c}
7	Glyphosate potassium 441 g/l (14 DAT water apply)	2205	55 ^{c}	70 ^{b}	65 ^{b}
8	Glyphosate potassium 441 g/l (21 DAT water apply)	2205	55 ^{c}	75 ^{b}	65 ^{b}
9	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (7 DAT water apply)	720+10	60 ^{bc}	60 ^{b}	60 ^{bc}
10	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (14 DAT water apply)	720+10	65 ^{b}	75 ^{b}	75 ^{b}
11	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (21 DAT water apply)	720+10	70 ^{b}	70 ^{b}	80 ^{b}
12	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC	720+300+700	95 ^{a}	90 ^{a}	100 ^{a}
13	Clomazone 480 EC+Oxadiazon 200 CS+Penoxsulam 25.2 OD	720+300+40,3	95 ^{a}	95 ^{a}	100 ^{a}
14	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD	720+300+1126	95 ^{a}	100 ^{a}	100 ^{a}
15	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+ Penoxsulam 25.2 OD +Bentazon-sodium 480 SL	720+300+1126+720	100 ^{a}	100 ^{a}	100 ^{a}
16	Control		0 ^{e}	0 ^{d}	0 ^{d}
	LSD (P<0.05)		11.08	10.21	7.18

*There is no difference at the level of (P<0.05) according to the Tukey test between the applications shown with the same letter in the columns.

Table 4. Statistical grouping of herbicides used in the integrated control of *E. oryzoides*, *E. crus-galli* and *C. differmis* populations according to efficacy percentages on the 28[th] day after application in the biological efficacy trial of herbicides.

No	Active ingredients and formulations	Dose (g a.i. ha^{-1})	*E. oryzoides*	*E. crus-galli*	*C. differmis*
1	Clomazone 480 EC	720	90 ^{a}	95 ^{a}	0 ^{c}
2	Clomazone 480 EC	960	95 ^{a}	100 ^{a}	0 ^{c}
3	Oxadiazon 200 CS	300	0 ^{d}	85 ^{a}	95 ^{a}
4	Oxadiazon 200 CS	400	0 ^{d}	85 ^{a}	10 ^{bc}
5	Clomazone 480 EC+Oxadiazon 200 CS	720+300	95 ^{a}	100 ^{a}	100 ^{a}
6	Glyphosate potassium 441 g/l (7 DAT water apply)	2205	10 ^{d}	30 ^{c}	20 ^{bc}
7	Glyphosate potassium 441 g/l (14 DAT water apply)	2205	35 ^{c}	45 ^{c}	20 ^{bc}
8	Glyphosate potassium 441 g/l (21 DAT water apply)	2205	45 ^{bc}	45 ^{c}	20 ^{bc}
9	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (7 DAT water apply)	720+10	35 ^{c}	35 ^{c}	15 ^{bc}
10	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (14 DAT water apply)	720+10	55 ^{b}	45 ^{c}	20 ^{bc}
11	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (21 DAT water apply)	720+10	55 ^{b}	60 ^{b}	30 ^{b}
12	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC	720+300+700	95 ^{a}	95 ^{a}	95 ^{a}
13	Clomazone 480 EC+Oxadiazon 200 CS+Penoxsulam 25.2 OD	720+300+40.3	95 ^{a}	100 ^{a}	95 ^{a}
14	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD	720+300+1126	95 ^{a}	100 ^{a}	100 ^{a}
15	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+ Penoxsulam 25.2 OD +Bentazon-sodium 480 SL	720+300+1126 +720	95 ^{a}	95 ^{a}	100 ^{a}
16	Control				
	LSD (P<0.05)		10.69	12.14	8.41

*There is no difference at the level of (P<0.05) according to the Tukey test between the applications shown with the same letter in the columns.

Table 5. Statistical grouping of herbicides used in the integrated control of *E. oryzoides*, *E. crus-galli* and *C. differmis* populations according to efficacy percentages on the 56th day after application in the biological efficacy trial of herbicides.

No	Active ingredients and formulations	Dose (g a.i. ha^{-1})	*E. oryzoides*	*E. crus-galli*	*C. differmis*
1	Clomazone 480 EC	720	40 ^{c}	60 ^{c}	0 ^{c}
2	Clomazone 480 EC	960	50 ^{c}	65 ^{bc}	0 ^{c}
3	Oxadiazon 200 CS	300	0 ^{d}	40 ^{d}	65 ^{b}
4	Oxadiazon 200 CS	400	0 ^{d}	50 ^{cd}	70 ^{b}
5	Clomazone 480 EC+Oxadiazon 200 CS	720+300	55 ^{c}	70 ^{b}	70 ^{b}
6	Glyphosate potassium 441 g/l (7 DAT water apply)	2205	0 ^{d}	0 ^{e}	0 ^{c}
7	Glyphosate potassium 441 g/l (14 DAT water apply)	2205	0 ^{d}	0 ^{e}	0 ^{c}
8	Glyphosate potassium 441 g/l (21 DAT water apply)	2205	0 ^{d}	0 ^{e}	0 ^{c}
9	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (7 DAT water apply)	720+10	0 ^{d}	0 ^{e}	0 ^{c}
10	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (14 DAT water apply)	720+10	0 ^{d}	0 ^{e}	0 ^{c}
11	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (21 DAT water apply)	720+10	0 ^{d}	0 ^{e}	0 ^{c}
12	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC	720+300+700	60 ^{b}	70 ^{b}	95 ^{a}
13	Clomazone 480 EC+Oxadiazon 200 CS+Penoxsulam 25.2 OD	720+300+40.3	70 ^{b}	70 ^{b}	80 ^{b}
14	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD	720+300+1126	100 ^{a}	100 ^{a}	100 ^{a}
15	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+ Penoxsulam 25.2 OD +Bentazon-sodium 480 SL	720+300+1126+720	100 ^{a}	95 ^{a}	100 ^{a}
16	Control		0 ^{d}	0 ^{e}	0 ^{c}
	LSD (P<0.05)		8.44	10.52	11.36

*There is no difference at the level of (P<0.05) according to the Tukey test between the applications shown with the same letter in the columns.

Table 6. Statistical grouping of herbicides used in the integrated control of *E. oryzoides*, *E. crus-galli* and *C. differmis* populations, according to pre-harvest percentages activity in the biological activity trial.

No	Active ingredients and formulations	Dose (g a.i. ha^{-1})	*E. oryzoides*	*E. crus-galli*	*C. differmis*
1	Clomazone 480 EC	720	0 ^{c}	0 ^{c}	0 ^{c}
2	Clomazone 480 EC	960	0 ^{c}	0 ^{c}	0 ^{c}
3	Oxadiazon 200 CS	300	0 ^{c}	0 ^{c}	30 ^{b}
4	Oxadiazon 200 CS	400	0 ^{c}	0 ^{c}	30 ^{b}
5	Clomazone 480 EC+Oxadiazon 200 CS	720+300	0 ^{c}	20 ^{b}	30 ^{b}
6	Glyphosate potassium 441 g/l (7 DAT water apply)	2205	0 ^{c}	0 ^{c}	0 ^{c}
7	Glyphosate potassium 441 g/l (14 DAT water apply)	2205	0 ^{c}	0 ^{c}	0 ^{c}
8	Glyphosate potassium 441 g/l (21 DAT water apply)	2205	0 ^{c}	0 ^{c}	0 ^{c}
9	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (7 DAT water apply)	720+10	0 ^{c}	0 ^{c}	0 ^{c}
10	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (14 DAT water apply)	720+10	0 ^{c}	0 ^{c}	0 ^{c}
11	Glyphosate IPA 360 g/lt+Carfentrazone-ethyl 5 g/l (21 DAT water apply)	720+10	0 ^{c}	0 ^{c}	0 ^{c}
12	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC	720+300+700	25 ^{b}	20 ^{b}	35 ^{b}
13	Clomazone 480 EC+Oxadiazon 200 CS+Penoxsulam 25.2 OD	720+300+40.3	30 ^{b}	30 ^{b}	30 ^{b}
14	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+Penoxsulam 25.2 OD	720+300+1126	95 ^{a}	95 ^{a}	100 ^{a}
15	Clomazone 480 EC+Oxadiazon 200 CS+Cyhalofop-butyl 200 EC+ Penoxsulam 25.2 OD +Bentazon-sodium 480 SL	720+300+1126+720	95 ^{a}	95 ^{a}	100 ^{a}
16	Control		0 ^{c}	0 ^{c}	0 ^{c}
	LSD (P<0.05)		4.52	11.63	9.58

*There is no difference at the level of (P<0.05) according to the Tukey test between the applications shown with the same letter in the columns.

It shows the level of endurance. 1500 cc ha^{-1} dose of penoxsulam following clomazone + oxadiazon application created a 5-10% difference compared to cyhalofop-butyl application. In another application, 5000 cc ha-1 dose of cyhalofop-butyl + penoxsulam followed by clomazone + oxadiazon application, and cyhalofop-butyl + penoxsulam + bentazone-sodium applications following clomazone + oxadiazon application were found

to be successful with an effect of over 90%.

In the last count made before harvest, 5000 cc ha^{-1} dose of cyhalofop-butyl + penoxsulam following clomazone + oxadiazon application and clomazone + oxadiazon application followed by cyhalofop-butyl + penoxsulam + bentazone-sodium applications, as can be seen in Figures 1 and 2 showed that 90% and a clean field before harvest (Table 5).

Figure 1. The view from cyhalofop-butyl penoxsulam parcels after pre-sowing clomazone + oxadiazon application.

Figure 2. The view from cyhalofop-butyl penoxsulam + bentazon-sodium parcels after pre-planting clomazone + oxadiazon application.

4. Discussion

Weed species that are a problem in rice cultivation areas also have adapted to this ecosystem (Holm et al., 1977). Rice is grown as a monoculture due to both the environment in which it is grown and its yield. The most natural result of years of monoculture production system and weed control in this product directly dependent on herbicides is the problem of resistance. As in many countries where rice is grown, Türkiye's herbicide resistance problem has peaked (Juliano et al., 2010; Mennan et al., 2011; Heap, 2012). Another critical problem in this regard is the prohibition of many herbicides within the European Union harmonisation laws. The lack of new herbicides with alternative mechanisms of action has made the situation more dramatic.

The development of integrated control systems that are less dependent on chemical control will make an important contribution to the control of weeds resistant to ALS and ACCase inhibitor herbicides (Itah et al., 1999; Fischer et al., 2000; Perron and Legere, 2000). The study's findings were supported by revealing that an integrated weed control approach, accompanied by an appropriate spraying program, provides efficacy in resistant weeds (Bajwa et al., 2015). It has been demonstrated that pre-sowing herbicides and combinations are highly effective, providing control over standard practices against *E. crus-galli*, *E. oryzoides,* and *C. difformis* resistant to ALS and ACCase inhibitor herbicides when supplemented with post-emergence herbicide treatment.

Implementing alternative integrated weed management practices is often more cumbersome and can increase emergency weed management costs. However, as the focus shifts from short-term to long-term economics and the potential for widespread, multi-domain resistance to nullify herbicides, the chemical costs of weed control will increase drastically (Davis and Frisvold, 2017). Implementing integrated weed management strategies for managing existing herbicide-resistant weeds and reducing future development of herbicide resistance is one of our most influential and economical long-term strategies. Educational campaigns are needed to increase adoption, emphasising the economic benefits of integrated weed management strategies (Llewellyn et al., 2004).

5. Conclusion

In studies on pre-planting herbicide applications and some herbicide combinations based on the pre-sowing herbicide applications of *C. difformis*, *E. oryzoides*, and *E. crus-galli* populations resistant to ALS and ACCase inhibitor herbicides, it was determined that pre-sowing applications such as clomazone and oxadiazon reduced weed pressure. The application of this herbicide mixture before sowing and after the emergence of cyhalofop-butyl + penoxsulam gave successful results in many cases.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.K.A.	H.M.
C	50	50
D	50	50
S	30	70
DCP	50	50
DAI	20	80
L	80	20
W	90	10
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection

and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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EFFECT OF SHORT-TERM STORAGE TEMPERATURE ON MECHANICAL PROPERTIES OF 'ISTANBUL' MEDLAR

Elçin YEŞİLOĞLU CEVHER[1]*, Ahmet ÖZTÜRK[2]

[1]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agriculture Machinery and Technologies Engineering, 55139, Samsun, Türkiye
[2]Ondokuz Mayıs University, Faculty of Agriculture, Department of Horticulture, 55139, Samsun, Türkiye

Abstract: The study investigated the effect of short-term storage time on the mechanical (rupture force, rupture energy, deformation) properties of the 'Istanbul' medlar cultivar grafted on Quince A (QA) and Quince Province BA29 (BA29) quince clone rootstock. The study gives the average values of the physical properties (weight, size, geometric mean diameter, sphericity, surface area, true density, bulk density, and porosity) of the 'Istanbul' medlar cultivar. The changes in breaking force, breaking energy, and deformation values of 'Istanbul' medlar varieties stored at room temperature (25±1 °C) and cold storage (4±1 °C) for 5 days were investigated. Separate measurements were made for skin and skinless fruits. The average rupture force value of fruits of the 'Istanbul' medlar cultivar stored at 25 °C was determined as 8.82 N, deformation 4.66 mm, and rupture energy 0.035 J. The average rupture force value of the fruits of the 'Istanbul' medlar cultivar stored at 4 °C was determined as 10.06 N, deformation 5.16 mm, and rupture energy 0.043 J. It was found that the storage conditions had a statistically significant effect on the rupture force, rupture energy, and deformation values of the QA and BA29 rootstocks of the 'Istanbul' medlar cultivar (P≤0.001).

Keywords: Medlar, Short-term storage temperature, Mechanical properties, Rupture force, Rupture energy

***Corresponding author:** Ondokuz Mayıs University, Faculty of Agriculture, Department of Agriculture Machinery and Technologies Engineering, 55139, Samsun, Türkiye
E mail: elciny@omu.edu.tr (E. YEŞİLOĞLU CEVHER)
Elçin YEŞİLOĞLU CEVHER https://orcid.org/0000-0001-9062-923X
Ahmet ÖZTÜRK https://orcid.org/0000-0002-8800-1248

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1. Introduction

Medlar (*Mespilus germanica* L.) is a perennial plant belonging to the Rosaceae family. Medlar, whose homeland is Europe and West Asia, grows wild in the Marmara and North Anatolian Mountains, Black Sea and Aegean regions in Türkiye (Uzun and Bostan, 2019; Żołnierczyk et al., 2021). It has found a natural habitat with other forest types in the Black Sea region of Türkiye. In the world commonly known as medlar, the names like döngel, töngel and beş bıyık are used in Türkiye (Uzun and Bostan, 2019; Aydın et al., 2020; Diler and Leblebicier, 2020). The cultivars named 'Istanbul' and 'Italian' were registered for the first time in Türkiye. It is rich in nutritional value, highly used for medical purposes, and evaluated as an ornamental plant. All these facts increase the cultivation area of medlar (Gürbüz and Bostan, 2020). The production amount of 5,278 tons was obtained from 259,000 medlar trees in 2022 in Türkiye (TSI, 2023). According to the regions, the highest medlar production in Türkiye is in the provinces of Samsun, Sinop, Trabzon, Düzce, Bartın, Çorum, and Giresun in the Black Sea Region; In the provinces of Afyonkarahisar, Manisa, Aydın, Burdur, Isparta, Kütahya and Uşak in the Aegean Region; It occurs in the provinces of Çanakkale, Bursa and Balıkesir in the Marmara Region (TSI, 2023).

The medlar fruit is very rich in amounts of B2, B1, C, and A vitamins, calcium, potassium, iron, and magnesium minerals. Also, it has phenolic acid, sugar, pectin, and organic acid content (Çakır and Öztürk 2019; Aydın et al., 2020). Medlar fruit is used in making jelly, sauce, and wine; it is consumed in the forms of marmalade, vinegar, pickles, and jelly (Canan et al., 2019; Uzun and Bostan, 2019; Glew et al., 2002). To be eaten as a fresh fruit, it should wait for the fruit flesh to turn brown because the newly harvested medlar fruit is light in color and has a hard texture and a bitter taste. Depending on the ripening period of the fruit, there are changes in its quality content (Ozturk et al., 2019 and Ozturk et al., 2022). The amount of fatty acids in medlar fruit containing twenty different fatty acids can decrease from a maximum of 6,121 ug g^{-1} to 2,583 ug g^{-1} during the ripening process. Unripe (raw) medlar fruit can treat intestinal inflammation, kidney diseases, and constipation (Uzun and Bostan, 2019; Gürbüz and Bostan, 2020; Diler and Leblebicier, 2020). Because of these features, medlar finds its place in markets and

markets (Aydın et al., 2020).

The effects of the processes in preserving the post-harvest product quality in fruits are quite high. The use Usingtapplied in post-harvest processes will reduce losses and provide quality products to the consumer (Sessiz and Özdemir, 2007). Experimental data are used to create models that predict agricultural products' quality and product behavior. These data are obtained from the determination of the physical and mechanical properties of the agricultural products. The effects that occur during the harvesting and processing stages of agricultural products can cause a decrease in the quality of horticultural commodities. In this sense, determining the parameters that are caused mechanical damage is important in reducing quality and quantity losses. In addition, in the processing industry, properties such as rupture resistance and hardness are among the parameters that should be known (Gül et al., 2020).

The study aimed to determine the effect of short-term storage time on the mechanical (rupture force, rupture energy, deformation) properties of the 'Istanbul' medlar cultivar, which is grafted on the QA and BA29 quince clonal rootstocks.

2. Materials and Methods

2.1. Plant Materials

In the study, fruits of the 'Istanbul' medlar cultivar, which was grafted on QA and BA29 rootstocks in the year 2018 at the Bafra (Samsun/Türkiye) research station of Ondokuz Mayis University (41° 33' 50'' N; 35° 52' 21'' E; altitude 20 m) were used as plant material. The grafted trees were planted at 3.5 x 3.0 m distances (952 tree ha^{-1}) and were pruned according to the modified leader system. The trees were pruned regularly every year, and irrigation was carried out usually twice a week in June - October, depending on the water requirement of the trees, with pressure compensating drippers at 1.20 m intervals, with two pipes per row on both sides of the trees. Weed control was performed regularly with a rotavator many times each year.

2.2. Climate and Soil Characteristics of the Research Area

The research orchard which fruits were provided from generally has a hot and humid climate in summer and a cool environment in winter. Most of the precipitation occurs in late autumn and early winter. According to the climate data of the Bafra district (Samsun), during the period which was done, the highest temperature was 35.1 °C, the lowest temperature was -4.5 °C, and the average annual temperature was observed at 14.1 °C. The soil of the research area has 2.73 - 10% clay, 13.21 - 20% silt, 6.5 - 20% sand, pH 7.5, 0.2 - 0.3 dS m^{-1} salt, 0.3 - 0.5 organic matter, 3 - 6% lime, 0.03 - 0.06 N, 5 - 10 ppm P level, and soil depth is more than 1 m.

2.3. Methods

Medlar fruits were harvested before they were fully ripe (physiological maturity = ready to harvest but not ready to eat) since they are used in different industrial areas other than fresh consumption. After harvesting, the fruits were immediately placed in the boxes and carried to the laboratory for preservation. The fruits were divided into two parts half was kept at ambient temperature (25±1 °C), and half of the fruits were stored at cold storage (4±1 °C) for 5 days.

2.4. Observations

The dimensions of the medlar fruits were measured with a digital caliper with a precision of 0.01 mm. Geometric mean diameter, sphericity, and surface area were calculated with the given formulas in Equation 1, 2 and 3 (Mohsenin, 1980):

$$D_g = (LWT)^{1/3} \quad (1)$$

D_g : Geometric mean diameter (mm), L: Length (mm), W: Width (mm), and T: Thickness (mm).

$$S = \pi D_g^2 \quad (2)$$

S: Surface area (mm^2)

$$\varphi = \frac{D_g}{L} 100 \quad (3)$$

φ: Sphericity (%).

The fruits' true density values were determined by the liquid displacement method. The measuring cylinder having a volume of 500 ml was used for measurement. Bulk density is the ratio of the medlar mass to its total volume. It was determined by weighing the medlar fruits after filling a cylindrical cardboard box with a height of 150 mm and a volume of 500 ml (Mohsenin, 1980; Mansouri et al., 2017; Lammari et al., 2022).

The given Equation 4 below was used to determine the porosity value of medlar fruits (Mohsenin, 1980):

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad (4)$$

P_t: True density (kg m^3), and P_b: Bulk density (kg m^3).

A universal material testing device (Lloyd Instrument LRX Plus, Lloyd Instruments Ltd, An AMATEK Company) was used to determine the rupture force, rupture energy, and deformation, which are the mechanical properties of the medlar. 100N load on the moving part of the device cell installed. The software program (NEXYGEN Plus) processed the obtained data. A force-deformation curve with a sudden decrease in the force value was obtained in the measurements. In the force-deformation curve obtained, the horizontal axis shows the deformation, and the vertical axis shows the force. The penetrating tip used in the trials has a diameter of 8 mm. The measurement values were obtained from the skin and skinless conditions of the medlar fruit. The fruit's skin was gently peeled off with a utility knife (Cevher and Öztekin, 2019).

2.5. Statistical Analysis

(Univariate) ANOVA test was performed to find the effect of short-term storage temperature on the mechanical properties of the 'Istanbul' medlar cultivar.

3. Results and Discussion

After harvesting the QA and BA29 rootstocks of the 'Istanbul' medlar cultivar used in the study, physical properties were determined to provide information about the product. The mean and standard deviation values of the physical characteristics of 'Istanbul' medlar cultivars are given in Table 1. Weight, length, width, thickness, geometric mean diameter, arithmetic values, mean diameter, sphericity, surface area, bulk density, true density, and porosity values are shown in the Table 1.

The largest and smallest values of the 'Istanbul' medlar on the QA rootstock were found to be 46.20 g – 30.23 g for weight, respectively; 49.95 mm – 42.82 mm for length; 44.33 mm – 38.61 mm for width; 45.03 mm - 38.32 for thickness; 45.51 mm-39.87 mm for geometric mean diameter; 45.55 mm – 39.92 mm for arithmetic mean diameter; 99.53% - 88.75% of sphericity; for surface area 6508.15 mm^2 – 4993.43 mm^2 ; bulk for density 390.40 kg m^3 – 380.60 kg m^3 ; true for density 1098.02 kg m^3 – 1046.94 kg m^3 ; It was determined as 64.72% - 63.29% for porosity.

The largest and smallest values of the 'Istanbul' medlar on the BA29 rootstock were acquired 38.41 g – 25.69 g for weight, respectively; 35.97 mm – 34.38 mm for length; 30.87 mm – 29.73 mm for width; for thickness 22.70 mm - 20.04 mm; for geometric mean diameter 28.95 mm - 27.55 mm; 29.41 mm – 28.29 mm for arithmetic mean diameter; for sphericity 83.96% - 78.51%; for surface area 2634.27 mm^2 – 2384.15 mm^2 ; bulk for density 397.20 kg m^3 – 393.6 0 kg m^3 ; true for density 1314.36 kg m^3 – 1101.56 kg m^3 ; 70.03% - 64.12% for porosity.

In addition, the study determined that the average physical properties values of the 'Istanbul' medlar on the QA rootstock were higher than the 'Istanbul' medlar on the BA29 rootstock.

Mechanical properties of medlar fruits of the 'Istanbul' cultivar on the QA and BA29 rootstocks were measured by considering the fruit peel and storage temperature conditions. The average values of the mechanical properties of the medlar fruit are given in Table 2. The statistical significance level of the effect of rootstocks and processes and interactions on the rupture force, rupture energy and deformation is shown in Table 2.

Significant factors and their interactions (for levels ≤0.01 and ≤0.05) are shown in bold in the table. According to the results of the analysis, the effects of rootstock (R), storage condition (S), processing (P), and rootstock x treatment (R x P) interaction on rupture force, rupture energy, and deformation were found to be statistically significant. In addition, the effect of storage condition (S) x process (S x P) interaction on shear force and rootstock x storage condition (R x S) interaction on deformation is statistically significant.

According to the measurement results of 'Istanbul' medlar on the BA29 rootstock stored in a 25°C warehouse environment and made with shell, the largest and smallest values for rupture force were obtained 16.38 N -14.88 N; 6.85 mm – 5.36 mm for deformation; it was observed between 0.062 J – 0.055 J for rupture energy. Under the same conditions, the highest and lowest values of 'Istanbul' medlar on the BA29 rootstock without skin were recorded 3.22 N-2.32 N for rupture force, respectively; 4.02 mm – 3.06 mm for deformation; it was measured as 0.016 J – 0.013 J for rupture energy.

The largest and smallest measurement values of the 'Istanbul' medlar on the QA rootstock, which is stored at 25°C with skin, were obtained 15.01 N - 13.55 N for rupture force, respectively; for 5.66 mm – 4.62 mm deformation; the rupture energy was found to be 0.059 J – 0.033 J. For the same condition, the largest and smallest values of the skinless fruits were recorded at 3.04 N -2.01 N for rupture force; 4.33 mm – 3.46 mm for deformation; it was found in the range of 0.022 J – 0.018 J for rupture energy.

As a result of the experiments, the rupture force value measured with the skin of the medlar fruit was determined as 21.32 N and the smallest 13.55 N. The skinless measurement values were the largest, 4.48 N, and the smallest, 2.02 N.

Table 1. Physical properties of the 'Istanbul' cultivar of medlar on the QA and BA29 rootstocks

Physical Properties	Rootstocks	
	QA	BA29
Weight (g)	38.21 ± 4.72	33.74 ± 4.61
Length (mm)	46.03 ± 2.42	34.96 ± 0.53
Width (mm)	41.56 ± 1.92	30.25 ± 0.16
Thickness (mm)	41.09 ± 2.21	21.51 ± 0.93
Geometric Mean Diameter (mm)	42.82 ± 1.74	28.33 ± 0.48
Arithmetic Mean Diameter (mm)	42.89 ± 1.75	28.91 ± 0.40
Sphericity (%)	93.12 ± 0.04	81.05 ± 0.02
Surface Area (mm^2)	5767.94 ± 466.90	2522.06 ± 84.49
Bulk Density (kg m^3)	383.68 ± 3.85	395.38 ± 1.32
True Density (kg m^3)	1068.88 ± 14.21	1171.26 ± 68.80
Porosity (%)	64.10 ± 0.04	66.14 ± 0.06

Table 2. Mechanical properties of the 'Istanbul' medlar cultivar on the QA and BA29 rootstocks

Rootstocks	Storage Conditions	Processing	Rupture Force (N)	Deformation (mm)	Rupture Energy (J)
BA29	25°C	With Skin	15.67 ±0.53	6.06 ±0.50	0.060 ±0.002
		Without Skin	2.73 ±0.33	3.51 ±0.38	0.014 ±0.001
	4°C	With Skin	17.80 ±2.45	6.37 ±1.85	0.068 ±0.006
		Without Skin	3.53 ±0.84	4.55 ±0.57	0.030 ±0.009
QA	25°C	With Skin	14.42 ±0.56	5.11 ±0.29	0.047 ±0.007
		Without Skin	2.48 ±0.40	3.95 ±0.35	0.020 ±0.001
	4°C	With Skin	15.84 ±0.82	5.48 ±0.45	0.052 ±0.004
		Without Skin	3.07 ±0.60	4.24 ±0.55	0.024 ±0.002
Main Effects					
BA29			9.93 ±7.05[a]	5.12 ±1.53[b]	0.047 ±0.007[b]
QA			8.95 ±6.31[b]	4.69 ±0.75[a]	0.036 ±0.15[a]
	25°C		8.83 ±6.33[b]	4.66 ±1.08 [a]	0.035 ±0.02 [a]
	4°C		10.06 ±7.01[a]	5.16 ±1.30 [b]	0.043 ±0.02 [b]
		With Skin	15.93 ±1.78[a]	5.76 ±1.08[b]	0.053 ±0.01[b]
		Without Skin	2.95 ±0.69[b]	4.06 ±0.60 [a]	0.021 ±0.01[a]
P-values					
Rootstock (R)			0.000	0.000	0.016
Storage condition (S)			0.000	0.000	0.040
Processing (P)			0.000	0.000	0.000
R x S			0.327	0.000	0.328
R x P			0.000	0.000	0.005
S x P			0.023	0.410	0.357
R x S x P			0.585	0.150	0.250

* the difference between the averages shown with different letters in the same column is statistically significant..

In the same case, the maximum deformation value was acquired at 9.44 mm, and the smallest was 3.28 mm. Skinless measurement values were the largest at 5.33 mm and the smallest at 3.06 mm.

A similar situation was the largest 0.06 J and the smallest 0.03 J in measurement values for rupture energy as skin. Skinless measurement results were determined as 0.04 J at the largest and 0.01 J at the smallest.

Regarding storage conditions, the rupture force value was measured at 25°C, the largest at 16.38 N and the smallest at 2.02 N. While at cold storage conditions (4°C), the maximum rupture force was recorded at 21.32 N and the minimum at 2.10 N. Rupture energy at 4°C was 0.08 J maximum and 0.02 J minimum. The deformation at 25°C was observed at the largest at 6.85 mm and the smallest at 3.06 mm when the storage condition was 4°C; the maximum deformation was measured as 9.44 mm and the smallest at 3.21 mm.

The study determined the mechanical properties of with and without skin medlar fruit. It was observed that the rupture force, rupture energy, and deformation values of medlar fruits on all the rootstocks stored at 25°C after harvest were lower than those stored at 4°C. This is because the texture of fruits is more softened at 25°C. Singh and Reddy (2006) investigated the physical-mechanical properties of orange fruit in ambient and refrigeration storage conditions. According to the results of the study, it was determined that the puncture force value of orange fruits under ambient conditions was lower than the fruits stored in the refrigerator. Similarly, it has been reported that the cutting energy values of fruits stored in ambient conditions are lower than those held in refrigeration conditions. This situation is compatible with the study.

This study investigated the effect of short-term storage temperature on the mechanical properties of 'Istanbul' medlar fruit grown in Türkiye. The obtained physical and mechanical properties data can contribute to information about the 'Istanbul' medlar fruit. The study determined parameters that can be used in the post-harvest design process of the 'Istanbul' medlar fruit and in the product processing industry. The information obtained may help maintain the product quality of the 'Istanbul' medlar fruit and prevent mechanical losses.

4. Conclusion

The effects of short-term storage temperature on the mechanical properties of the fruits of the 'Istanbul' medlar cultivar were investigated. The results obtained are summarized below.

1. Storage conditions had a statistically significant effect on the rupture force, rupture energy, and deformation values of QA and BA29 rootstocks of the 'Istanbul' medlar cultivar (P≤0.001).
2. The effect of being with or without skin on the mechanical properties of fruits is statistically significant (P≤0.001)
3. The effect of rootstock, storage condition, and processing interaction on mechanical properties is statistically significant (P≤0.001).

4. Average rupture force value of fruits of the 'Istanbul' medlar cultivar stored at 25 °C was determined as 8.82 N, deformation 4.66 mm, and rupture energy 0.035 J.
5. The average rupture force value of the fruits of the 'Istanbul' medlar cultivar stored at 4 °C was determined as 10.06 N, deformation 5.16 mm, and rupture energy 0.043 J

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.Y.C.	A.Ö.
C	50	50
D	100	
S		100
DCP	50	50
DAI	50	50
L	30	70
W	80	20
CR	20	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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DEVELOPMENTAL RESPONSE OF BERSEEM (*Trifolium alexandrinum* L.) TO BORON

Hüseyin BULUT[1]*

[1]*Ministry of Environment, Urbanization and Climate Change, General Directorate of Meteorology, 16120, Ankara, Türkiye*

Abstract: The aim of the study was to determine the effect of boron fertilization on agromorphological properties of berseem (*Trifolium alexandrinum* L.). The field experiment was arranged in randomized blocks with divided parcels. It was applied with 3 replications in 2017 and 2018 years in Ankara conditions. Three different berseem varieties (Derya, Erix, Mario) were sown to main parcels and 5 different boron fertilizer doses (0, 100, 200, 400, 800 g da^{-1}) were applied to sub-parcels. According to the research results; plant height was varied between 67.20 and 98.33 cm. Stem diameter was 3.43-4.45 mm, stem numbers were 6.17-11.67 pieces and head numbers were 7.28-15.02 pieces. The highest plant height and stem diameter were exhibited by variety of Mario. Erix displayed the highest development in terms of stem numbers, Derya showed the highest development in terms of head numbers. When boron doses were compared with control parcel, the highest plant height, stem diameter, stem numbers and head numbers were obtained at 100 g da^{-1} boron dose.

Keywords: Agromorphological properties, Berseem varieties, Boron doses

***Corresponding author:** Ministry of Environment, Urbanization and Climate Change, General Directorate of Meteorology, 16120, Ankara, Türkiye
E mail: huseyinbulut03@gmail.com (H. BULUT)
Hüseyin BULUT https://orcid.org/0000-0002-9862-9776

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1. Introduction

The role of the nutrients taken by the plant from the soil in the emergence of yield power is very important. Gezgin and Hamurcu (2006) stated that micronutrients perform important functions, although the amounts taken by plants are very small. To develop healthy plants and obtain a product with good quality, the boron element must be at a sufficient level in the soil. In plants, boron deficiency occurs most often in acidic sandy soils with low organic matter. Boron deficiency can even occur in plants despite of sufficiently high amounts of boron in soils or in fully-expanded leaves which is usually common under conditions where the humidity is high and the transpiration is low (Gunes et al., 2017). The lack of boron in the soil can be solved quickly with new fertilizer types developed today. The amount of boron that plants need to complete their development is very low. Soil boron analysis is important in determining whether plants need boron fertilization. The balance between nutrients in the soil is maintained in the fertilization applied by performing soil boron analysis. Correct fertilization contributes to the balanced nutrition of the plant and increases its resistance to diseases and pests. The amount of boron that causes deficiency symptoms and the amount of toxic effects are very close to each other (Adriano, 1986). There are big differences between field crops in terms of boron requirement. It is important to know the boron requirement of the plant before boron fertilization.

Forage crops agriculture in Türkiye consists of very few plant species which are alfalfa, sainfoin, and vetch and silage corn. Therefore, it is necessary to include new forage plants that may be an alternative considering the climate, soil conditions and agricultural systems of the regions. Also these forage crops are insufficient in terms of sustainable animal husbandry. For this reason, factors affecting yield in forage crop production are extremely important. Forage crops, especially trifoliums require more boron than many plants (Gupta, 2007). In these plants, if the boron amount in the leaves is below 20 mg kg^{-1}, boron fertilization is recommended (Gunes et al., 2017). Correct fertilization of these plants increases yield and quality. On the other hand, alternative species must be included in every period of forage crop production. The inclusion of one-year forage crops, which can be grown as short or second crops in the vegetation period, will contribute to the reduction of the roughage deficit. Berseem clover is one of the alternative species that can adapt to different regions. Berseem (*Trifolium alexandrinum* L.) is a one-year, legume forage plant that adapts well to semi-arid climatic conditions (Putievsky and Katznelson, 1970). The plant develops well in places where annual precipitation is more than 400 mm or where there is sufficient irrigation (Soya, 2009). Genckan (1983) reported that the first product of this forage plant is used for herb production and other products are used for grazing purposes in the vegetation period.

This study was carried out to determine the boron

requirement of berseem clover grown in regions with irrigation facilities in semi-arid climatic conditions. In the study, the yield changes in the agromorphological characteristics of the plant were evaluated by the amounts of boron given to berseem clover at different rates.

2. Materials and Methods

The field experiment was conducted at Ankara University, Faculty of Agriculture, Department of Field Crops parcels in 2017 and 2018. The elevation of the research area from the sea was 860m. Ankara has a semi-arid and less humid climate. The summers are hot and dry, the winters are cool and rainy. Vegetation period of the plant was between April and August. In vegetation period of 2017 and 2018, total precipitation was 161.7 mm and 174.1 mm, average temperature was 19.5 °C and 20.9 °C and average humidity was 49.7% and 48.1%. Precipitation was showed a regular distribution in 2017, but irregular in 2018 (Table 1).

Soil of the research area was clayey with moderately alkaline. It was insufficient in terms of total nitrogen and organic matter. Soil had enough phosphorus and potassium. The salt level of the soil was harmless. The amount of boron of the soil was measured as 1.13 mg kg^{-1} for 2017 and 0.96 mg kg^{-1} for 2018 (Table 2). The amount of available boron in the soil changes every year depending on the precipitation situation.

Three different varieties were used in the study. These were Derya, Erix and Mario. Derya was developed by Eastern Mediterranean Agricultural Research Institute. Erix and Mario were certified foreign varieties. 3 kg of seeds were sown per decare by hand to a depth of 1-1.5 cm. The field experiment was set up in a randomized complete block design with a split plot arrangement having 3 replications in 2017 and 2018 in Ankara conditions. Berseem varieties were sown to main parcels and 5 different boron fertilizer doses (D1: Control, D2:100, D3:200, D4:400, D5:800 g da^{-1}) were applied to sub-parcels. In the study, each parcel area had 4.5 m^2 in 5 rows and each row is 3 m in length. 20 kg da^{-1} of 18-46-0 (Diammonium Phosphate) was applied to all parcels. Etidot-67 (Disodium Octaborate Tetrahydrate) with 20% pure boron content was preferred for boron fertilization. Boron fertilizer was melted in water and applied to the soil. The parcels were irrigated 2 times and weed struggle took place during the growing period.

In the study, agromorphological properties which are plant height, stem diameter, stem numbers per plant and head numbers per plant were investigated. The study of Erac (1982) was used for the plant height and stem diameter calculations. While the plants were in the flowering phase, the length between the soil surface and the far end of the plant was measured with a ruler. In each parcel, 5 plants were randomly selected from the middle rows and the average of them was accepted as the average plant length of the parcel. Stem diameter was found by measuring the distance between the 2nd and 3rd knots of the main stem of 5 plants in the flowering phase. In the measurements, 0.1 mm split compass was used. The study of Guncan (1992) was used for the stem numbers and head numbers. The stems and heads of 5 plants randomly selected from the parcels that have reached harvest maturity were counted and calculated the average of these for the stem numbers and head numbers.

Table 1. Climate factors of 2017, 2018 and long years at Ankara location (Anonymous, 2019)

Climate Factors	Years	Months												
		Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Avg.
Average Temperature (°C)	2017	-1.3	2.9	8.1	11.0	15.8	20.4	25.6	24.7	22.6	12.6	7.1	4.7	12.9
	2018	3.1	6.5	10.0	15.3	18.0	21.4	24.5	25.1	20.1	14.9	9.0	3.3	14.3
	Long Years	0.1	1.7	5.7	11.2	16.0	19.9	23.4	23.3	18.8	13.1	7.2	2.4	11.9
Average Humidity (%)	2017	76.8	66.7	60.2	50.6	55.9	58.0	38.1	45.8	34.2	56.3	69.7	78.3	57.6
	2018	77.0	73.1	63.3	44.9	59.6	53.4	45.4	37.4	45.3	58.9	64.4	81.4	58.6
	Long Years	77.3	73.0	64.6	58.8	57.3	52.0	44.5	43.6	48.2	58.9	70.0	77.6	60.5
														Tot.
Total Precipitation (mm)	2017	36.3	8.7	49.4	22.8	53.8	56.3	11.6	17.2	4.4	31.5	41.3	46.3	379.6
	2018	54.8	37.3	84.6	3.8	102.7	45.0	11.0	11.6	4.3	55.1	25.1	70.5	505.8
	Long Years	40.4	35.2	39.2	43.3	51.8	34.7	14.1	12.5	18.8	28.0	31.7	44.3	394.0

Table 2. Soil factors of 2017 and 2018 of the research area (Anonymous, 2018)

	Year	pH	Salt (%)	Total N (%)	Organic Matter (%)	P_2O_5 ($kgda^{-1}$)	K_2O ($kgda^{-1}$)	B ($mgkg^{-1}$)
Soil Factors (0-30cm)	2017	8.08	0.04	0.09	1.06	7.96	119.99	1.13
	2018	7.88	0.03	0.07	0.91	6.64	96.02	0.96

The statistical analysis of variance for all the measured properties was performed by using the Mstat-C package program. Duncan multiple comparison test was applied to compare the means.

3. Results

3.1. The Analysis of Variance

The analysis of variance for three berseem varieties and five boron doses in 2017, 2018 and both years was given in Table 3. In terms of plant height, variety was significant at 5% and 1% level in 2017 and 2018. Boron dose was significant at 1% level in both years. The means of 2017 and 2018 were grouped because of year factor was insignificant. When stem diameter was examined, variety was significant at 5% level in 2018. The boron dose was significant at 1% level in both years. The means of 2017 and 2018 were grouped separately because of year factor was significant at 5% level.

In terms of stem numbers per plant, variety was significant at 5% and 1% level in 2017 and 2018. The boron dose was significant at 1% level in both years. The means of 2017 and 2018 were grouped together because of year factor was insignificant. When head numbers per plant was examined, variety was significant at 5% level in 2017. The boron dose was significant at 1% level in both years. The means of 2017 and 2018 were grouped because of year factor was insignificant (Table 3).

3.2. Plant Height

Plant height means for 2017, 2018 and both years were given separately in Table 4. Erix had 89.24 cm as two-year means. The lowest plant height means were obtained from Derya with 74.85 cm. The highest plant height mean of two years was observed in Mario with 92.44 cm.

Plant height means for different boron doses were ranged between 80.78 and 91.58 cm according to the average of the two years. Compared to the control parcels, the highest plant height mean was reached at 91.58 cm at 100 g da^{-1} boron dose (Table 4).

3.3. Stem Diameter

Stem diameter means for 2017, 2018 and both years were given separately in Table 5. Erix had 4.05 mm as two-year means. The lowest stem diameter means were obtained from Derya with 3.74 mm. The highest stem diameter mean of two years was observed in Mario with 4.06 mm.

Stem diameter means for different boron doses were ranged between 3.78-4.26 mm according to the average of the two years. Compared to the control parcels, the highest stem diameter mean was reached with 4.26 mm at 100 g da^{-1} boron dose (Table 5).

Table 3. Analysis of variance for varieties and boron doses in 2017, 2018 and both years

Source of Variation	Plant Height	Stem Diameter	Stem Numbers/Plant	Head Numbers/Plant
		2017		
Variety	1533.91*	0.35	37.31*	82.86*
Error	125.80	0.09	2.22	6.87
Dose	165.86**	0.51**	4.56**	12.77**
Variety x Dose	6.16	0.02	0.07	0.40
Error	5.59	0.02	0.34	0.28
		2018		
Variety	1119.36**	0.73*	50.44**	6.40
Error	5.85	0.07	0.73	2.76
Dose	145.66**	0.45**	4.32**	7.08**
Variety x Dose	2.76	0.01	0.19	0.13
Error	1.72	0.01	0.60	0.11
		2017 and 2018		
Year	64.52	0.11*	3.44	1.07
Replicate	100.91	0.59	13.73	21.21
Error	96.5	0.01	0.69	8.09
Variety	2634.67**	1.04**	82.56**	67.63**
Year x Variety	18.60	0.04	5.19	21.64
Error	65.82	0.08	1.47	4.81
Dose	304.63**	0.95**	8.71**	19.23**
Year x Dose	6.89	0.01	0.16	0.62
Variety x Dose	7.39	0.01	0.16	0.34
YearxVarietyxDose	1.52	0.01	0.10	0.19
Error	3.65	0.01	0.20	0.20

*= 0.05, **= 0.01 shows significant probability level.

Table 4. Plant height means of different varieties and boron doses in 2017, 2018 and both years (cm)

2017					
Dose	Derya	Erix	Mario	Means	
D1	73.60±5.74	86.60±6.71	91.28±6.79	83.82±6.55	
D2	81.00±6.32	94.87±7.41	97.28±7.57	91.04±6.76	
D3	74.93±5.77	91.33±6.79	93.00±7.26	86.42±6.68	
D4	68.80±5.31	86.80±6.70	90.80±7.09	82.13±6.43	
D5	67.20±5.29	85.33±6.69	87.13±6.77	79.89±6.29	
Means	73.11±5.69	88.99±6.73	91.89±6.83		
2018					
Dose	Derya	Erix	Mario	Means	
D1	74.27±5.96	87.27±6.77	90.53±6.68	84.02±6.59	
D2	83.53±6.51	94.47±7.96	98.33±7.83	92.11±7.11	
D3	79.40±6.26	91.08±7.14	94.40±7.87	88.29±6.63	
D4	75.07±5.79	88.87±6.70	93.13±7.35	85.69±6.79	
D5	70.67±5.38	85.80±6.73	88.53±6.62	81.67±6.42	
Means	76.59±5.93	89.49±6.99	92.99±7.25		
2017-2018 Variety Means		Derya	Erix	Mario	
		74.85±6.69^{c}	89.24±6.96^{b}	92.44±7.13^{a}	
2017-2018 Dose Means		D1	D2	D3	D4	D5
		83.92±6.58^{c}	91.58±7.34^{a}	87.36±6.85^{b}	83.91±6.57^{c}	80.78±6.29^{d}

* Means with the same letter are not significantly different at 5% probability level.

Table 5. Stem diameter means of different varieties and boron doses in 2017, 2018 and both years (mm)

2017					
Dose	Derya	Erix	Mario	Means	
D1	3.71±0.25	3.81±0.26	3.90±0.27	3.81±0.26^{cd}	
D2	4.04±0.28	4.45±0.31	4.37±0.31	4.29±0.30^{a}	
D3	3.97±0.27	4.23±0.30	4.19±0.29	4.13±0.29^{ab}	
D4	3.84±0.26	4.10±0.29	3.99±0.27	3.98±0.27^{bc}	
D5	3.47±0.23	3.78±0.26	3.86±0.26	3.70±0.26^{d}	
Means	3.81±0.26	4.07±0.29	4.06±0.28		
2018					
Dose	Derya	Erix	Mario	Means	
D1	3.52±0.24	3.82±0.26	3.90±0.27	3.75±0.26^{c}	
D2	3.93±0.27	4.35±0.30	4.43±0.31	4.24±0.30^{a}	
D3	3.77±0.26	4.10±0.29	4.14±0.29	4.01±0.28^{b}	
D4	3.66±0.25	4.06±0.29	4.04±0.28	3.92±0.27^{b}	
D5	3.43±0.24	3.78±0.26	3.78±0.26	3.66±0.25^{c}	
Means	3.66±0.25^{b}	4.02±0.28^{a}	4.06±0.29^{a}		
2017-2018 Variety Means		Derya	Erix	Mario	
		3.74±0.26	4.05±0.28	4.06±0.28	
2017-2018 Dose Means		D1	D2	D3	D4	D5
		3.78±0.26	4.26±0.29	4.07±0.28	3.95±0.27	3.78±0.26

* Means with the same letter are not significantly different at 5% probability level.

3.4. Number of Stem

Stem means for 2017, 2018 and both years were given separately in Table 6. Mario had 9.66 pieces as two-year means. The lowest stem means were obtained from Derya with 6.96 pieces in both years. The highest stem mean of two years was observed in Mario and Erix with 9.66 and 9.98 pieces. Stem means for different boron doses were ranged between 8.06-9.89 pieces according to the average of the two years. Compared to the control parcels, the highest stem mean was reached with 9.89 pieces at 100 g da^{-1} boron dose (Table 6).

3.5. Number of Head

Head means for 2017, 2018 and both years were given separately in Table 7. Erix had 12.08 pieces as two-year means. The lowest head means were obtained from Mario with 9.94 pieces in both years. The highest head mean of two years was observed in Derya with 12.89 pieces. Head means for different boron doses were ranged between 10.43-13.04 pieces according to the average of the two years. Compared to the control parcels, the highest head mean was reached with 13.04 pieces at 100 g da^{-1} boron dose (Table 7).

Table 6. Stem numbers/plant means of different varieties and boron doses in 2017, 2018 and both years (piece)

2017					
Dose	Derya	Erix	Mario	Means	
D1	6.93±0.90	9.85±1.27	8.87±1.15	8.55±1.11	
D2	7.95±1.03	11.13±1.43	10.06±1.31	9.71±1.26	
D3	7.41±0.96	10.27±1.32	9.17±1.18	8.95±1.16	
D4	6.33±0.82	9.84±1.27	8.72±1.13	8.30±1.08	
D5	6.17±0.80	9.21±1.19	8.06±1.05	7.81±1.01	
Means	6.96±0.91	10.06±1.31	8.98±1.16		
2018					
Dose	Derya	Erix	Mario	Means	
D1	6.67±0.86	9.62±1.25	9.66±1.26	8.65±1.11	
D2	7.79±1.01	10.73±1.39	11.67±1.51	10.06±1.31	
D3	7.26±0.97	10.13±1.31	10.83±1.42	9.41±1.22	
D4	6.74±0.89	9.70±1.27	10.35±1.36	8.93±1.16	
D5	6.36±0.82	9.33±1.21	9.20±1.18	8.30±1.08	
Means	6.96±0.89	9.90±1.28	10.34±1.35		
2017-2018 Variety Means		Derya	Erix	Mario	
		6.96±0.89[b]	9.98±1.27[a]	9.66±1.25[a]	
2017-2018 Dose Means		D1	D2	D3	D4	D5
		8.60±1.12[c]	9.89±1.29[a]	9.18±1.18[b]	8.62±1.12[c]	8.06±1.05[d]

* Means with the same letter are not significantly different at 5% probability level.

Table 7. Head numbers/plant means of different varieties and boron doses in 2017, 2018 and both years (piece)

2017					
Dose	Derya	Erix	Mario	Means	
D1	12.73±3.68	11.79±3.39	8.27±2.38	10.93±3.16	
D2	15.02±4.35	13.61±3.94	11.07±3.21	13.23±3.83	
D3	14.02±4.06	12.72±3.68	9.73±2.81	12.16±3.54	
D4	13.24±3.83	12.16±3.51	8.04±2.34	11.15±3.25	
D5	12.46±3.62	11.11±3.22	7.28±2.12	10.28±2.98	
Means	13.49±3.91	12.28±3.57	8.88±2.55		
2018					
Dose	Derya	Erix	Mario	Means	
D1	11.87±3.42	11.15±3.22	10.93±3.16	11.32±3.28	
D2	13.38±3.88	13.05±3.77	12.13±3.51	12.85±3.71	
D3	12.81±3.71	12.62±3.65	11.47±3.34	12.30±3.57	
D4	12.10±3.51	11.84±3.42	10.69±3.10	11.54±3.34	
D5	11.27±3.68	10.69±3.10	9.78±2.84	10.58±3.07	
Means	12.29±3.27	11.87±3.42	11.00±3.19		
2017-2018 Variety Means		Derya	Erix	Mario	
		12.89±3.74[a]	12.08±3.51[b]	9.94±2.87[c]	
2017-2018 Dose Means		D1	D2	D3	D4	D5
		11.13±3.22[c]	13.04±3.77[a]	12.23±3.54[b]	11.35±3.31[c]	10.43±3.02[d]

* Means with the same letter are not significantly different at 5% probability level.

4. Discussion

4.1. Plant Height

Plant height means of the varieties were varied between 74.85-92.44 cm in 2017 and 2018 (Table 4). Celen (1998) reported that plant height varied between 64.23 and 80.42 cm in a study on seed yield characteristics of some berseem clover varieties under Izmir conditions. The means obtained from varieties in the study were higher than the reported values.

4.2. Stem Diameter

Stem diameter means of the varieties were varied between 3.74-4.06 mm in 2017 and 2018 (Table 5). Demirok (1993) reported that stem diameter varied between 4.09 and 4.47 mm in a study on weed yield characteristics of some berseem clover varieties under Ankara conditions. The means obtained from varieties in the study were consistent with reported values.

4.3. Number of Stem

Stem means of the varieties were varied between 6.96-10.34 pieces in 2017 and 2018 (Table 6). Celen (1998) reported that number of stem varied between 2.50 and 4.30 pieces in a study on some seed yield and yield

characteristics of some berseem clover varieties under Izmir conditions. The means obtained from varieties in the study were higher than the reported values.

4.4. Number of Head

Head means of the varieties were varied between 9.94-12.89 pieces in 2017 and 2018 (Table 7). Guncan (1992) reported that number of head varied between 5.90 and 12.00 pieces in a study on seed yield characteristics of some berseem clover varieties under Ankara conditions. The means obtained from varieties in the study were consistent with reported values.

5. Conclusion

Considering the different boron doses applied in berseem clover varieties; Mario has reached the highest plant height and stem diameter means. The highest development in terms of stem numbers per plant was observed in Erix. Derya was showed the highest development in terms of head numbers per plant. When boron doses were compared with control parcels, the highest plant height, stem diameter, stem numbers and head numbers were obtained at 100 g da^{-1} boron dose. It was determined as the best boron dose for high yield and quality**.**

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.B.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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EFFECTS OF SELENIUM BIOFORTIFICATION ON PHYTOCHEMICAL CHARACTERISTICS OF SOME TABLE GRAPE CULTIVARS

Seda SUCU[1]*, Neval TOPCU ALTINCI[1], Adem YAĞCI[1], Duran KILIÇ[2], Sezer ŞAHİN[3], Rüstem CANGİ[1]

[1]*Tokat Gaziosmanpasa University, Agricultural Faculty, Department of Horticulture, 60250, Tokat, Türkiye*
[2]*Republic of Türkiye Ministry of Agriculture and Forestry, Middle Black Sea Transitional Zone Agricultural Research İnstitute, 60200, Tokat, Türkiye*
[3]*Tokat Gaziosmanpasa University, Agricultural Faculty, Department of Soil Science, 60250, Tokat, Türkiye*

Abstract: Table grapes, rich in vitamins and minerals, play an important role in human nutrition, thus largely used in daily diets. Selenium (Se) with positive impacts on human health and anticancerogenic effects, has recently become prominent in human nutrition and animal feeding. In this study, selenium fortifications were made at different doses (control, 4 ppm and 8 ppm) to 9 different table grape cultivars (Alphonse Lavallée, Bilecik İrikarası, Cardinal, Sultani Seedless, Tekirdağ Seedless, Italia, Lival, Victoria, Royal) and total phenolics, anthocyanins and flavonoids of the cultivars were determined. While total phenolics of the whole berry was presented, skin and pulp total anthocyanins and total flavonoids were presented separately. The greatest total phenolic amount was obtained from 4 ppm selenium treatment in Bilecik İrikara (157.31 mg/g) cultivar. The greatest total anthocyanin contents were obtained from the skin of with 8 ppm selenium treatment in Alphonse Lavallée (11.22 mg/g). Selenium treatments increased total flavonoids of Bilecik İrikarası, Lival, Royal and Sultani Seedless cultivars. It was concluded based on findings that Se treatments influenced phytochemical characteristics of the table grapes.

Keywords: Selenium, Total phenolic, Total anthocyanin, Italia, Alphonse Lavallée

***Corresponding author:** Tokat Gaziosmanpasa University, Agricultural Faculty, Department of Horticulture, 60250, Tokat, Türkiye
E mail: seda.sucu@gop.edu.tr (S. SUCU)

Seda SUCU	https://orcid.org/0000-0002-5187-5048
Neval TOPCU ALTINCI	https://orcid.org/0000-0002-4734-7832
Adem YAĞCI	https://orcid.org/0000-0002-3650-4679
Duran KILIÇ	https://orcid.org/0000-0002-8851-5214
Sezer ŞAHİN	https://orcid.org/0000-0002-0520-3945
Rüstem CANGİ	https://orcid.org/0000-0002-8264-9844

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1. Introduction

Table grapes with a high allure, a wide range of nutrients and pharmacological characteristics are mostly available for fresh consumptions (Yadav et al., 2009). Compared to several other fruit species, grapes are quite rich in phenolic compounds (Xia et al., 2010).

Grape quality largely depends on vineyard management, cultivar and harvest time (Rizzuti et al., 2015). Phenolic compounds, responsible for color, taste and aroma of grapes, are the most important quality components and have supplementary effects on human nutrition and health (Kunter et al., 2013). Until recently, phenolic substances of wine grapes have been analyzed, but such compounds are also important quality traits in table grapes, especially in colored cultivars. Table grapes were reported as an important source of phenolics (catechin, flavonols, phenolic acids, anthocyanins) (Rolle et al., 2010). Following sugars and organic acids, phenolic compounds constitute the third greatest compound group in grapes. Existence and relative ratios of certain phenolic substances in a grape berry are genetically controlled species and cultivar characteristics. However, the quantity of these substances is mainly dependent on climate and soil conditions, maturity stage and cultural practices (Ribéreau-Gayon et al., 2000). In terms of total phenolics, black grape cultivars were reported to be richer than white cultivars (Yang and Xiao, 2013). Anthocyanins constitute the largest sub-group of phenolic substances. Anthocyanins exist in berry skin and are defined as natural pigments giving specific red, blue and purple tones of the grapes (Ho et al., 2001). Anthocyanins begin to form at veraison stage, accumulate in berry skin throughout the maturity and reach maximum levels at the end of maturity.

Potential market value of different grape cultivars has gradually been discovered. Exploring grape quality and special medicinal effects will play significant theoretical and practical roles in the future of table grape cultivation (Zhu et al., 2017).

Selenium is a trace element. In human nutrition,

selenium (Se) reduces the risk of cancer (Kiskova et al., 2014), scavenges free radicals (Bors and Saran, 1987), exhibits resistance against membrane lipid peroxidation and slows down the aging process (Rice-Evans, 2001) and boosts the immune system (Keskinen et al., 2009). Plants are the primary source of selenium. However, fundamental of Se is still ambiguous. Plants play a vital role in Se deficiency and toxicity. Therefore, there is always a need for detailed studies about selenium mechanism (Gupta and Gupta, 2017). Previous studies on selenium biofortification revealed that selenium treatments influenced phytochemical contents of the plants differently. It was reported that total phenolics of broccoli increased with selenium treatments (Bachiega et al., 2016), total phenolics of onion and tomato decreased with increasing selenium doses (Pöldma et al., 2013; Schiavan et al., 2013), total phenolics of apples generally decreased with selenium treatments (Groth et al., 2020).

In this study, effects of different doses of selenium biofortifications on phytochemicals contents of 9 different table grape cultivars were investigated.

2. Materials and Methods

2.1. Experimental Design

Experiment was conducted in 2017 in adaptation vineyard of Middle Black Sea Transitional Zone Agricultural Research Institute in Tokat province of Türkiye. Experimental grapevines are 7 years old and the plot was established at 3.00 x 1.75 m (row spacing x on-row vine spacing) spacing. The trunks are 70 cm high and double-arm ed training system was used in the vineyard. The cultivars used in the present experiment included Alphonse Lavallée, Italia, Lival, Victoria, Royal, Bilecik İrikarası, Cardinal, Prima, Trakya İlkeren, Flame Seedless, Sultani Seedless, Tekirdağ Seedless. All the cultivars were grafted on 1103 Paulsen rootstocks. Soil samples were taken from 0-30 cm soil profile to determine physical and chemical properties of vineyard soils. The characteristics of the vineyard siol are as follows: Sand ratio was 54.02%, clay ratio was 31.58%, silt ratio was 14.39%, salt content was 0.02%, organic matter content was 1.18%, degree of saturation was 56%, soil texture was CL, EC was 0.57, pH was 7.78, P ratio was 5.72 and K ratio was 102.4. Selenium content of experimental soil was around 1.15 μg kg^{-1}.

2.2. Measurement Methods of Grape Samples

Pruning, chemical treatments, irrigation, soil tillage, cluster thinning, removal and cluster tipping were practiced in accordance with the relevant standards (Anonymous, 1992; Ateş and Kısmalı, 2007). Fertilization was practiced to have 12 kg N/da, 8 kg P2O5/da and 8 kg K2O/da at two different periods. Besides, sufficient quantity of micro elements was applied half to soil and half to leaves. Selenium fertilization was practiced 3 times to spray the entire canopy in 10-20-30 day intervals from the berry set period according to Zhu et al. (2017). Treatment doses were selected as 0 (control), 4, 8 mg kg^{-1} of Selenium (Sodium Selenate fertilizer). Cluster samples were taken at harvest maturity. Samples were brought to laboratory and cold-stored until total phenolics, total anthocyanin and total flavonoid analyses.

Total Phenolics contents; About 5 g sample was taken from each replicate of each cultivar. Berry samples were divided into small pieces using a bistoury. Samples were extracted with 50% ethanol, completely homogenized, filtered through Whatman filter papers and final volume was completed to 25 ml with 50% ethanol. Samples were kept in a fridge until the time of analysis. Total phenolics of the samples was determined according to Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Spectrophotometer readings were performed at 765 nm wavelength. Results were expressed as gallic acid equivalent mg/g with the use of a standard curve prepared by using standard as gallic acid solutions (Harmankaya, 2003).

Total Anthocyanin and Flavonoids Contents; Samples prepared according to Bino et al. (2005) and analysis was conducted in accordance with Di Stefano and Cravero (1991). Results were expressed in mg/g. For 10 berry skins of present cultivars (about 10 g), 40 ml solution was prepared (Skin:solution=1:4), berry skins were placed into the solution, kept at 30 °C for 72 hours, then preserved at -20 °C until the time of analysis. Before spectrophotometer reading, samples were diluted with hydrochloric ethanol at 1/10 ratio and readings were performed at 280 nm and 520 nm wavelengths.

2.3. Statistical Analysis

Experiment was conducted in randomized blocks – split plots experimental design with 3 replications and 3 grapevines in each replicate. A total of 243 grapevines (9 cultivars x 3 doses x 3 replicates x 3 grapevines in each replicate) were used in the experiment. Experimental data were subjected to analysis of variance and means were compared with the LSD (0.05) test.

3. Results and Discussion

Effects of 0, 4 and 8 ppm selenium (Se) biofortifications on total phenolics, skin-pulp anthocyanins and skin-pulp flavonoids of 9 different grape cultivars were investigated in this study. Selenium treatments influenced total phenolics of the cultivars (Table 1) (Figure 1). The greatest total phenolics was obtained from 4 ppm Se treatment of Lival cultivar (214.67 mg/g) and the lowest from 4 ppm Se treatment of Victoria cultivar (68.01 mg/g). In Tekirdağ Seedless cultivar, increasing total phenolics were observed with increasing treatment doses, the total phenolics of 80.29 mg/g in control treatment increase to 82.03 mg/g at 4 ppm Se treatment and 108.90 mg/g at 8 ppm Se treatment. In Bilecik İrikarası cultivar, Se treatments positively influenced total phenolics, the value of 98.81 mg/g in control treatment increased to 157.31 mg/g at 4 mg/g Se and to 134.81 mg/g 8 ppm Se treatments. In Royal cultivars, decreasing total phenolics were observed with increasing treatment doses, the total phenolics of 170.61 mg/g in control treatment decreased to 157.05 mg/g 4

mg/g Se and to 91.99 mg/g at 8 ppm treatments. In Victoria cultivar, selenium biofortifications reduced total phenolics. Effects of selenium treatments on total phenolics of Alphonse Lavaellee, Cardinal, Lival and Sultani Seedless cultivars were not found to be significant. Skin anthocyanin contents of the cultivars are shown in Table 2. The greatest value was observed in control treatment of Lival cultivar (12.20 mg/g) and the lowest in 4 ppm Se treatment of Tekirdağ Seedless cultivar (2.05 mg/g). In Alphonse Lavallée, Bilecik İrikarası and Cardinal cultivars, skin anthocyanin contents increased with increasing treatment doses and the values respectively reached 160.07, 134.81 and 213.17 mg/g 8 mg/g Se treatment. In Lival and Tekirdağ Seedless cultivars, selenium treatments decreased skin anthocyanins. In Alphonse Lavallée, Bilecik İrikarası, Royal and Tekirdağ Seedless cultivars, selenium biofortification reduced pulp anthocyanin contents.

Table 1. Effects of selenium treatments of total phenolics (mg/g) of the cultivars

Selenium Treatments	Cultivar								
	Alphonse Lavallé	Bilecik İrikarası	Cardinal	Lival	Royal	Tekirdağ Seedless	Victoria	Italia	Sultani Seedless
CONTROL	156.99	98.81 ^{b}	211.88	202.24	170.61 ^{a}	80.29 ^{b}	111.86 ^{a}	75.34 ^{b}	105.75
4 PPM	144.64	157.31 ^{a}	199.41	214.67	157.05 ^{a}	82.03 ^{b}	68.01 ^{b}	93.34 ^{a}	101.64
8 PPM	160.07	134.81 ^{ab}	213.17	205.20	91.99 ^{b}	108.90 ^{a}	101.06 ^{a}	70.01 ^{c}	129.60
LSD (0.05)	N.S.	35.93	N.S.	N.S.	39.64	4.74	12.77	2.85	N.S.

NS= non-significant.

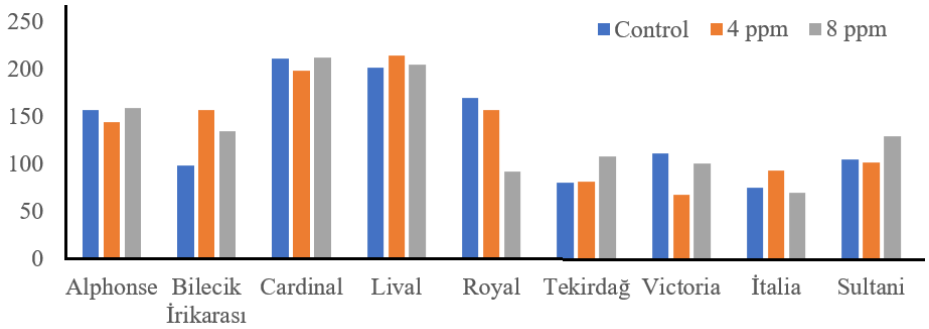

Figure 1. Total phenolics of the cultivars at different selenium doses.

Table 2. Effects of selenium treatments on skin-pulp anthocyanin contents of the cultivars

	Selenium treatments	Cultivars								
		Alphonse Lavallée	Bilecik İrikarası	Cardinal	Lival	Royal	Tekirdağ Seedless	Victoria	Italia	Sultani Seedless
Skin (mg/g)	Control	6,71 ^{b}	6,22 ^{b}	3,18 ^{c}	12,20 ^{a}	8,06	5,98 ^{a}	-	-	-
	4 ppm	6,71 ^{b}	6,76 ^{b}	4,42 ^{b}	11,38 ^{b}	8,38	2,05 ^{b}	-	-	-
	8 ppm	11,22 ^{a}	8,17 ^{a}	7,22 ^{a}	7,71 ^{c}	6,15	2,14 ^{b}	-	-	-
	LSD (0.05)	3,66	1,41	1,14	0,72	N.S.	2,38	-	-	-
Pulp (mg/g)	Control	2,50 ^{a}	1,39	1,19	1,14	1,11 ^{a}	2,83 ^{a}	-	-	-
	4 ppm	1,50 ^{b}	1,17	3,30	2,23	0,77 ^{b}	0,55 ^{b}	-	-	-
	8 ppm	2,43 ^{a}	0,93	2,92	1,21	0,70 ^{b}	0,90 ^{b}	-	-	-
	LSD (0.05)	0,72	N.S.	N.S.	N.S.	0,17	1,52	-	-	-

The greatest skin flavonoid content (Table 3) was obtained from 4 ppm treatment of Royal cultivar (177.41 mg/g) and the lowest from the control treatment of Sultani Seedless cultivar (87.60 mg/g). Compared to the control treatments, the greatest skin flavonoid contents of the cultivars were observed in 4 ppm Se treatment of Alphonse Lavallee cultivar (139.00 mg/g), 4 ppm Se treatment of Bilecik İrikarası cultivar (141.31 ppm), 8 ppm Se treatment of Cardinal cultivar (140.59 mg/g), 8 ppm Se treatment of Lival cultivar (131.50 mg/g), 4 ppm treatment of Royal cultivar (177.41 mg/g) and 4 ppm Se treatment of Sultani Seedless cultivar (96.25 mg/g). In Alphonse Lavallée cultivar, 8 ppm Se treatments reduced skin flavonoid content (120.50 mg/g). The greatest pulp flavonoid content was observed in the control treatment of Sultani Seedless cultivar (39.15 mg/g) and the lowest in 4 ppm Se treatment of Alphonse Lavallée cultivar (7.94 mg/g). In Bilecik İrikarası cultivar, pulp flavonoid contents increased with increasing treatment doses and the pulp flavonoid content of 17.54 mg/g in control treatment increased to 19.37 mg /g at 4 ppm Se treatment and 21.36 mg/g at 8 ppm Se treatment. In Sultani Seedless cultivar, pulp flavonoid contents decreased with increasing treatment doses and the pulp flavonoid content of 39.25 mg/g in control treatment decreased to 31.95 mg/g at 4 ppm Se treatment and 21.44 mg/g at 8 ppm treatment.

Table 3. Effects of selenium treatments on skin-pulp flavonoid contents of the cultivars

	Selenium treatments	Cultivars								
		Alphonse Lavallée	Bilecik İrikarası	Cardinal	Lival	Royal	Tekirdağ Seedless	Victoria	Italia	Sultani Seedless
Skin (mg/g)	Control	133,00 [a]	112,90 [b]	117,50 [b]	118,00 c	124,00 [c]	119,15	98,85	106,53	87,60 [b]
	4 ppm	139,00 [a]	141,31 [a]	108,06 [c]	150,31 a	177,41 [a]	107,63	97,70	103,66	96,25 [a]
	8 ppm	120,50 [b]	128,54 [ab]	140,59 [a]	131,50 b	141,35 [b]	118,36	99,70	103,81	95,75 [a]
	LSD (0.05)	10,80	24,54	7,17	8,06	12,80	N.S.	N.S.	N.S.	3,24
Pulp (mg/g)	Control	12,41 [a]	17,54	17,58	21,32	21,78	18,26	13,92	12,77 [b]	39,25 [a]
	4 ppm	7,94 [b]	19,37	23,71	19,93	16,72	18,03	16,99	16,66 [a]	31,95 [b]
	8 ppm	9,68 [ab]	21,36	14,58	23,65	17,87	17,73	12,60	11,23 [b]	21,44 [c]
	LSD (0.05)	2,94	0,00	N.S.	N.S.	N.S.	N.S.	N.S.	3,35	3,99

Present findings about the effects of selenium biofortification on total phenolics of different table grape cultivars comply with the findings of previous studies conducted with other plants (onion, broccoli) (Pöldma et al., 2013; Bachiega et al., 2016). Effects of selenium biofortification in selenate and selenite forms on total phenolics of apples were not found to be significant (Groth et al., 2020). Present findings obtained from Alphonse Lavellee, Cardinal, Lival and Sultani Seedless cultivars comply with those earlier findings.

Health benefits of foodstuffs have recently become prominent issues in Türkiye and the world. Plants offer such benefits through several secondary metabolites (total phenols, antioxidants, anthocyanins, etc.) they contain. Selenium is an important source of antioxidants. It was reported that selenium reduced risk of cancer, cardiovascular diseases, scavenged free radicals, improved resistance against lipid peroxidation, boosted immune system and slowed down the aging process (Bors and Saran, 1987; Ip et al., 1992; Rice – Evens, 2001; Whanger, 2004; Flores-Mateo et al., 2006; Keskinen et al., 2009; Perez-Corona et al., 2011; Kiskova et al., 2014). Such health benefits of selenium come from being a component of peroxidase and iodothyronine deiodinase enzymes (WHO, 2003; Gül, 2000). Selenium is at the forefront of defense mechanism and such a case is practiced through fighting against hydrogen peroxides with oxidative damage on cells and protecting cell membrane (Djanaguiraman et al., 2005; Gong et al., 2005; Germ et al., 2007; Yao et al., 2009; Cartes et al., 2010).

In the present study, total phenolics, total anthocyanins and total flavonoids of 9 different grape cultivars were investigated. While total phenolics of the whole berry was presented, skin and pulp total anthocyanins and total flavonoids were presented separately. With selenium treatments, total phenolics increased in Italia, Tekirdağ Seedless, Lival and Bilecik İrikarası cultivars. Skin total anthocyanins were high in Alphonse Lavalle, Bilecik İrikarası and Cardinal cultivars. This was an expected case since anthocyanins are metabolites mostly encountered in colored species and present cultivars were not included in cultivars with colored flesh. Selenium treatments increased skin flavonoids in Bilecik İrikarası, Lival, Royal and Sultani Seedless cultivars. Such an increase is quite significant since vitamin E is activated only with the existence of trace quantity of selenium with a great contribution to antioxidant mechanism (Cakmak and Marschner, 1988; Uluozlu, 2005; Kabirov et al., 2008). Zhu et al. (2017) reported that selenium treatments did not change resveratrol compound of the grapes, but increased procyanidin contents. Flavonoids are among the key stones of procyanidin, thus present findings comply with the results of that study. Assuncao et al. (2018) reported increasing antioxidant enzyme activity of wine yeasts with selenium treatments. In another study with selenium treatments to wine yeasts, increases were reported in SOD, CAT and GPX enzyme activities and as a negative case, dose-dependent increases were also observed in lipid peroxidation (Talbi et al., 2018). Although total phenolics, total anthocyanins and total flavonoids were not compared one-on-one, these compound represent one another since all of them are oxidation-preventing compounds.

4. Conclusion

In present study, effects of selenium biofortification on total phenolics, total anthocyanins and total flavonoids of 9 different grape cultivars were investigated. Selenium treatment doses differently influenced total phenolics, skin-pulp total anthocyanins and flavonoids of the cultivars. Differences in findings may have resulted from treatments doses or selenium treatments forms or cultivar-specific characteristics.

In total phenolics analyses, Victoria, Royal (control treatment), Bilecik İrikarası, Italia (4 ppm selenium treatment), Tekirdağ Seedless (8 ppm selenium treatments) cultivars were found to be prominent. In total anthocyanin analyses, it was observed that selenium treatments increased total skin anthocyanins of Tekirdağ Seedless and Lival (control), Alphonse Lavallé, Bilecik İrikarası and Cardinal (8 ppm selenium treatment) cultivars.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	S.S.	N.T.A.	A.Y.	D.K.	S.Ş.	R.C.
C	30	10	30	10	10	10
D	80		20			
S	100					
DCP	40	20	20	5	15	5
DAI	50	10	10	10	10	10
L	40	30	15	5	5	5
W	35	35	10	5	10	5
CR	30	10	30	10	10	10
SR	70	10	5	5	5	5
PM	60	10	10	10	5	5

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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THE IMPACT OF PERSIAN CLOVER (*Trifolium resupinatum* L.) ON SOIL HEALTH

Mandana MIRBAKHSH[1]*, Seyedeh Sara SOHRABI SEDEH[2], Zahra ZAHED[2]

[1]*Purdue University, Department of Agronomy, 47907, West Lafayette, IN, USA*
[2]*Alzahra University Department of Plant physiology, Vanak Village-street, 1993893973, Tehran, Iran*

Abstract: Persian clover (*Trifolium resupinatum*) is an exception as it is a promising legume species due to its adaptation to environmental stress and waterlogged soil. Soil health indicator link to environment services such as nutrient management, crop practices, and biodiversity. The main comparisons in this study were conducted between annual covers (Corn and sorghum), and two grass cultivars (*Miscanthus sinensis* and *Miscanthus junceus*) that are native to East Asia, while tillage system and fertilization legume Persian clover (*Trifolium resupinatum*), were also applied in the analysis. The regression analysis revealed improved soil carbon was consistently associated with greater moisture, soil aggregate stability (WAS), and carbon mineralization under legume covers. While grasses and legume recorded sequestering more carbon, corn and corn-sorghum in rotation crops showed the worse impact on soil pH and bulk density (BD). Non-tillage practices significantly increased soil aggregate stability and soil moisture under grasses and legume. The combination of total carbon (TC) field measurements with cropping systems information has improved our understanding of how different cropping practices influence soil health improvement in full profile. The main factor for clustering treatments based on indicators was fertilization and tilling operation according to Euclidean distance that was applied to measure similarity of the groups. It can develop appropriate and cost-effective agricultural management activities, maintains or improves carbon soil accumulation to guide farmer decision making and ultimately advancing food and nutritional security.

Keywords: Soil health indicators, Perennial, Cover cropping, Total organic carbon (TOC), Soil quality

***Corresponding author:** Purdue University, Department of Agronomy, 47907, West Lafayette, IN, USA
E mail: mmirbakh@purdue.edu (M. MIRBAKHSH)
Mandana MIRBAKHSH https://orcid.org/0000-0002-2712-2193
Seyedeh Sara SOHRABI SEDEH https://orcid.org/0009-0002-8068-0423
Zahra ZAHED https://orcid.org/0009-0002-4675-1582

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1. Introduction

Soil health indicators play significant role especially when sustainable agriculture faced the problem of massive production to cover malnutrition and feed the rising population of the world. Concerns about environmental sustainability of annual biofuel cropping systems such as corn warrant the need for an improved understanding of soil health under different agroecosystems. Corn production requires great amount of N fertilizer, which causes environmental contamination, greenhouse gases (GHGs) emission, and raise the risk of human exposure to nitrate from contaminated drinking water as well Ward et al. (2005). The significant increase of GHGs in the atmosphere also has increased the global mean temperature by ~1°C over the last century that affects quality of agricultural crops Zhao et al. (2017). Un-balanced presence of nano-particles makes this scenario even worse, because it alters photosynthesis and induces oxidative stress by Reactive oxygen species (ROS) generation that results in DNA degradation, cell death, and Antioxidant enzymes inhibition Mirbakhsh (2023). In this situation soil can play a key role to protect health and reduce environmental contamination. Soil health indicators are responsive to different cropping system to proper sufficient food to combat with malnutrition and hunger Augustine and Lane (2014).

Integrated or coupled system-level modeling has the potential to alleviate this situation and enhance soil health. The great interest of scientists to decrease the use of fertilizer and feed the rising population simultaneously have stimulated research on perennial grasses that considered as second-generation biofuel systems as well Blanco-Canqui (2010). Evaluation of the potential benefits of biofuels over fossil fuels have greatly relied on land use conversion to perennial or cover crops (Monti et al., 2012). The conversion of cropland to biofuel cropping has been proposed to potentially enhance soil health and mitigate GHGs (Anderson-Teixeira et al., 2009). Cover cropping has also been recognized as a cost-effective management tool and beneficial practice for enhancing soil health while also providing multiple agroecosystem function such as improving water quality and use efficiency of N by reducing N leachate and preventing further environmental contamination (Tonitto et al., 2006). Cover cropping's success depends on soil conditions, climate, and management approaches (Gomez, 2017).

Cover crops are annual, biennial and perennial species

grown on to protect the soil and to its fertility (Hartwig and Ammon, 2002). Persian winter legume (*Trifolium resupinatum* L.) as a cover crop has shown substantial biomass production due to a good germination and establishment beside its beneficial characteristics including weed suppression, soil aggregation and water retention improvement in Midwest. *Trifolium resupinatum* is also considered as a beneficial cover crop for minimizing nitrate leaching losses from lands under intensive grain production (Valkama et al., 2015).

Management practices can affect soil properties (Ashworth et al., 2018). The sustainability of soil resources can be maintained by promoting soil health through improved management practices (Norris et al., 2020). The choice of suitable cropping practices for increasing soil organic matter content should focus on several factors such as GHGs emissions mitigation, carbon capture improvement, and temporal yield stability (Somerville et al., 2010; Knapp and van der Heijden, 2018). For example; well-managed arable land conversion to perennial or cover cropping beside annual plots have been resulted in accumulation of SOC, due to their low nutrient requirements, extensive fibrous root systems, root exudates, and subsequent turnover of aboveground biomass (Ledo et al., 2020; Chen et al., 2022). On the other side, no-tillage reduced soil pH, but increased organic matter and Ca, Mg, and K concentrations compared to conventional tillage (Tarkalson et al., 2006). Currently maximizing stabilization and accumulation of soil C in deep horizons is a central component of successful attempts to reverse SOC loss in agricultural lands to combat the accelerated climate change and enhance soil health (Lal et al., 2013; Crow et al., 2018; Sun et al., 2021; Chen et al., 2022).

Our objective is to examine the effect of Persian winter legume, grasses, and corn with different management combination on potential of soil health indicators. We hypothesized that grass and legumes will enhance soil health indicators and crop yield compared to conventional tillage annual crop–fallow. Our experimental site has 6 treatments that have been continuously applied in a randomized complete block design with four replicates. For annual practices we have continuous corn, corn/ sorghum and sorghum/corn in rotation with conventional tillage. For cover cropping we have Persian winter clover (*Trifolium resupinatum* L) (Figure 1a), for perennial covers we have *Miscanthus sinensis* (Silver-grass) (Figure 1b) and *Miscanthus junceus* (Okavango Delta grass) (Figure 1c). Data for all soil health indicators and annualized crop yield were analyzed using regression analysis of soil components and to distinguish between 6 treatments with different cropping practices. Cluster analysis was used to split the soil health indicators to recognize the similarity of different cropping systems and cultivars.

2. Materials and Methods

2.1. Field Experiment

The experiment was conducted at a Research Farm (Islamic Azad University, Chalus Branch), located at 40° 55′ N and 53° 72′ E with an altitude of 4 m above sea level, during 2019-2020 crop year to test our alternative hypothesis. Soil samples with 0.15 m depth were collected in mid-April 2019 before tillage, planting, and fertilization. Measurements were made for intact and repacked soil cores. Mean temperature, relative humidity, and precipitation of study site are presented in Table 1.

The experimental design includes 6 treatments in completely randomized block design that have been applied since 2000. For perennial grasses there was silver-grass (*Miscanthus sinensis*) and Okavango Delta grass (*Miscanthus junceus*) with no tillage system and zero fertilizer. For cover cropping winter clover (*Trifolium resupinatum* L.) was added to corn in different plots with zero fertilizer and no tilling. The subset of three treatment chose for annual that contains corn (CC) and corn/sorghum-sorghum/corn (CS-SC) in rotation with tillage.

Figure 1. a. Persian Clover (*Trifolium resupinatum*) in a non-hairy winter annual clover with toothed margins and small pink flowers that produces olive green purple seeds. **b**. Chinese silver grass (*Miscanthus sinensis)* is used as ornamental grasses traditionally used in Japan with erect, airy, plumed seed heads that are usually produced in late summer. **c**. Okavango Delta grass (*Miscanthus junceus*) is produced by seasonal flooding and is found in permanent marshland. It is home to some of the world's most engaged species of large mammals.

Table 1. Meteorological parameters for the field sites during experiment (Mazandaran province Meteorological Office)

Months	Mean temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	2019	2020	2019	2020	2019	2020
November	12.98	14.06	79.87	84.32	148.36	193.12
December	10.52	12.13	85.10	79.80	185.30	122.90
January	8.90	10.60	80.30	82.40	95.20	94.30
February	10.30	12.20	84.70	88.20	87.90	94.30
March	8.10	11.20	83.40	86.50	110.10	118.40
April	11.40	14.15	83.10	79.90	70.30	125.10
May	16.40	18.90	80.70	82.10	18.10	41.20
June	24.20	21.50	73.20	79.30	10.30	26.30

The N sources for corn treatments were urea-ammonium nitrate 38% (w/w) N (UAN) side-dressed at corn growth stage V5 at rates of 185 and 115 kg N ha^{-1} yr^{-1} for CC and CS, respectively, and liquid swine manure (80% [w/w] of N as $NH4^{+}$) injected into CC at a rate of 255 ± 24 kg N ha^{-1} yr^{-1} in either the spring (SM) or the fall (FM). Tillage operations were chisel in the fall and chisel plus disk in the spring for all cropped plots except perennials. Seeds were planted at a population density of 182 and 560 seeds ha^{-1}, respectively. Soils were tested each fall for general fertility using recommended protocols.

Soil samplings occurred within the week of corn planting in early July (11 July 2019 and 3 July 2020) and again at corn growth stage R1 in late September (25 September 2019 or 27 September 2020).

2.2. Soil Sampling and Analysis

A slice of soil (4 m wide by 1.5 cm thick by 15 cm depth) was removed from three sides of the hole using a soil knife. Soils from three sides of six holes were placed in a plastic bag to prepare a composite sample for analysis and the bag was put in a cooler packed with ice for transportation to the laboratory. A subsample of 400 g soil from each plot was sealed in a plastic bag, put in a cooler packed with ice, and shipped to soil testing laboratories for analysis of biological properties. The remaining soil was shipped to other laboratories where soils were air-dried, ground, and sieved to 2 mm prior to the analysis of physical and chemical properties.

Measurements were made for intact and repacked soil cores. For total C and N concentration (g Kg^{-1}) in the soil the samples sieved through 2 mm sieve then 0.5 gram sub-sample from each core was combusted (CN analyzer) to quantify C:N ratio. The soil C data was square root transformed prior to the analysis to make the data more normally distributed. The accurate measurement of bulk density that is directly influenced by organic matter (OM) content is critical for converting SOC on a weight basis to content per unit volume. Cores were oven dried to get a dry mass for measuring bulk density. Bulk density was calculated by dividing the oven-dried soil samples with the volume of soil core Grossman and Reinsch (2001). Samples were taken from the center of each depth interval to represent depth increment.

Aggregation is a complex process of the interactions between roots and mycorrhizal fungi in relation to plant community composition which is improved by cover cropping/fresh root exudation and its influences on SOC sequestration Lal (2015). Samples for measuring wet aggregate stability (WAS) of the soil in this part of the project were taken by using hydraulic probe 5.3 cm in diameter from four mentioned depths. Samples were push through 8 mm sieve while still is moist and then dried and sieved to remove 2 mm fraction. From each sample two 25 g subsample were derived for analyzing using wet aggregate size distribution method. Aggregate stability was determined using the sprinkle infiltrometer as the amount of 0.25–2.00 mm aggregates remaining in a 0.25 mm sieve after a 5 min simulated hard rainfall (Schindelbeck et al., 2016). The average mean weight diameter was calculated for each depth.

Soil pH and electrical conductivity were determined using 1:2 soil/water ratio with a pH meter. Soil P, Ca, N, K concentrations were determined by extracting the soil with Mehlich-3 solution and quantification by inductive coupled plasma-atomic emission spectroscopy (ICP-AES) (Sikora and Moore, 2014).

2.3. Data Analysis

Data for all soil health indicators and different treatments were analyzed using R version 4.0. The regression analysis of the soil health components at particular time of soil sampling provides an estimate of relative influence of each soil health indicators. Hierarchical clustering with Ward's algorithm was performed to assess common soil health indicators among different treatments that was analyzed by correlation matrix to delineate the relationships between treatments. This type of clustering shows which treatments tend to have similar variations in concentrations.

3. Results and Discussion

The regression and correlation patterns for soil health indicators in 2019 showed high positive correlation of soil moisture with total Carbon (TC), Carbon mineralization, Wet aggregate stability (WAS) under winter clover and silver-grass. WAS significantly influenced by carbon and nitrogen mineralization under winter clover (Figure 2). Since, soil aggregates are formed through physical and biochemical activities treatment and tillage effect had strong and direct impact on it. The positive correlation also recorded between

total N (TN) and TC under treatments with winter clover and silver-grass. WAS recorded high relation to carbon content of the soil and C-mineralization in grasses and legume (Table 2). The results indicates that physical protection of SOC can be achieve through positive interaction between SOC and soil structure such as WAS and moisture. On the other site the positive interaction of TN and TC reveals the main mechanism of soil organic matter (SOM) protection with TN and TC (Zhang et al., 2020). Accumulation of greater SOC under perennials and no-tillage system effectively promotes soil aggregate, which can store SOC for long-term and increase moisture to protect roots (Aziz et al., 2013; Hernandez et al., 2019). These finding is so important, especially regarding soil moisture that has a direct impact on other health factors and associated for agro-meteorological variables at different time-frequency domains Jamshidi et al. (2021).

Correlation patterns for 2020 soil health indicators showed correlation of soil moisture with C-mineralization, wet aggregate, N-mineralization, TC (Figure 2). It was pretty the same as 2019 and did not have a change (Table 2). Retaining crop residues in perennials and cover crops reduce exposure of the soil to external environmental factors such as heat and increase moisture, which has direct positive impact on TC Zheng et al. (2018). The negative correlation between TC and bulk density was recorded in 2020 as well (Figure 2). Increased SOC content under perennial or/and cover cropping with no-tillage system can subsequently improve soil physical properties such as bulk density and WAS Maiga et al. (2019). Carbon mineralization showed correlation to TC, TN, N-mineralization, and wet aggregate in all treatments. High correlation was recorded between C-mineralization and N-mineralization and also TN and TC specially under legume (Table 2). Glomalin that is produced from cover crops root might be one of the main reasons in adhesion and stabilization of soil under cover crops treatments Liu et al. (2020).

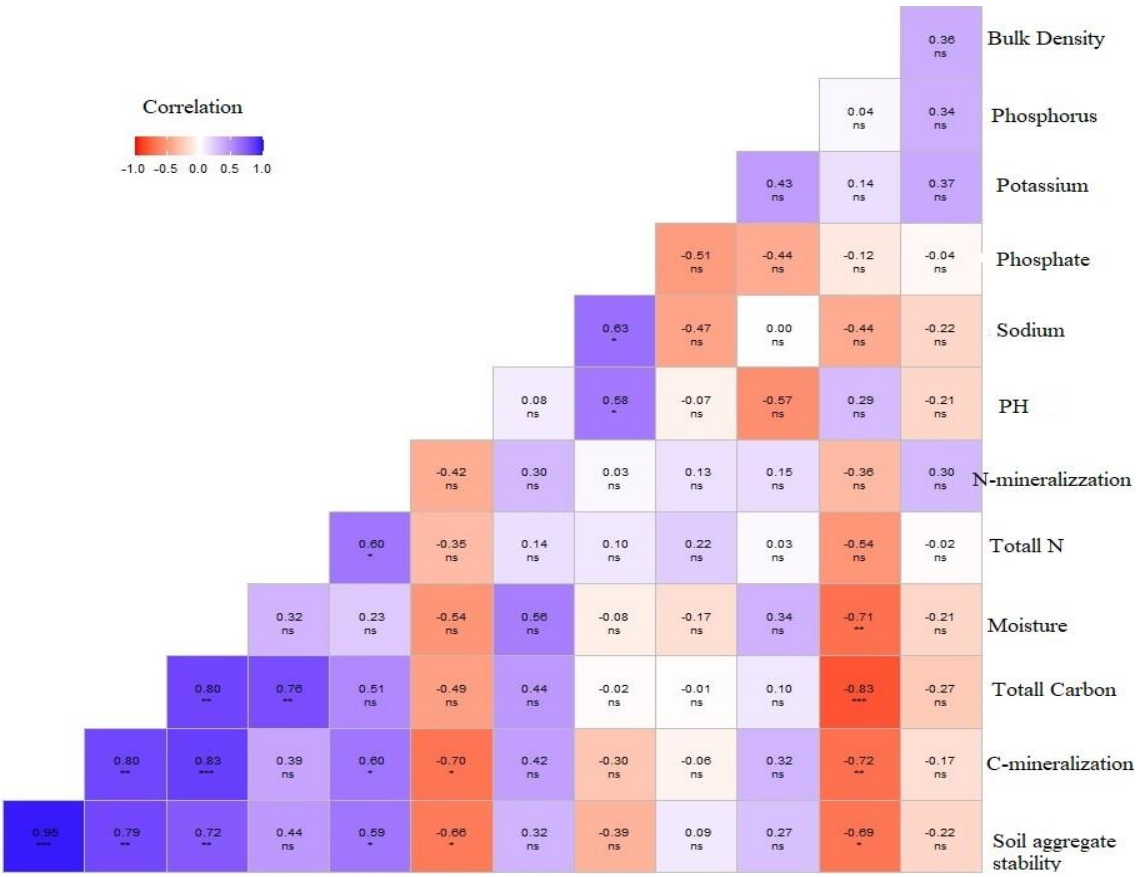

Figure 2. Correlation patterns between soil health components for 2019 and 2020 in average. The results recorded strong relation between soil aggregate stability and mineralization that increase total carbon as well in both year, and strong negative relation between Bulk density with total N and total C is shown.

Moisture had greater impact on TC in grasses than corn, which increased over time and its greatest impact was recorded in 2020 that was about ten-fold compares to annual (Table 2). The impact of TN on TC was greater in perennial than annual, which increased over the time. However, the impact of TC on TN regarding (perennial and legume: annual) was not constant and the highest record was shown in 2019. (Table 2). Wet aggregate recorded higher impact on TC in perennial than annual, which increased over time and in 2020 and was greater in perennial than annual. However, regarding the impact of wet aggregate on TN comparing perennial to annual;

although perennial recorded greater than annual but it decreased to equal ratio over time (Table 2). The impact of respiration on TC was greater in perennial compares to annuals, which increased over time and the highest record was shown in 2020. However, carbon mineralization showed pretty constant impact on TN in both perennial and annual (Table 2). The impact of Nitrogen mineralization on TN was greater than on TC, but in both ratios, perennial recorded higher impact on TN and/or TC than annual. Although perennial showed the potential for storing C in ground and reducing atmospheric CO_2 concentration, management practices such as reduced- or no-tillage, residue retention may reduce SOC losses and increase soil health. Plant residue management is also so important in retaining soil moisture and increase WAS. Our results agree with the idea of using winter legume and grasses for restoration of degraded agriculture lands, either on their own such as our grasses or in combination with annual such as our Persian clover that was dressed with corn.

Hierarchical clustering with Ward's minimum variance algorithm creates a hierarchical decomposition of the given set of data objects forming a dendrogram – a tree which splits the database recursively into smaller subsets. Euclidean distance was applied to measure similarity of the groups. The dendrogram shows two main big cluster around 20,000 Euclidean distances. Fertilizer application had significant impact on clustering our treatments. No fertilizer was added to legume and two type of grasses that are clustered separately from other treatments that were under fertilization. Moreover, all perennial grasses and legume are grouped in this cluster and are separated from annual. However, silver and delta perennial grasses recorded the highest similarity with minimum Euclidean distances, then legume joined them. Tillage effect was significant in grouping treatments because half of treatments in each group were under no-tillage and the rest of them were with conventional tillage (corn), (corn and sorghum in rotation), and (sorghum and corn in rotation) (Figure 3).

Table 2. Pearson correlation among comprehensive assessment of soil health indicators for all 6 treatments among 209 and 2020. Correlation patterns for legume clover showed high correlation of soil moisture with TC, C-mineralization, wet aggregate. Positive correlation recorded between total N and total C. Carbon mineralization showed correlation to total C and wet aggregate. High correlation was recorded between wet-aggregate and C-mineralization and TC under silver-grass. High correlation was recorded between C-mineralization and N-mineralization and also TN and TC in Persian legume. Factors that showed correlation to TC and TN are; Wet aggregate, N-mineralization and bulk density (with negative correlation)

Indicators	Moisture	PH	Bulk density	Aggregate	Nmineralization	P	K	P-	Na	TN	TC	Cmineralization
Moisture	1	-0.03278522	-0.665762172	0.772172028	0.199550022	-0.41995	0.548526	0.349383	-0.18667	0.188424764	0.904864	0.749973426
PH	-0.032785216	1	0.337759339	0.017979778	0.430068968	0.38453	0.004185	0.515873	-0.06595	-0.197605264	-0.28602	0.008516348
Bulk density	-0.665762172	0.33775934	1	-0.687174436	-0.388247529	0.410672	-0.45417	-0.00097	-0.05399	-0.398160841	-0.86577	-0.692139627
Aggregate	0.772172028	0.01797978	-0.687174436	1	0.568231388	-0.01269	0.284375	0.377384	-0.42219	0.396793951	0.797971	0.907894344
Nmineralization	0.199550022	0.43006897	-0.388247529	0.568231388	1	0.310394	0.36138	0.202081	0.034686	0.136273095	0.231185	0.617660213
Phosphorus	-0.419946781	0.3845304	0.410672252	-0.012690605	0.310393568	1	-0.53205	0.313879	-0.35585	0.131682043	-0.39645	-0.094194469
Potassium	0.548526284	0.00418546	-0.45417183	0.28437489	0.36137954	-0.53205	1	0.025232	0.58453	-0.067479694	0.431537	0.356391514
Phosphate	0.349382822	0.51587298	-0.000968516	0.377383827	0.202081312	0.313879	0.025232	1	-0.38589	-0.046972493	0.123176	0.323750712
Sodium	-0.186665491	-0.0659472	-0.053989705	-0.422187574	0.034685981	-0.35585	0.58453	-0.38589	1	0.124212903	-0.13811	-0.418843826
Total N	0.188424764	-0.19760526	-0.398160841	0.396793951	0.136273095	0.131682	-0.06748	-0.04697	0.124213	1	0.434136	0.084956843
Total C	0.904864244	-0.28602188	-0.865773618	0.797970977	0.231185015	-0.39645	0.431537	0.123176	-0.13811	0.434135841	1	0.739562375
Cmineralization	0.749973426	0.00851635	-0.692139627	0.907894344	0.617660213	-0.09419	0.356392	0.323751	-0.41884	0.084956843	0.739562	1

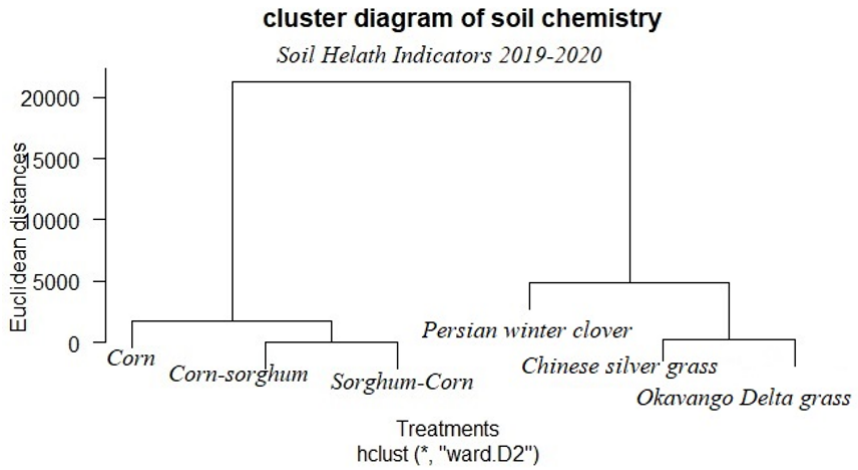

Figure3. According to the dendrogram first legume and Miscanthus (Silver grass and Okavango), then Corn and Sorghum in rotation are the first and second two lines that merged. Rather than different cropping system there are two other factors that impact the separation in this dendrogram, which are fertilization and tilling system that should be considered in improving agriculture systems. According the range of similarity corn and sorghum in rotation and Silver-grass and Delta-grass recorded the highest rate.

The results recorded the most similarity between corn and sorghum in rotation and Silver with Delta grasses. The results agree with the theory that when perennial and legumes are deployed on the landscape it is generally assumed that soil health increase in comparison to annual. However, there exists a critical need to synthesize and evaluate data on greenhouse gas emissions and SOC sequestration at meaningful temporal and spatial scales following land use changes to second-generation biofuel cropping systems as well. Thus, *Miscanthus,* and winter clover have potential to improve Soil health contents while supplying feedstock for biofuel production.

4. Conclusion

Our results demonstrate that routine soil test should include analysis of total carbon/ nitrogen for the long-term sustainability under different cropping practices and agricultural system. The most promising soil health indicator that related to different cropping practices were organic Carbon, total carbon, followed by total Nitrogen, bulk density, moisture. Our study of different indicators of soil health that included physical and chemical properties under different cropping systems experiments showed that no-fertilization in combination to perennial and covers caused the most promising distinction between treatments. Cover cropping such as Persian winter clover side dress in corn had similarity to perennial grasses (*Miscanthus sinensis* and *Miscanthus junceus*) regarding improving soil health as well. Our results indicates that perennial covers and legume cultivated with corn increase soil health and quality, where organic carbon, total carbon and nitrogen has highest contribution to these cropping practices in the most top soil layers. Grasses and legumes have therefore the potential to be a climate change mitigator, by resulting a negative C balance system or at least storing some C in the ground and thus reducing atmospheric CO_2 concentration. C stored in ground during perennial and cover cropping may be lower than C and other GHGs emitted during combustion, which resulting in net positive emission from plantation and potentially contributing to food security and local economic. The full potential of these biofuel cropping systems to increase soil health can be best realized with proper N fertilization, particularly, when the land use conversion involves grasslands.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.M.	S.S.S.S.	Z.Z.
C	40	30	30
D	70	20	10
S	50	50	
DCP	30	40	30
DAI	40	30	30
L	60	20	20
W	50	50	
CR	70	20	10
SR	50	50	
PM	40	40	20
FA	40	40	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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CHARACTERIZATION AND IN VITRO EVALUATION OF CARROT PULP ENSILED WITH DIFFERENT DRY ROUGHAGES

Ali Faruk KANDEMİR[1], Canan KOP BOZBAY[1]*

[1]*Eskisehir Osmangazi University, Faculty of Agriculture, Department of Animal Science, 26480, Eskisehir, Türkiye*

Abstract: This study investigated the nutrient content, the digestibility of organic matter (OMD), and the metabolic energy (ME) values of silages prepared from carrot pulp, a by-product of the food industry. Five different silages with five replicates were prepared by adding wheat straw (CP + WS), vetch/oat hay (CP + VOH), maize stalk straw (CP + CS), alfalfa hay (CP + AH), and barley straw (CP + BS) as 20% absorption material to 80% carrot pulp. The silages were opened after 60 days, and physical and chemical analyses were performed. The dry matter content was highest in the CP+BS silage. Crude protein content was highest in the CP+AH and CP+VOH groups. CP+AH silage had the highest ADF and NDF. The CP+AH and CP+VOH groups had high-quality relative feed values, while all groups had very good Flieg quality values. There were no differences between the groups for OMD and ME. To conclude, it was found that carrot pulp silage can be used as an alternative feed source in animal nutrition.

Keywords: Silage, Carrot pulp, In vitro, Alternative roughage

***Corresponding author:** Eskisehir Osmangazi University, Faculty of Agriculture, Department of Animal Science, 26480, Eskisehir, Türkiye
E mail: cbozbay@ogu.edu.tr (C. KOP BOZBAY)
Ali Faruk KANDEMİR https://orcid.org/0000-0002-4611-3428
Canan KOP BOZBAY https://orcid.org/0000-0002-8071-5860

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1. Introduction

Agro-industrial by-products result from various physical, chemical and biological processes of animal or plant products that are not used as raw materials in the production chain (Rosas et al., 2016). These by-products have attracted considerable interest due to their potential use in animal feed. Due to their local availability and low prices, they are used as the main components of livestock rations in many developing countries (Mugerwa et al., 2012). In Türkiye today, there is an incredible biodiversity of products that have gone through the industrialisation process; these produce non-traditional wastes (by-products, residues) (Mirzaei-Aghsaghali and Maheri-Sis, 2008; Özdemir and Okumuş, 2022; Özdemir and Ülger, 2022) that can be incorporated into the diets of dairy and beef cattle in the region. Among the industrialised products, fruits, vegetables, roots, etc., are sold fresh, while others are processed into nectars, juices, jams, wines, and canned food. The nutritional value and nutritional management of the resulting agro-industrial wastes and by-products have been discussed, and some studies have been conducted on the level at which these non-conventional feed resources can be used in the feed of different animal species (Tekin, 2017; Başar and Atalay, 2020). Studies have shown that fruit and vegetable wastes can be used as an alternative feed source due to the inadequacy of our existing feed resources in rational terms (quantity-quality-price) (Günal et al., 2017; Ülger et al., 2018; Gharehbagh et al., 2020; Özdemir and Ülger, 2022; Ülger and Özdemir, 2023).

The carrot (*Daucus carota* L.) is one of the most popular root vegetables grown worldwide (Torronen et al., 1996). In addition to their roots, carrots can be commercially processed into nutrient-rich processed products such as juice, canned food, pickles, etc. In 2022, 788,578 tonnes of carrots were produced in Türkiye (TÜİK, 2022). Up to 50% of the raw material in carrot juice production becomes waste in the form of pulp (Nocolle et al., 2003). If these data are evaluated, a large amount of carrot pulp is generated annually as waste from fruit juice factories in our country. As fruit and vegetable pulp deteriorates rapidly due to its high water content, it can be used fresh, dried, or ensiled for use as animal feed.

Today, many fruit pulps are no longer used in the industrialisation process. Therefore, evaluating the chemical composition and feed value of these non-traditional by-products, such as carrot pulp, is necessary and should be used as a reference. For this purpose, the possibilities of ensiling carrot pulp and the nutrient values, organic matter digestibility, and metabolic energy values of the silages were investigated in the present study.

2. Materials and Methods

The carrot (*Daucus carota* L.), alfalfa hay (*Medicago sativa*), wheat straw (*Triticum*), vetch/oat hay (*Vicia*

sativa-Avena sativa L.), barley straw (*Hordeum vulgare* L.) and corn stalk straw (*Z. mays*) used in the study were obtained from livestock farms in Eskisehir and brought to Eskisehir Osmangazi University, Faculty of Agriculture, Laboratory of Animal Science. Carrot pulp was obtained using a juicer (Mehtap Reyyan MSM-3). Dry roughages were cut into approximately 1.5-2.2 cm long pieces. The rumen fluid was obtained from male Simmental cattle with a live weight of 600-650 kg, which was fed with a fattening diet + wheat + barley straw from a slaughterhouse (Keskin Et Entegre Kombinası) in Eskisehir immediately after slaughter.

The dry matter (DM) contents of carrot pulp and dry roughages were determined (AOAC, 2006) and five silage groups were prepared in 1 kg glass jars in 5 replications. The silages were prepared by mixing 80% carrot pulp + 20% dry roughage, resulting in 30% DM in the silage. The treatment groups were as follows: carrot pulp+wheat straw silage (CP+WS), carrot pulp+vetch/oat hay silage (CP+VOH), carrot pulp+corn stalk-straw silage (CP+CS), carrot pulp+alfalfa hay silage (CP+AH) and carrot pulp+barley straw silage (CP+BS). After 60 days, the silages were opened and dried (AOAC, 2006). To determine the dry matter (DM) content of the silages, they were dried in an oven at 55 ^{0}C for 96 hours until they reached a constant weight. After determining the dry weight of the silages, the samples were ground and prepared for analysis. Subsequently, ash, crude protein, and ether extract (AOAC, 2006) and cell wall components (crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF)). Van Soest et al. (1991) measured in the milled silage samples (1 mm particle size). Organic matter (OM) and nitrogen-free extract (NFE) values were calculated. Relative feed value (RFV, Rohvveder et al., 1978) and RFV grade, used to determine the feed quality of carrot pulp silages, were calculated from dry matter digestibility (DMD) and dry matter intake (DMI) data (Van Dyke and Anderson, 2000). Flieg quality score and grade were calculated according to Kılıç (1986). pH values were measured using an electronic pH meter (HANNA Instruments I-2211). Organic matter digestibility (OMD) and metabolic energy (ME) of the samples were determined using the ANKOM^{RF} gas production system according to the gas production method reported by Menke and Steingass (1988).

The data were analyzed in SPSS 17.0 package program. The Kolmogorov-Simirnov test was applied for the data's normality assumption, and the variances' homogeneity was evaluated with the Levene test. The data of the study were subjected to a one-way analysis of variance. Duncan test, one of the multiple comparison tests, was used.

3. Results

3.1. Characteristics of Carrot Pulp and Dry Roughages

The nutrient composition and cell wall components of the carrot pulp and dry roughages used in the study are given in Table 1. The OMD and ME values of carrot pulp were higher than the dry roughages used for ensiling (Table 2).

3.2. Characteristics of Carrot Pulp Silages

The nutrient composition and cell wall components of the carrot pulp silage groups are shown in Table 3. DM was higher in the CP+BS group than in the CP+AH group and in the CP+AH group than in the other groups ($P<0.001$).

Table 1. Nutrient composition and cell wall components of carrot pulp and dry forages (%)

Material	DM	Ash	CP	EE	OM[1]	NFE[2]	ADF	NDF	CF
Carrot pulp	93.16	5.59	5.26	0.60	87.57	72.58	19.52	19.32	9.13
Wheat straw	91.25	10.53	4.58	1.67	80.72	45.62	44.03	68.09	28.85
Vetch/oat hay	89.81	8.31	15.84	0.75	81.50	44.63	28.85	39.13	20.28
Corn stalk-straw	89.91	7.61	5.31	1.52	82.30	46.76	43.68	72.35	28.71
Alfalfa hay	91.05	11.74	17.9	1.31	79.31	35.92	32.01	39.22	24.18
Barley straw	91.98	7.54	4.58	1.26	84.44	52.08	37.76	67.32	26.52

DM= dry matter, CP= crude protein, EE= ether extract, OM= organic matter, NFE= nitrogen-free extract, ADF= acid detergent fiber, NDF= neutral detergent fiber, CF= crude fiber. [1]OM= %DM-%Ash, [2]NFE= %DM – (%CP + %EE + %CS + %Ash).

Table 2. Gas production (ml), organic matter digestibility (OMD, %), and metabolic energy (ME, MJ/kg DM) values of carrot pulp and dry roughages

Material	Hour 3	Hour 6	Hour 12	Hour 24	Hour 48	Hour 72	OMD	ME
Carrot pulp	49.53	100.04	185.51	241.12	266.38	257.64	60.66	9.71
Wheat straw	13.58	27.89	50.45	98.48	142.87	146.03	39.63	6.34
Vetch/oat hay	31.14	65.72	111.57	159.39	187.34	184.65	45.91	7.35
Corn stalk-straw	22.12	48.86	91.41	159.48	202.99	199.35	49.48	7.92
Alfalfa hay	21.46	54.14	90.23	121.20	136.81	130.71	37.58	6.10
Barley straw	19.69	52.02	90.42	138.55	166.75	163.83	42.53	6.81

OMD= organic matter digestibility, ME= metabolic energy

Table 3. Nutrient composition and cell wall components of carrot pulp silage groups (%)

Silage	DM	Ash	CP	EE	OM[1]	NFE[2]	ADF	NDF	CF
CP+WS	26.35^{c}	9.51^{b}	5.55^{c}	0.47^{b}	83.07^{c}	50.05^{c}	40.69^{a}	53.25^{a}	27.01^{a}
CP+VOH	25.97^{c}	9.58^{b}	9.25^{a}	1.76^{a}	82.10^{d}	50.43^{c}	29.80^{c}	39.48^{b}	20.50^{c}
CP+CS	26.40^{c}	7.76^{c}	5.55^{c}	0.37^{b}	83.88^{b}	54.20^{a}	37.12^{b}	48.26^{a}	24.06^{b}
CP+AH	27.16^{b}	11.48^{a}	9.50^{a}	1.75^{a}	79.09^{e}	48.08^{d}	28.29^{c}	32.78^{c}	19.57^{c}
CP+BS	27.93^{a}	7.71^{c}	6.59^{b}	1.64^{b}	85.47^{a}	51.73^{b}	34.75^{c}	51.42^{a}	25.69^{ab}
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM	0.167	0.465	0.650	0.142	0.711	0.659	1.002	1.718	0.649

CP+WS= carrot pulp+wheat straw silage, CP+VOH= carrot pulp+vetch/oat hay silage, CP+CS= carrot pulp+corn stalk-straw silage, CP+AH= carrot pulp+alfalfa hay silage, CP+BS= carrot pulp+barley straw silage, DM= dry matter, CP= crude protein, EE= ether extract, OM= organic matter, NFE= nitrogen-free extract, ADF= acid detergent fiber, NDF= neutral detergent fiber, CF= crude fiber. [1]OM= %DM-%Ash, [2]NFE= %DM – (%CP + %EE + %CS + %Ash), SEM= standard error of the mean, a,b= within a row, means with different superscripts differ significantly (P<0.05).

Table 4. Dry matter digestibility (DMD, %), dry matter intake (DMI, %), relative feed values (RFV, %), pH and Flieg quality score values of carrot pulp silage groups

Silage	DMD	DMI	RFV	RFV grade	pH	Flieg quality score	Flieg quality score grade
CP+WS	57.30^{e}	2.27^{c}	100.77^{d}	3rd quality	4.2	89.77	Very good
CP+VOH	65.39^{b}	3.04^{b}	154.15^{b}	High	4.2	87.02	Very good
CP+CS	59.85^{d}	2.49^{c}	115.74^{c}	2nd quality	4.2	89.08	Very good
CP+AH	67.36^{a}	3.67^{a}	191.66^{a}	High	4.3	86.04	Very good
CP+BS	61.80^{c}	2.38^{c}	113.97^{c}	2nd quality	4.2	91.01	Very good
P	<0.001	<0.001	<0.001		0.835	0.401	
SEM	0.555	0.081	5.147		0.036	1.545	

Silage group descriptions are the same as Table 4, [1] RFV grade value, [2] Flieg quality score grade value, SEM= standard error of the mean; a,b: Within a row, means with different superscripts differ significantly (P<0.05).

Table 5. Gas production (ml), organic matter digestibility (OMD, %), and metabolic energy (ME, MJ/kg DM) values of carrot pulp silage groups

Silage	Hour 3	Hour 6	Hour 12	Hour 24	Hour 48	Hour 72	OMD	ME
CP+WS	24.06	47.64	81.43	129.31	184.73	201.99	49.74	7.61
CP+VOH	26.82	77.30	136.56	168.75	197.28	205.08	50.40	7.72
CP+CS	26.25	50.08	95.05	156.31	211.98	232.16	55.11	8.41
CP+AH	38.08	84.41	138.51	168.58	190.17	193.57	48.52	7.43
CP+BS	34.82	68.41	116.48	166.99	213.82	225.84	53.75	8.21
P	0.690	0.280	0.090	0.420	0.740	0.270	0.279	0.298
SEM	3.244	6.412	8.696	7.229	7.254	6.432	1.093	0.164

Silage group descriptions are the same as Table 4, SEM= standard error of the mean, a,b: Within a row, means with different superscripts differ significantly (P<0.05).

The CP+AH silage had a higher ash content than the CP+WS and CP+VOH silages, and the CP+WS and CP+VOH silages had a higher ash content than the CP+CS and CP+BS silages (P<0.001). The highest crude protein values were obtained when alfalfa and vetch-oat hay were added as adsorbents to carrot pulp, followed by barley and wheat/corn stalk grass (P<0.001). EE was higher in CP+AH and CP+VOH silages than in the other silages (P<0.001). The organic matter content in the silage groups was higher in the CP+BS, CP+CS, CP+WS, CP+VOH and CP+AH groups (P<0.001). NFE was higher in the CP+CS group than in all groups, in the CP+BS group than in the CP+VOH, CP+AH and CP+WS groups, and in the CP+VOH and CP+WS groups than in the CP+AH group (P<0.001).

As shown in Table 3, the effect of treatments on cell wall components was significant (P<0.001). The ADF value was highest in the CP+WS silage and higher in the CP+CS silage than in the other groups. The NDF values were higher in the CP+WS, CP+CS and CP+BS silages than in the CP+VOH and CP+AH silages (P<0.001). The lowest CC content was found in CP+VOH and CP+AH silages, followed by CP+CS and CP+BS and CP+WS silages, respectively (P<0.001). According to Table 4, as a result of the calculation using DMD and DMI values, the highest RFV was found in CP+AH silage, followed by CP+VOH, CP+CS/CP+BS and CP+WS groups, respectively (P<0.001). There was no significant effect of treatments on pH and Flieg quality scores (P>0.05; Table 4). The factors had no effect on OMD, ME and gas production (P>0.05; Table 5).

4. Discussion

4.1. Characteristics of Carrot Pulp and Dry Roughages

All forages can be ensiled, but their suitability for ensiling varies with their dry matter and carbohydrate content (Yitbarek and Tamir, 2004; Büyükkılıç et al., 2018). Absorbents (absorption enhancers) are among the feed additives that are effective in silage preservation. In fact, the sugar (61.3%; Ramos-Andrés et al., 2020) and moisture (84.67%) contents of carrot pulp are high. For this reason, it can be ensiled with absorbent additives and used as an alternative feed material in animal nutrition. The dry matter content of dry roughage used as an absorbent in the current study showed a value between 89.91-91.98% and was compatible with the literature. Similarly, the nutrient composition of the carrot pulp and dry roughage used in the current study was consistent with the literature (Jagtap et al., 2000; Wadhwa et al., 2015; Ergün et al., 2020).

When fibre content, OMD, and ME values of all silage materials used in the present study are considered, carrot pulp had much lower fibre and higher OMD and ME values than dry roughages used as absorbents. This situation allows carrot pulp to be supplemented with dry roughages for silage production. Indeed, Azizi et al. (2019) compared the chemical composition and nutritional value of carrot pulp with wheat straw and alfalfa and observed the highest and lowest gas production volume and constant gas production rate after 16 and 24 h of incubation in carrot pulp and wheat straw incubation, respectively. However, the highest gas production volume and gas production potential were observed for alfalfa, while the lowest amount was observed for wheat straw. The positive correlation between organic matter digestibility and the ME value of a feed explains why the ME value of carrot pulp was higher than that of dry roughages in the study. Since this situation gives information about the nutritional value of feeds, the nutritive value of carrot pulp will be high.

4.2. Characteristics of Carrot Pulp Silages

Depending on the chemical composition of the nutrients in the forages, there may be differences in their nutritional value, and these differences may be reflected in the quality of the silages. Indeed, in the present study, the different roughages added to the carrot pulp reflected the differences in their chemical composition in the silages.

The DM content, an important indicator of silage quality, indicates the amount of nutrients available to the animal in the silage. The DM content varies according to the raw materials used in fruit and vegetable pulp silages. Santos et al. (2014) reported 33.7% DM in grape pulp silages, and Başar and Atalay (2020) reported 6.76-9.66% DM in different citrus pulp silages. Considering that the DM content of the highest quality corn silages is between 25-32% (Kılıç, 1986), all silages obtained in the present study can be considered as good quality in terms of DM content (25.97-27.93%). This situation shows that the ensiling potential of carrot pulp is high.

The ash content, which is a measure of the ability to meet the mineral requirements of animals, was higher in carrot pulp silages (7.71-11.48%) than in corn silage (4.81%) (Tharangani et al., 2021) and citrus silage (3.26-5.33%) (Başar and Atalay, 2020). This may be due to the different mineral contents of dry roughage as absorbents for silages. In fact, the carrot pulp silage to which the highest ash content alfalfa hay was added had the highest ash content. Similarly, the differences between the groups in terms of protein content showed that the raw materials used were linearly influenced by the protein content. The CP+AH and CP+VOH silages contained 9.50% and 9.25% protein respectively, while the other silage groups gave values close to corn silage. In the studies carried out with different fruit pulps, the protein ratio showed a wide range. Özdemir and Ülger (2022) reported protein values of 6.20-7.80% in peach pulp, Başar and Atalay (2020) reported 6.50-11.46% in different citrus pulps and Massaro Junior et al. (2022) reported 13.98% in grape pulp. The use of different absorbent materials, as well as different varieties and species, can accentuate this difference. Indeed, legume grasses, which are difficult to ensilage due to their high crude protein content, low concentration of water-soluble carbohydrates and high buffering capacity, are prone to proteolysis (Muck, 2010). Therefore, the addition of high-protein forages to carrot pulp with a high water-soluble carbohydrate content resulted in successful ensiling and increased protein content of silage. The amount of protein in a feed is the most important indicator of feed quality (Gillen and Berg, 1998). As the digestibility of roughage increases with the protein content of the feed, ruminant diets should contain at least 7-8% crude protein on a dry matter basis for healthy microbial activity (Van Soest, 1994). Therefore, it can be said that carrot pulp silages with alfalfa hay and vetch-oat hay are high quality diets. When the silage groups were analysed for EE content, despite the differences, the EE content (0.37-1.76%) was lower than that of corn silage, which may be because both carrot pulp (0.6%) and absorbent additives had low EE content (0.74-1.67%).

The highest OM content was in the CP+BS silages (85.47%). In comparison, the lowest value was found in the CP+AH group (79.09%). The reason for the lower OM content compared to corn silage is the high ash content of the silage materials used in this study. The amount of NFE calculated to determine the content of easily soluble carbohydrates was found to be the lowest in CP+AH silage, although there was a difference between the groups (48.08-54.20%) compared to corn silage (59%) (Sarıçiçek et al., 2016). This is because the crude protein content is higher in CP+AH silage than in corn silage and other carrot pulp silages.

ADF and NDF content negatively correlate with feed digestibility and intake capacity, respectively. Roughages with high NDF content reduce feed intake in high yielding dairy cows. Indeed, in the present study, the NDF, ADF,

and fiber contents of carrot pulp silages made by adding vetch-oat and alfalfa hay were found to be at the desired levels in the diet of dairy cows. The ADF content was higher, especially in the groups containing straw. The higher ADF content of roughage, such as straw, can explain this. There is a positive relationship between the NDF content and the water-holding capacity of a feed (Razak et al., 2012). This suggests that the dry roughage used as an absorbent in the current study may be a potential supplement. In addition, it has been reported that NDF reduces the in vitro digestibility and, thus the dry matter content of diets with a high NDF content (Mahyuddin, 2008; Mertens et al., 2012). The fact that the dry roughage used in this study did not reduce digestibility indicates that ensiling was successful.

Relative feed value (RFV) is widely used as an index to assess quality and compare feed types and prices. Higher RFVs indicate higher feed quality (Jeranyama and Garcia, 2004). The protein content of the feed is not taken into account when calculating the RFV, but higher RFV values are generally associated with higher protein content (Stallings, 2006). Indeed, the present results confirm this information. The CP+AH and CP+VOH groups, which had lower ADF and NDF contents, had the highest RFV values, while their protein contents were higher than the other groups. In view of this information, it can be said that the silages obtained by adding alfalfa and vetch-oat hay to carrot pulp have a high feed quality.

One of the most important factors influencing fermentation during ensiling is pH. Good silage should have a pH of 3.8-4.2. Although there was no difference between silage pH values in the present study, it can be said that the pH values of all silages except the CP+AH group were within the accepted limits for good silages (Ergün et al., 2020). Similarly, there was no difference between groups in the Flieg quality scores used to determine silage quality, but all our groups were considered very good quality (85-100).

The nutritional value of a ruminant feed is determined by the concentration of chemical constituents and the rate and extent of their digestion. It is very important to determine not only the nutrient content and quality but also the digestibility of the feed (Ayaşan et al., 2020). In our silages obtained in the study, OMD was found between 93.65-52.50%, while ME values were found between 6.94-8.02 MJ/kg DM, and there was no statistical difference between the groups. The effect of the factors on gas production was also found to be insignificant. When the data obtained were compared with the OMD values of adsorbent feeds used in the preparation of carrot pulp silage, it was found that the addition of adsorbent to carrot pulp did not affect ensiling; on the contrary, OMD digestibility increased (Table 6). The differences in nutrient composition, cell wall components, and NDF between groups were not reflected in OMD, ME, and gas production. Studies report that dietary NDF and HP are both negatively and positively associated with gas production (Ndlovu and Nherera, 1997; Larbi et al., 1998). Several factors influence the fermentation of feed by rumen microorganisms and, thus, gas production. Therefore, the lack of difference in our in vitro digestibility results in this study may be due to feed components such as EE and protein that contribute little or no gas production but are degraded in vitro.

Table 6. Effect of adding carrot pulp to adsorbents on OMD (%)

Material	OMD
CP+WS	49.74
Wheat straw	39.63
CP+VOH	50.4
Vetch/oat hay	45.91
CP+CS	55.11
Corn stalk-straw	49.48
CP+AH	48.52
Alfalfa hay	37.58
CP+BS	53.75
Barley straw	42.53

CP+WS= carrot pulp+wheat straw silage, CP+VOH= carrot pulp+vetch/oat hay silage, CP+CS= carrot pulp+corn stalk-straw silage, CP+AH= carrot pulp+alfalfa hay silage, CP+BS= carrot pulp+barley straw silage.

5. Conclusion

In general, the wide variation in the chemical composition of different feeds offers users flexibility in formulating rations according to the productive performance of the target animals. Considering the properties studied for this purpose, carrot pulp, like other agro-industrial by-products, has the potential to be ensiled and used as animal feed.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.F.K.	C.K.B.
C		100
D		100
S		100
DCP	90	10
DAI	90	10
L	60	40
W	50	50
CR		100
SR		100
PM		100
FA		100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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FERMENTATION PROPERTIES AND NUTRITIVE VALUE OF SUNFLOWER ENSILED WITH DIFFERENT MIXING RATIOS OF SILAGE MAIZE, SWEET CORN AND SWEET SORGHUM

İbrahim ERTEKİN[1]*, Ersin CAN[1]

[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, 31060, Hatay, Türkiye*

Abstract: In recent years, with the effect of global warming, researches on the potential of plants that are more resistant to drought as forage crops and their ensiling properties have become increasingly widespread. Current study aimed to investigate the ability of ensiling, fermentation quality and nutritive value of a sunflower variety, which is more drought resistant than many forage crops, with silage corn, sweet corn and sweet sorghum at different mixing ratios. Plant species used as silage material in present study were grown simultaneously in separate plots. All plants were harvested by hand in the range of about 25-32% dry matter and these harvested crops were theoretically chopped with 2-3 cm electric shredding machine for silage. In addition to pure silages of all crop materials, mixed silages of 25%+75%, 50%+50% and 75%+25% were made in 3 replications. Besides some properties of silage beginning materials, some fermentation properties and feed quality parameters of resulted silages were investigated. As a result of this study, 50%+50% mixed silage of the sunflower variety used as silage material with maize, sweet corn and sweet sorghum increased the silage fermentation quality compared to the pure sunflower silages and improved the silage feed quality compared to the pure cereal (corn, sweet corn and sweet sorghum) silages.

Keywords: Silage, Mixed silage, Fermentation, Nutritive value, Sunflower

***Corresponding author:** Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, 31060, Hatay, Türkiye
E mail: ibrahim.ertkn@hotmail.com (İ ERTEKİN)
İbrahim ERTEKİN https://orcid.org/0000-0003-1393-8084
Ersin CAN https://orcid.org/0000-0003-3530-6010

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1. Introduction

Ensiling is described as preserving technology of fresh crops and their by-products based on lactic acid fermentation process under anaerobic conditions (Ertekin and Kızılşimşek, 2020; Ertekin et al., 2022). Preserved materials can be presented to livestocks when attainability to fresh forages is difficult especially in winter period. Lactic acid bacteria found on ensilaged fresh material surface produce the organic acids mainly lactic acid by using water soluble carbohydrates (WSC) and it is preserved fresh crops (Ertekin and Kızılşimşek, 2020) in the silo. Silage fresh materials which has a low water soluble carbohydrates and not produce effectively suitable fermentation end products can be ensiled with sugar sources like molasses, glucose and crops with high WSC etc. (Kung et al., 2018). Crops with high WSC have been commonly used as sugar source in mixed silages.

Sunflower (H*elianthus annus* L.) is a mainly important oil crop around the World due to its physical, chemical and nutritive properties (Souza et al., 2005). This plant has become substantial today, when we feel the effect of global warming even more in terms of using the water in the soil efficiently and being more resistant to drought (Tomich et al., 2003). It is inevitable to have an alternative plant, especially in agricultural lands and marginal areas where water is limited. On the other hand, many studies have been carried out to evaluate this plant as silage and to include it as an alternative forage plant (Ozduven et al., 2009; Cruvinel et al., 2017; Temür et al., 2021; Yıldız et al., 2022). As a result of these studies, it has been reported that the plant can be an alternative forage source, especially in areas where irrigation is limited or no irrigation possibility is available. In addition, it has been suggested to ensilage the plant with various WSC sources to be used as silage.

In recent years, silage of plants as a mixture has become a practical method for silage fermentation quality and nutritional value. In this context, there are many current studies in the literature (Wang et al., 2019; Wang et al., 2020; Zeng et al., 2020; Mu et al., 2021; Wang et al., 2021 Li et al., 2022). It is accepted as a practical method to ensilage the plants with high WSC content as a source of WSC during ensiling, by mixing them with plants which have various cultivation and feeding advantages. Silage maize, sweet sorghum and sweet maize have adequate WSC content to obtain good quality silage (Kizilsimsek et al., 2017; Wang et al., 2019; Ertekin 2021; Ertekin and Yilmaz 2022). Ensiling these crops with crops containing low WSC can create a quality fermentation process.

Present study was carried out to determine the effect of

mixtures of sunflower with silage maize, sweet maize and sweet sorghum on silage fermentation quality and nutritive value.

2. Materials and Methods

Cultivars of hybrid snack sunflower (cv. F400), hybrid silage maize (cv. BATEM 7255) sweet maize (cv. BATEM tatli) and sweet sorghum (cv. Erdurmuş) were cultivated on separate parcels in Field 49 area of Hatay Mustafa Kemal University in 2022 growing season. Cultivated all these crops were harvested at same date (July 30, 2022). Cultivars of silage maize, sweet maize and sweet sorghum were harvested about 30±2% dry matter content while hybrid sunflower cultivar was harvested in dough stage with about 25% dry matter. Harvested crops were chopped theoretically 2-3 cm theoretical lenght via chopping machine and silage maize, sweet maize and sweet sorghum crops were ensilaged with snack sunflower by mixing at rate of 25%+75, 50%+50% and 75%+25%. In addition, pure silages of all component crops were also made to compare the pure silages with mixture. Before ensiling, 500 g fresh material was taken from both mixture and pure samples to obtain dried samples for chemical analysis of the initial materials. Chopped fresh material was ensiled to vacuum bag as 300±50 g via vacuum packaging machine. The laboratory type plastic silos were stored in dark conditions at room temperature during 75 days. At the end of fermentation, silage samples were opened and about 100±20 g of samples were taken for chemical composition analysis. According to Yan et al. (2019), 20 g fresh silage was weighted and 180 ml ringer solution was added to weighted silages. Samples mixed with silage and ringer solution were blended during 1 minutes. It was obtained filtered samples from blended samples. These filtered samples were used for some analysis.

At the end of fermentation process, the silages obtained mixtures and pure fresh material and initial materials were analyzed to determine dry matter (DM), crude ash (CA) and crude protein (CP) according to procedure of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with ANKOM technology according to methods of Van Soest et al. (1991). Acid detergent lignin (ADL) contents of the silage and fresh materials were evaluated baker method using 72% sulfuric acid. Water soluble carbohydrates (WSC) of fresh materials were determined according to phenol-sulfuric acid method described by Dubois et al. (1956). Contents of lactic acid (LA), acetic acid (AA), propionic acid (PA) butyric acid (BA) and ethanol (ETOH) were analyzed by using high pressure liquid chromatography at 42°C, 0.6 mL min^{-1} flow rate and by using refractive index detector described by de Quiros et al. (2009) after the sample cleaning procedure. The silage pH was determined via table typed pH meter in filtered liquid samples isolated from fresh silages according to the Yan et al., (2019). Ammonia nitrogen (NH_3-N) contents of silages were analyzed with distillation unit of Kjeldahl apparatus using 100 mL filtered silage liquid samples.

Opened silage samples and fresh silage material were investigated in terms of silage microbial content. In this contexts, Filtered samples isolated from both fresh material and silage material were diluted from 10^{-1} to 10^{-10}. Diluted samples were spread in disposable sterile plastic petri dishes and to determine the lactic acid bacteria and yeast and mold, it was used MRS agar (De Man Ragosa and Sharpe) and MEA (Malt Extract Agar) nutrient media, respectively. Prepared petri dishes with filtered samples and nutrient media were put in an incubator. These samples were allowed to proliferate for lactic acid bacteria and yeast and mold during 48 hours at 37°C.

All data obtained from current study were evaluated according to general linear model using SAS JMP 13.0 statistical package programme. Traits found significant in 0.05 probability level were compared Tukey pairwise test.

3. Results and Discussion

The dry matter (DM), chemical compositions and microbial counts of silage beginning materials are given in Table 1. Investigated properties for DM, chemical compositions and microbial counts expect for mold were significant among plant species. These results indicated that there are vital differences among plant species in terms of silage beginning characteristics. The most quality crop in terms of nutritive value was sunflower. The highest WSC content and LAB count were determined in sweet sorghum.

Results given for NDF, ADF, ADL, CA and CP properties of silages evaluated in terms of nutritive value are in Table 2. All characteristics were found to be significant due to effects of pure and mixture silage treatments. The NDF contents of the silages ranged from 50.02 to 65.16 %. The highest NDF value was obtained from 25SF+75SS treatment while the lowest was in 50SF+50M treatment. It has been reported that excessively high NDF content in feeds can cause various ailments in animals fed with these feeds (Beauchemin, 1996). On the other hand, NDF content of less than 32% is known to be a disadvantage in terms of animal nutrition (Broderick, 2003). The NDF contents of pure M, SM and SS were higher than pure SS treatment. However, as the sunflower ratio increased, the NDF content did not increase. Presumably, the presence of microorganisms that degrade cell wall substances in pure sunflower silages may have caused this situation. The ADF content ranged from 20.21 to 40.81 % (Table 2). The highest ADF value was detected in 75SF+25M treatment, whereas the lowest ADF was found in 25SF+75M treatment. It has been stated that as the ADF content in the feeds decreases, the digestibility of the feeds increases (Ball et al., 2001). Therefore, it can be said that 25SF+75M treatment has a more suitable ADF content in terms of animal nutrition. The ADL contents of the silages obtained from pure and mixture silage materials ranged from 3.31 to 9.20 % (Table 2). The

highest ADL value was obtained from pure SF while the lowest ADL content was in pure M. High ADL content is undesirable since it negatively affects the digestion in terms of animal nutrition and does not contribute energy to the fed animals (Manaye et al., 2009). As the proportion of sunflower in mixed silages increased the ADL content of the silages increased. The CA contents of pure and mixed silages ranged from 16.01 and 26.02%. The highest CA was obtained from 50SF+50SS treatment while the lowest value was in Pure SC silage. The crude ash content for feeds is very important trait because of its nutritional value (Quirino et al., 2023). Nurk et al. (2017) reported that the CA of mixed silages changes according to mixing ratios in a research used as silage material of maize and common bean similar to results of current study. The CP ratio significantly affected from the treatments and ranged from 5.43 and 10.56%. The highest CP content was determined in 75SF+25SC treatment whereas the lowest CP was in Pure M. The CP content in feeds is one of the most important nutrients that meet the basic nutritional needs of animals fed with feed (Rezende et al., 2023). Nurk et al. (2017) stated that the CP of mixed silages made from maize and common bean improve similar to results obtained from present study.

Table 1. Dry matter, chemical compositions and microbial counts of fresh material before ensiling

Properties	Plant species				P values
	Maize	Sunflower	Sweet Corn	Sweet Sorghum	
DM (%)	31.03±0.68 a	25.69±0.40 b	32.16±0.75 a	30.17±0.46 a	0.0003
NDF (% DM)	50.62±0.49 c	53.59±0.64 bc	57.09±0.95 b	62.20±1.73 a	0.0003
ADF (% DM)	24.48±0.20 d	36.74±0.37 a	27.10±0.46 c	34.35±0.74 b	<.0001
ADL (% DM)	2.08±0.08 c	7.92±0.15 a	1.69±0.13 c	3.77±0.20 b	<.0001
CA (% DM)	6.41±0.06 d	11.08±0.09 a	7.19±0.06 b	6.80±0.05 c	<.0001
CP (% DM)	5.62±0.18 c	12.45±0.16 a	9.03±1.35 b	5.09±0.08 c	0.0002
WSC (% DM)	19.15±0.54 b	5.25±0.12 c	22.27±0.58 a	23.38±0.63 a	<.0001
LAB (log_{10} cfu g^{-1} DM)	3.18±0.09 b	2.68±0.12 c	3.11±0.03 bc	3.83±0.14 a	0.0004
Yeast (log_{10} cfu g^{-1} DM)	3.86±0.07 b	2.22±0.03 c	4.12±0.06 a	4.26±0.05 a	<.0001
Mold (log_{10} cfu g^{-1} DM)	1.12±0.07	1.23±0.03	1.24±0.07	1.10±0.07	ns

DM= dry matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, CA= crude ash, CP= crude protein, WSC= water soluble carbohydrate, LAB= lactic acid bacteria, ns: not significant

Table 2. Chemical compositions of pure and mixed silages obtained from different plant species

Treatments	Properties				
	NDF (% DM)	ADF (% DM)	ADL (% DM)	CA (% DM)	CP (% DM)
Pure M	55.79±0.84 cd	30.26±0.59 de	3.31±0.08 e	21.95±5.25 abc	5.43±0.03 f
Pure SC	58.89±0.05 bc	33.67±0.57 cd	3.63±0.49 e	16.01±0.50 c	8.80±0.09 c
Pure SF	51.65±0.08 ef	38.97±0.02 ab	9.20±0.04 a	16.98±2.92 bc	9.58±0.20 abc
Pure SS	65.13±1.19 a	39.73±0.92 a	4.93±0.04 cde	16.13±0.61 c	5.67±0.03 f
25SF+75M	50.91±0.31 ef	29.21±0.23 e	3.61±0.24 e	25.55±0.14 a	6.34±0.12 ef
25SF+75SC	56.92±0.71 cd	33.42±0.36 cd	4.66±0.13 de	22.85±2.06 abc	9.29±0.06 bc
25SF+75SS	65.16±0.27 a	37.75±0.29 ab	5.70±0.12 cd	23.08±3.59 ab	7.75±0.24 d
50SF+50M	50.02±0.45 f	30.23±0.70 de	4.67±0.14 de	24.61±1.17 a	7.39±0.09 d
50SF+50SC	54.50±1.18 de	34.14±1.06 c	6.38±0.38 bc	23.46±1.48 ab	10.33±0.11 a
50SF+50SS	56.91±0.81 cd	36.15±0.68 bc	6.57±0.36 bc	26.02±0.72 a	8.98±0.10 c
75SF+25M	61.81±1.27 ab	40.81±1.14 a	8.70±0.47 a	16.99±2.91 bc	6.94±0.51 de
75SF+25SC	56.59±0.19 cd	37.76±0.75 ab	7.92±0.66 ab	23.14±1.37 ab	10.56±0.18 a
75SF+25SS	57.40±0.16 cd	38.01±0.54 ab	7.66±0.18 ab	22.68±1.96 abc	10.20±0.23 ab
P values	<.0001	<.0001	<.0001	0.0312	<.0001

DM= dry matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, CA= crude ash, CP= crude protein, M= maize, SC= sweet corn, SF= sunflower, SS= sweet sorghum

Parameters of silage pH, ammonia nitrogen (NH_3-N), lactic acid bacteria (LAB), yeast and mold counts significantly affected from treatments (Table 3). The silage pH was between 3.54 and 4.22. As sunflower ratio increased in mixed silages, silage pH ascended. Many studies informed that the silage pH in mixed silages increased as the ratio of effortless ensilaged crops increased (Wang et al., 2019; Zeng et al., 2020; Mu et al., 2021). The NH_3-N content of silages obtained from pure and mixed treatments was between 4.22 and 7.09%. The highest NH_3-N were found in Pure SF while the lowest value was in Pure M. As the SF ratio in mixed silages

increased the NH_3-N content increased. It was stated that the NH_3-N content of silage materials with high N content is higher than that of low N content similar to results obtained from present study (Zeng et al., 2020). The LAB count ranged from 3.32 to 5.54 log_{10} cfu g^{-1} DM. While the lowest LAB count was obtained from pure SF treatment the highest LAB was detected in Pure SC. As the sunflower ratio in the mixed silages decreased, the LAB number of the silages increased. Similar to results obtained from current study, some researchers reported LAB count in pure and mixed silages (Wang et al., 2019; Zeng et al., 2020). Yeast and mold count of pure and mixed silages were between 1.43-2.44 log_{10} cfu g^{-1} DM and 1.25-1.70 log_{10} cfu g^{-1} DM. For both yeast and mold count, the highest value was obtained from pure SS the lowest value was in pure SF. As the sunflower ratio in the mixed silages increased, both the yeast and mold counts of the silages decreased. Similar to the results obtained from this study, it was reported that both yeast and mold numbers decreased as the ratio of other plants in the cereal silage increased (Pursiainen and Tuori 2008).

Table 3. Silage pH, microbial counts and ammonia nitrogen contents of the silages obtained from pure and mixed of different plant species

Treatments	Properties				
	pH	NH_3-N (% DM)	LAB (log_{10} cfu g^{-1} DM)	Yeast (log_{10} cfu g^{-1} DM)	Mold (log_{10} cfu g^{-1} DM)
Pure M	3.73±0.01 h	4.22±0.19 e	4.37±0.08 cd	2.02±0.03 bcd	1.54±0.06 abc
Pure SC	3.65±0.02 i	4.39±0.21 de	4.75±0.06 bc	2.10±0.04 bc	1.46±0.09 abc
Pure SF	4.22±0.01 a	7.09±0.33 a	3.32±0.15 g	1.43±0.05 g	1.25±0.04 c
Pure SS	3.54±0.01 j	4.29±0.26 de	5.54±0.16 a	2.44±0.05 a	1.70±0.13 a
25SF+75M	3.93±0.02 f	5.39±0.22 cde	3.99±0.08 def	2.02±0.02 bcd	1.47±0.04 abc
25SF+75SC	3.86±0.01 fg	5.52±0.13 cd	4.75±0.08 bc	1.90±0.04 b-e	1.41±0.07 abc
25SF+75SS	3.73±0.01 h	5.74±0.21 bc	5.24±0.12 ab	2.40±0.05 a	1.59±0.09 ab
50SF+50M	4.09±0.02 cd	6.11±0.26 abc	3.83±0.05 ef	1.87±0.01 c-f	1.40±0.03 abc
50SF+50SC	4.00±0.01 e	6.19±0.14 abc	4.41±0.08 cd	1.82±0.06 def	1.36±0.06 bc
50SF+50SS	3.85±0.00 g	6.44±0.21 abc	4.75±0.06 bc	2.11±0.06 b	1.48±0.05 abc
75SF+25M	4.16±0.01 ab	6.82±0.30 ab	3.53±0.07 fg	1.63±0.06 fg	1.33±0.03 bc
75SF+25SC	4.12±0.01 bc	6.87±0.22 ab	3.86±0.11 ef	1.74±0.06 ef	1.30±0.05 bc
75SF+25SS	4.03±0.01 de	6.89±0.36 ab	4.18±0.08 de	2.02±0.05 bcd	1.37±0.01 abc
P values	<.0001	<.0001	<.0001	<.0001	0.0032

DM= dry matter, pH= power of hydrogen, NH_3-N= ammonia nitrogen, LAB= lactic acid bacteria, M= maize, SC= sweet corn, SF= sunflower, SS= sweet sorghum

The lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ethanol (ETOH) properties were affected by pure and mixture silages obtained from maize, sweet corn, sunflower and sweet sorghum crops (Table 4). When the all silage end products were investigated, the highest LA, AA, PA, BA and ETOH were obtained from Pure SS treatment whereas the lowest value for all silage end products were determined in Pure SF. As the proportion of the sunflower in mixed silages increased, Values for all silage end product characteristics decreased. Such results in studied on mixture silages stated and it was emphasized that plants that promote lactic acid production should be used to improve lactic acid content in silages (Pursiainen and Tuori 2008; Kennedy et al., 2018; Di Miceli et al., 2023).

5. Conclusion

This study was carried out to determine the nutritive value and silage fermentation quality of pure and mixed silages of maize, sweet corn, sunflower and sweet sorghum plants. Compared to pure cereal silages, the nutritive value of all mixture silages improved because sunflower crop offered a higher quality nutritive value. On the other hand, as the proportion of cereal in mixed silages increased, silage fermentation quality improved. When all the results obtained from the current study are evaluated, the use of 50%+50% sunflower and corn, sweet corn or sweet sorghum in silages may provide a better quality silage in terms of both nutritive value and silage fermentation quality.

Table 4. Lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ethanol (ETOH) contents of the silages obtained from pure and mixed of different plant species

Treatments	Properties				
	LA (% DM)	AA (% DM)	PA (% DM)	BA (% DM)	ETOH (% DM)
Pure M	4.23±0.06 abc	1.33±0.04 bcd	0.54±0.03 b-f	0.36±0.02 cd	0.88±0.02 cde
Pure SC	4.34±0.16 ab	1.48±0.02 ab	0.69±0.04 ab	0.42±0.03 abc	1.05±0.04 bc
Pure SF	2.00±0.08 j	0.72±0.05 i	0.29±0.02 g	0.25±0.01 d	0.53±0.05 f
Pure SS	4.49±0.19 a	1.56±0.06 a	0.77±0.06 a	0.53±0.05 a	1.43±0.11 a
25SF+75M	3.77±0.05 cde	1.23±0.04 cde	0.50±0.03 def	0.35±0.02 cd	0.86±0.01 cde
25SF+75SC	3.86±0.10 bcd	1.35±0.02 bcd	0.62±0.03 a-d	0.40±0.02 bc	0.99±0.04 cd
25SF+75SS	3.97±0.16 abc	1.40±0.05 abc	0.68±0.04 abc	0.48±0.04 ab	1.28±0.09 ab
50SF+50M	3.21±0.05 fgh	1.08±0.04 e-h	0.44±0.03 efg	0.33±0.01 cd	0.78±0.02 c-f
50SF+50SC	3.27±0.05 efg	1.15±0.03 d-g	0.52±0.02 c-f	0.36±0.01 cd	0.86±0.04 cde
50SF+50SS	3.35±0.13 def	1.19±0.04 c-f	0.56±0.03 b-e	0.41±0.03 abc	1.05±0.07 bc
75SF+25M	2.66±0.06 i	0.93±0.04 hi	0.38±0.02 fg	0.30±0.01 cd	0.69±0.03 ef
75SF+25SC	2.68±0.03 hi	0.97±0.04 gh	0.42±0.02 efg	0.31±0.01 cd	0.73±0.05 def
75SF+25SS	2.72±0.10 ghi	0.98±0.04 fgh	0.44±0.02 efg	0.34±0.02 cd	0.82±0.06 cde
P values	<.0001	<.0001	<.0001	<.0001	0.0032

DM= dry matter, LA= lactic acid, AA= acetic acid, PA= propionic acid, BA= butyric acid, ETOH= ethanol, M= maize, SC= sweet corn, SF= sunflower, SS= sweet sorghum

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	İ.E.	E.C.
C	50	50
D	100	
S		100
DCP	90	10
DAI	60	40
L	80	20
W	50	50
CR	20	80
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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JUMBO BROWN AND GOLDEN ITALIAN JAPANESE QUAIL: A COMPARATIVE EXAMINATION OF EGG QUALITY, EGG YOLK LIPID PEROXIDATION AND FATTY ACID PROFILES

Enfal ÖNCÜ GÜL[1], Hasan Hüseyin İPÇAK[1]*

[1]*Dicle University, Faculty of Agriculture, Department of Animal Science, 21280, Diyarbakır, Türkiye*

Abstract: This study was conducted to compare two different Japanese quail (*Coturnix coturnix japonica*) breed lines in terms of egg quality, egg yolk lipid peroxidation, and fatty acid profiles. The research was carried out with Jumbo Brown (Jumbo Brown *Coturnix japonica*) and Golden Italian (Golden Italian *Coturnix japonica*) Japanese quail breed lines with dark brown and golden yellow plumage colors at an average body weight of 200±10 g and 10 weeks of age. The experimental groups consisted of Jumbo Brown Japanese quail breed and Golden Italian Japanese quail breed, each containing 80 Japanese quails fed a standard quail diet. Each group was divided into 20 subgroups, with 4 quails housed in each subgroup. The study lasted for 10 weeks (11–20 weeks) according to the randomized plot experimental design. The egg quality data were recorded over an overall period, divided into two periods of 5 weeks each: period 1 (11 to 15 weeks) and period 2 (16 to 20 weeks). Egg and eggshell weights of the Golden Italian breeds were higher in the overall period ($P<0.05$). The eggshell proportion increased only in the 2nd period in the Golden Italian breed ($P<0.05$). No significant differences were observed between the breed lines in terms of egg-specific gravity, egg shape index, eggshell thickness, albumen index, yolk index, Haugh unit, and egg yolk color (L, a, b) values ($P>0.05$). However, the egg yolk of the Jumbo Brown breed had a higher crude protein content ($P<0.05$). No difference was observed in yolk malondialdehyde values between breeds in fresh and stored eggs ($P>0.05$). Moreover, the egg yolk ΣPUFA/ΣSFA ratio, ΣPUFA, and Σn-6 values were higher in the Jumbo Brown breed than in the Golden Italian breed ($P<0.05$). In conclusion, Jumbo Brown breed eggs may be an alternative to traditionally consumed chicken eggs because of their high yolk crude protein content and favorable fatty acid profile.

Keywords: Japanese quails, Breed, Egg quality, Oxidative stability, Fatty acid profiles, Alternative food

***Corresponding author:** Dicle University, Faculty of Agriculture, Department of Animal Science, 21280, Diyarbakır, Türkiye
E mail: huseyinipcak@gmail.com (H. H. İPÇAK)
Enfal ÖNCÜ GÜL https://orcid.org/0009-0009-9439-0727
Hasan Hüseyin İPÇAK https://orcid.org/0000-0002-6807-8870

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1. Introduction

Japanese quails (*Coturnix coturnix japonica*) are the smallest birds among poultry species commonly raised for meat and eggs worldwide (Mizutani, 2003; Chang et al., 2005). The breeding and production systems of Japanese quails are similar to those observed in laying hens, and the rearing phase can occur in floor systems or battery cages. Japanese quails can start laying at 42 days of age, and usually continue laying for up to 60 weeks. Egg production peaks at over 95% and maintains high egg production levels (more than 90%) for longer than hens (Bertechini and Oviedo-Rondon, 2023). Therefore, quail farming has become increasingly popular in the poultry industry. However, non-chicken egg production remained at a certain level. It has been reported that 87 million tons of eggs are produced from laying hens worldwide, while only 6.3 million tons of eggs are produced from quails, accounting for 10% of all table eggs (TRIDGE, 2020; FAO, 2021). This can be attributed to the fact that chicken egg production and consumption is a widespread and large-scale industry worldwide, with consumer habits in general. However, the interest of today's consumers in healthy nutrition, food safety, and quality has forced market boundaries to change, and quail farming for egg production has gained momentum, especially in Asian countries such as China, Brazil, and Russia (TRIDGE, 2020). Although quail eggs are smaller and lighter than chicken eggs, they are richer in certain vitamins (B12 sources) and minerals (Fe, P, Se, and Zn) (Tunsaringkarn et al., 2013; Shoemaker, 2020). Therefore, it is important to determine egg quality characteristics and the factors affecting these characteristics to increase the acceptability of quail eggs as an alternative to chicken eggs in human diets.

Egg external and internal quality characteristics can be associated with genetics, feeding, management, storage conditions, stocking density, egg processing, and stage of the production cycle (Douglas, 2013). Punya Kumari et al. (2008) determined that the quality characteristics of Japanese quail eggs have high heritability. In contrast, several studies have reported that plumage color or genetic diversity can play a significant role in quail egg

quality (Hrnčár et al., 2014; İnci et al., 2015; Hassan et al., 2017). Moreover, Cahyadi et al. (2019) found that the eggs of the brown plumage quail line possessed superior external and internal characteristics compared to the eggs of the black plumage line. Petek et al. (2022), however, found that eggs from the cross-line black coloured quail line are heavier and possess higher breaking strength compared to the eggs of the wild-type and recessive white quail lines. They also determined that eggs from the white line of quails had a shorter egg length and the highest shape index values, along with a lighter egg yolk color. However, quail eggs have a low saturated fat ratio and contain high unsaturated fatty acids, thus having an appropriate PUFA/SFA and n-6/n-3 ratio (Cufadar et al., 2021; Göçmen et al., 2021). Previous studies have found that quail eggs have a fatty acid profile above the 0.45 PUFA/SFA ratio and 4:1 n-6/n-3 ratio recommended by the HMSO (1994) for a healthy human diet (Özbilgin et al., 2021). Moreover, quail eggs contain twice as much docosahexaenoic acid (DHA) as chicken eggs do (Kazmierka et al., 2005). Indeed, a healthy fatty acid composition not only serves as a significant energy source in the human body but also plays other biological roles, such as regulating membrane structure and function, modulating intracellular signal pathways, transcription factor activity, gene expression, and production of bioactive lipid mediators (Calder, 2015). Therefore, quail eggs can be promoted in the human diet as an alternative to healthy and balanced diets because of their rich nutrient contents. However, consumers generally have a limited understanding of yolk fat content and composition in eggs, and are unaware of the potential nutritional and health benefits of including quail eggs in human diets. However, the lack of studies on yolk oxidative stability in quail eggs indicates that research in this area is largely limited to chicken eggs. Therefore, further research is needed to characterize the specific nutrient profiles of quail eggs. In this context, this study aimed to comparatively investigate the external and internal egg quality, yolk oxidative stability, and fatty acid profiles of the Jumbo Brown and Golden Italian quail breeds.

2. Materials and Methods

2.1. Bird, Diets, Housing and Management

This research was carried out at Dicle University, Faculty of Agriculture, Department of Animal Science, Poultry Research and Application Facilities. Jumbo brown (Jumbo Brown Coturnix japonica) and golden Italian (Golden Italian Coturnix japonica) Japanese quail (Coturnix coturnix japonica) breed lines with dark brown and golden yellow plumage colors with an average body weight of 200±10 g and 10 weeks of age were used as animal materials. The experiment consisted of groups of Jumbo Brown Japanese quail fed standard quail egg feed and Golden Italian Japanese quail fed standard quail egg feed, each containing 80 laying quails. Each group was divided into 20 subgroups, and four quails were housed in each subgroup. At the beginning of the experiment, the body weights and egg production levels of the quails were determined, and the quails were randomly distributed to the battery cage system according to their similar body weights and egg production levels. Quails were housed in a battery cage system made of galvanized material with a nipple drinker and four compartments (1 m x 0.4 = 0.4 m2) on each floor. The study was conducted for 10 weeks (11 to 20 weeks) according to the randomized plot experimental design, and feed and water were provided ad libitum during the experiment. During the experiment, 16 h of light and 8 h of dark were applied. Lighting was performed using light bulbs at night. The temperature inside the poultry house was maintained at 22-24 °C and the relative humidity was 55-60%. Temperature and humidity were monitored daily during the experiment using a digital temperature and humidity meter (VZN, Türkiye).

The quails were fed laying quail diet throughout the experiment. The diets of the experimental groups were prepared in mash form at the feed production facility of Dicle University, Faculty of Agriculture, Department of Animal Science, in accordance with the nutrient requirements of laying quail, as reported by NRC (1994). To prepare the diets, the nutrient composition of the major ingredients was determined prior to the experiment. The composition (g/kg), nutrient content (%), and Metabolizable Energy (kcal ME/kg) levels of the diets used in this study are presented in Table 1. In addition, the nutrient content of the diets was determined using the Weende analysis method (AOAC 2000). Crude protein (CP) determination in the diet was performed using an automatic nitrogen/protein analyzer (Leco FP-528, USA). Ether extract (EE) analysis was performed using an automatic fat determination (Soxhlet) device (Velp Scientifica, Italy), and crude fiber (CF) content was determined according to the Lepper method (Bulgurlu and Ergül, 1978). Starch determination was carried out in two stages according to the TS ISO 6493 standard (TSE, 2004), and the Luff-Schoorl method reported in the TS 12232 standard was used to determine the sugar content (TSE, 1997). In addition, the following regression equations reported by Carpenter and Clegg (1956) and TSE (1991) (TSE No: 9610) were used to calculate the ME content of the laying quail diet (Equation 1).

$$\text{ME kcal/kg} = 38*[(CP\times1) + (EE\times2.25) + (\text{Starch}\times1.1) + (\text{Sugar}\times1.05)]+53 \quad (1)$$

2.2. Egg Quality Measurements

In order to determine egg quality in the study, throughout the 10-week experiment, 10 eggs were collected weekly from each group on the same day and hour, and were brought to the Dicle University Faculty of Agriculture Department of Animal Science feed/food analysis laboratory. However, egg quality data were recorded over an overall period of 10 weeks, divided into

two periods of 5 weeks each: period 1 (11–15 weeks) and period 2 (16-20 weeks). In this context, first, the weights (g) of the eggs were determined using a balance with a precision of 0.01 g (Dikomsan FGH series, Türkiye). Subsequently, the width (mm) and length (mm) of the eggs were measured using a digital caliper (Mitutoyo, Japan) and their specific gravity was measured using a RADWAG balance (AS 220.R2, Poland).

Table 1. Ingredients, Composition (%), nutrient content (%), and Metabolizable Energy (kcal/kg) levels of the laying quail diet

Items	%
Corn	52.50
Soybean meal (44% CP)	23.00
Sunflower meal (32% CP)	10.00
Bone meal (27 % Ca, 18 % CP)	2.00
Sunflower oil (8800 Kcal/kg)	3.45
Limestone ($CaCO_3$)	7.20
Dicalciumphosphate	1.10
DL-Methionine	0.10
Vitamin - Mineral Premix[1]	0.25
NaCl	0.40
Analysed chemical composition (%)	
Dry matter	90.5
Crude protein	18.20
Ether extract	5.11
Crude fiber	5.10
Crude ash	13.00
Starch	36.01
Sugar	3.34
Calculated values	
Metabolizable energy (ME poultry), kcal/kg	2819.99
Lysine, %	0.86
Methionine+cystine,	0.70
Ca, %	3.20
Available P,	0.50

[1]Vitamin and mineral premix providing per 2.5 kg of diet: vitamin A, 12000000 IU; Vitamin D3, 2500000 IU; Vitamin E, 30000 mg; Vitamin K3, 4000 mg; Vitamin B1, 3000 mg; Vitamin B2, 7000 mg; Vitamin B6, 5000 mg; Vitamin B12, 15 mg; Ca-D pantothenate, 10000 mg; Biotin, 45 mg; Vitamin C, 50000 mg; Folic acid, 1000 mg; Niacin amide, 30000 mg; Choline chloride, 200000 mg; Mn (II) oxide, 80000 mg; Fe (II) sulphate, 50000 mg; Cu (II) sulphate, 5000 mg; Zn sulphate, 60000 mg; Ca iodide, 1000 mg; Na selenite, 150 mg,; Apo karotenoik ester 10%, 500 mg; and Kantaksantin 10% 2000 mg.

Shell weights (g) were weighed on a balance, and shell proportions (%) were determined by relating shell weights to egg weights. Shell thickness (mm) was measured using an electronic digital micrometer (Mitutoyo, Japan) with a range of 0-25 mm and a precision of 0.001 mm. Egg shape index was calculated according to the equation reported by Anderson et al. (2004) (Equation 2).

$$\text{Egg shape index, \%} = (\text{egg width}/\text{egg length}) \times 100 \quad (2)$$

Egg albumen length (mm) and width (mm) and yolk diameter (mm) were measured with a digital caliper (Mitutoyo, Japan). Egg albumen and yolk heights (mm) were determined using a mechanical pedestal micrometer (Accud, Türkiye). The egg albumen index and egg yolk index were determined using the following equations (Equations 3-4) reported for laying hens by Heiman and Carver (1936) and Funk (1948):

$$\text{Albumen index, \%} = \frac{AH}{\left[\frac{(LDA+SDA)}{2}\right]} 100 \quad (3)$$

here, AH is Albumen height, LDA is long diameter of albumen and SDA is short diameter of albumen.

$$\text{Yolk index, \%} = \frac{Yolk\ height}{Yolk\ diameter} 100 \quad (4)$$

Haugh units were calculated using the following formula based on the values of white height (mm) and egg weight (g) per egg (Haugh, 1937) (Equation 5):

$$\text{Haugh unit} = 100 \times \log(\text{albumen height} + 7.57 - 1.7 \times \text{egg weight}^{0.37}) \quad (5)$$

Egg yolk color was determined by measuring brightness (L*), redness (a*), and yellowness (b*) using a colorimeter (3nh, China). The L* values ranged from 0 (completely black) to 100 (completely white). A lower L value indicates a darker color, whereas a higher L value indicates a lighter color. The a* value indicates where the color lies in the spectrum, from green (negative values) to red (positive values). This value ranges from -50 to +50, with -50 being exactly green and +50 being exactly red. b* indicates where the color lies in the spectrum from blue (negative values) to yellow (positive values). This value ranges from -50 to +50, with -50 being exactly blue and +50 being exactly yellow. Three readings were taken for each sample, and the instrument was calibrated with white and black tiles before each measurement.

2.3. Determination of Egg Nutrient Composition

Dry matter (DM), crude ash (CA), crude protein (CP), and ether extract (EE) analyses of egg albumens, yolks, and edible parts were performed according to the AOAC (2000). The DM values of the egg samples were determined by drying a certain amount of samples at 105 °C overnight, and the CA values were determined by incineration at 550 °C overnight. CP values were measured using an automatic nitrogen/protein analyzer (Leco FP-528, USA). EE was determined by extracting the samples with hexane (Tekkim, Türkiye) for a certain period in an automatic oil determination (Soxhlet) device

(Velp Scientifica, Italy). The determined values were obtained on a dry-matter basis.

2.4. Analysis of Egg Yolk Lipid Peroxidation

The amount of malondialdehyde (MDA), a secondary decomposition product of lipid peroxidation, in yolk samples from eggs stored at room temperature (22±2°C) on different days (0, 14, and 28) was determined using the TBARS (thiobarbituric acid-reactive substance) method developed by Witte et al. (1970). The absorbance of the pink complex formed as a result of the reaction between MDA and thiobarbituric acid (TBA; Merck, Darmstadt, Germany) was measured spectrophotometrically (UV-1201, Shimadzu, Kyoto, Japan) at 532 nm. A solution of trichloroacetic acid (TCA; Merck, Darmstadt, Germany) (20% weight/volume) was used for extraction. The MDA standard graph was drawn by preparing dilutions of 1.1.3.3 tetraethoxypropane (Merck, Darmstadt, Germany) at 0.5, 1, 2, 4, 5, 10, and 20 µmol/L. TBARS values were expressed in mg MDA/kg of egg yolk.

2.5. Determination of Egg Yolk Fatty Acid Profile

For the fatty acid profile of egg yolk, fats were extracted from 20 egg yolks, 10 from each group, using the solvent method with a 2:1 ratio of chloroform/methanol (Anonymous, 1987). Fatty acid methyl esters of egg yolks were determined according to the Turkish Food Codex, European Communities Commission Regulation (Announcement No.2014/53) using a gas chromatography device (Agilent 7890 GC/FID, USA) equipped with a flame ionization detector and a silica capillary column (Anonymous, 2014). Nitrogen was used as the carrier gas and the flow rate was set to 35 mL/min. The hydrogen and air pressures were set at 0.5 kg/cm. The oven temperature was initially maintained at 165 °C for 15 min and then raised to 200 °C at a rate of 5 °C/min, and the injector and detector temperatures were held constant at 250 °C. The injection volume was 1µl. The fatty acid composition of egg yolk was expressed as a % of the total fatty acids.

2.6. Statistical Analysis

Statistical analyses of the data from the experimental groups were performed using SPSS (version 22.0; SPSS, 2013). The normality was checked using the Shapiro-Wilk test, and Levene's test was for homogeneity of variances. Statistical calculations of the groups were evaluated using an independent sample t-test. ANOVA was used to determine the differences in MDA data according to the storage periods. Values with a significance level of $P<0.05$ were considered statistically significant, and $0.05<P<0.10$ were considered as trends.

3. Results

3.1. External and Internal Egg Quality

The weekly egg external quality values of the two quail breeds are summarized in Table 2. Eggs of Golden Italian quail breeds in the same age group were heavier than Jumbo Brown quail eggs in the 1st period (11–15 weeks) and overall period (11–20 weeks) ($P<0.05$).

Table 2. External egg quality values of two different Japanese quail breeds

Items	Jumbo Brown	Golden Italian	SEM[1]	*P*-value
Egg weight, g				
11 to 15 wk	12.30[b]	12.97[a]	0.162	0.027
16 to 20 wk	11.60	12.32	0.238	0.133
11 to 20 wk	11.95[b]	12.65[a]	0.160	0.024
Egg specific gravity				
11 to 15 wk	1.062	1.063	0.001	0.390
16 to 20 wk	1.053	1.058	0.003	0.355
11 to 20 wk	1.057	1.061	0.002	0.288
Egg shape index, %				
11 to 15 wk	76.57	77.87	0.564	0.287
16 to 20 wk	78.79	78.46	0.617	0.803
11 to 20 wk	77.68	78.16	0.438	0.599
Eggshell weight, g				
11 to 15 wk	1.39	1.44	0.032	0.471
16 to 20 wk	1.18[b]	1.44[a]	0.049	<0.001
11 to 20 wk	1.29[b]	1.44[a]	0.031	0.008
Eggshell proportion, %				
11 to 15 wk	11.30	11.16	0.250	0.800
16 to 20 wk	10.29[b]	11.72[a]	0.292	0.014
11 to 20 wk	10.74	11.44	0.190	0.063
Eggshell thickness, mm				
11 to 15 wk	0.291	0.284	0.005	0.563
16 to 20 wk	0.282	0.302	0.011	0.411
11 to 20 wk	0.286	0.293	0.006	0.603

Differences between mean values with different letters (a-b) in the same row are statistically significant at $P<0.05$ level. [1]SEM, standard error of means.

In addition, eggshell weight in the 2nd period (16 to 20 weeks) and overall period and eggshell proportion in the 2nd period were higher in Golden Italian quail breeds (P<0.05). In contrast, egg specific gravity, egg shape index, and egg shell thickness were not significantly different among the quail breeds (P>0.05). However, the proportion of eggshells tended to increase in the Golden Italian quail breeds for the overall period.

There was no difference in the egg albumen index, egg yolk index, Haugh unit, and yolk L, a, and b values between quail breeds in all periods (P>0.05; Table 3).

3.2. Egg Nutrient Composition

The nutrient compositions of egg yolk, egg albumen, and edible egg parts of the quail breeds are listed in Table 4. Accordingly, only the yolk CP content of the Jumbo Brown quail breed was significantly higher than that of the Golden Italian quail breeds (P<0.05). In contrast, it was found that yolk DM and egg albumen EE of Golden Italian quail breeds showed an increasing trend compared to Jumbo Brown quail breeds.

Table 3. Internal egg quality values of two different Japanese quail breeds

Items		Jumbo Brown	Golden Italian	SEM[1]	*P*-value
Albumen index, %					
11 to 15 wk		9.05	8.39	0.307	0.306
16 to 20 wk		10.08	9.21	0.525	0.438
11 to 20 wk		9.57	8.80	0.315	0.231
Yolk index, %					
11 to 15 wk		42.15	44.00	0.716	0.213
16 to 20 wk		42.03	41.35	0.978	0.750
11 to 20 wk		42.09	42.67	0.611	0.644
Haugh unit					
11 to 15 wk		87.07	84.25	0.960	0.171
16 to 20 wk		86.48	85.37	1.233	0.678
11 to 20 wk		86.45	84.81	0.708	0.257
Egg yolk color					
	L	57.34	58.41	2.665	0.854
11 to 15 wk	a	28.11	27.15	1.980	0.826
	b	32.43	32.98	2.421	0.917
	L	50.32	52.20	3.997	0.828
16 to 20 wk	a	20.25	21.15	1.001	0.681
	b	30.60	30.93	1.848	0.935
	L	53.83	55.31	2.451	0.772
11 to 20 wk	a	24.18	24.15	1.341	0.992
	b	31.51	31.95	1.499	0.888

L*= brightness, a*= redness, b*= yellowness, [1]SEM, standard error of means. P<0.05.

Table 4. Nutrient content of eggs of two different Japanese quail breeds (%, in DM)

Egg nutrient content		Jumbo Brown	Golden Italian	SEM[1]	*P*-value
Egg yolk	DM(Fresh)	50.00	52.74	0.819	0.094
	CP	33.68[a]	30.24[b]	0.896	0.046
	EE	53.71	51.92	1.030	0.417
	CA	3.15	3.49	0.211	0.453
Egg albumen	DM(Fresh)	11.81	12.62	0.595	0.528
	CP	83.99	86.39	1.566	0.476
	EE	1.74	1.78	0.215	0.932
	CA	5.57	7.01	0.424	0.088
Edible part (whole)	DM(Fresh)	25.82	26.79	0.705	0.124
	CP	44.88	43.83	1.212	0.228
	EE	43.52	42.72	0.662	0.118
	CA	4.60	5.41	0.348	0.269

Differences between mean values with different letters (a-b) in the same row are statistically significant at P<0.05 level. DM= dry matter, CP= crude protein, EE= ether extract, CA= crude ash, [1]SEM, standard error of means.

3.3. Egg Yolk Lipid Peroxidation

The effects of genotype, storage time, and genotype*storage time on yolk MDA values in quail breed eggs stored at room temperature on days 0, 14, and 28 were found to be insignificant (P>0.05) (Table 5).

3.4. Egg Yolk Lipid Profile

The fatty acid profiles of quail egg yolks are presented in Table 6. Accordingly, linolelaidic acid was higher in Jumbo Brown quail eggs than in Golden Italian quail eggs (P<0.05). In contrast, gadoleic acid and cis 11,14-eicosadienoic acid values were higher in Golden Italian quail eggs (P<0.05). In addition, when the eggs of quails were evaluated in terms of yolk total fatty acid content, it was observed that ΣPUFA and Σn-6 values and ΣPUFA/ΣSFA ratio were higher in the Jumbo Brown quail breeds (P<0.05).

Table 5. TBARs (mg MDA/kg) values of fresh and stored egg yolks from two different Japanese quail breeds

	*TBARs value	SEM[1]	*P*-value
Genotype			
Jumbo Brown	0.79	0.013	0.991
Golden Italian	0.79		
Storage time			
Day 0	0.78		
Day 14	0.77	0.013	0.639
Day 28	0.81		
Genotype*Storage time			
Jumbo Brown d0	0.78		
Jumbo Brown d14	0.80		
Jumbo Brown d28	0.79	0.037	0.572
Golden Italian d0	0.78		
Golden Italian d14	0.75		
Golden Italian d28	0.83		

TBARs= thiobarbituric acid reactive substances, MDA= malondialdehyde, [1]SEM, standard error of means. P<0.05.

Table 6. Fatty acid composition of egg yolks of two different Japanese quail breeds (g/100 g total fat)

Fatty acids, %	Jumbo Brown	Golden Italian	SEM[1]	*P*-value
Myristic acid (C14:0)	0.48	0.48	0.054	0.968
Methyl myristoleate (C14:1)	0.60	0.85	0.116	0.304
Pentadecanoic acid (C15:0)	0.04	0.04	0.006	0.641
Palmitic acid (C16:0)	22.60	23.06	0.519	0.688
Palmitoleic acid (C16:1)	3.23	3.74	0.217	0.267
Heptadecanoic/Margaric acid (C17:0)	0.15	0.15	0.024	0.932
Heptadecanoic acid (C17:1)	0.08	0.09	0.015	0.922
Stearic acid (C18:0)	6.63	6.74	0.309	0.872
Trans-Octadecanoic acid (C18:1N9T)	46.43	46.72	0.589	0.825
Linolelaidic acid (C18:2N6T)	14.17[a]	11.39[b]	0.622	0.014
Alpha-Linolenic acid (C18:3N6)	0.19	0.16	0.025	0.551
Arachidic acid (C20:0)	0.14	0.21	0.028	0.283
Gadoleic acid (C20:1)	0.09[b]	0.35[a]	0.054	0.016
Cis 11,14-Eicosadienoic acid (C20:2)	0.03[b]	0.31[a]	0.051	0.003
Cis 11,14,17eicosatrienoic acid (C20:3N3)	0.49	0.41	0.063	0.558
Heneicosanoic acid (C21:0)	0.03	0.02	0.005	0.225
ΣSFA	30.07	30.69	0.692	0.683
ΣUFA	65.32	64.02	1.026	0.558
ΣMUFA	50.44	51.75	0.661	0.351
ΣPUFA	14.88[a]	12.28[b]	0.599	0.017
ΣPUFA/ΣSFA	0.50[a]	0.40[b]	0.019	0.003
Σn-9	49.84	50.90	0.675	0.466
Σn-6	14.36[a]	11.55[b]	0.625	0.013
Σn-3	0.49	0.41	0.063	0.558
Σn-6/Σn-3	29.29	28.17	1.038	0.618

Differences between mean values with different letters (a-b) in the same row are statistically significant at P<0.05 level. SFA= saturated fatty acids, UFA= unsaturated fatty acids, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, n-9= omega 9 fatty acids, n-6= omega 6 fatty acids, n-3= omega 3 fatty acids, [1]SEM, standard error of means.

No difference was observed in egg yolk ΣSFA, ΣUFA, ΣMUFA, Σn-9, Σn-3, and Σn-6/Σn-3 values between the quail breeds (P>0.05).

4. Discussion

The physicochemical properties of eggs are the characteristics that are most valued by consumers. Genchey (2012) reported that egg weight is among the most important parameters, not only for consumers but also for egg producers. In the present study, we found that eggs from Golden Italian quail breeds were heavier than those from Jumbo Brown quail in both the first and overall periods. Previous studies have reported significant differences in egg weights between quail breeds with different plumage colors or genotypes (Hrnčár et al., 2014; Eratalar and Okur, 2020). In another study, eggs from quails with original (wild-type) feather color were reported to be heavier than those from white, dark brown, and golden feathered quail breeds (İnci et al., 2015). Therefore, this difference among breeds may be attributed to genetic structure, age of the animal, laying cycle, diet composition, and environmental temperatures. On the other hand, the eggshell is an important feature in terms of the acceptability of eggs for hatching or table use and for keeping bacteria outside the egg. In the present study, it was found that the average eggshell proportion in weeks 16-20, and eggshell weight in the overall period were higher in Golden Italian quail breeds than in Jumbo Brown quail breeds. Hrnčár et al. (2014) also reported that eggshell weight was higher in meat-type quail breeds than in laying types, but eggshell proportion was not affected by genotype. In another study, İnci et al. (2015) found that eggshell weight was higher in quails with the original (wild type) feather color than in quails with other feather colors. Therefore, an increase in eggshell weight may be associated with an increase in egg weight. In addition, eggshell weight may vary with age, breed, anatomical structure (medullary bone development), dietary composition (presence of P, Zn, Mn, and vitamin D3), and environmental and physiological factors (disease, stress, etc.).

Eggs consist of water, proteins, lipids, minerals, and a small amount of carbohydrates. The chemical composition of eggs is another characteristic, given the importance of producers and consumers. As in chicken eggs, the most nutrient-dense part of quail eggs is yolk. In the present study, the CP content of jumbo brown quail egg yolk was higher than that of golden Italian egg yolk, and the nutrient contents of all other egg parts were similar. These results are consistent with those of previous studies (Zeweil et al., 2006; Dudusola, 2010). Jeke et al. (2018) reported that the CP content in whole eggs is similar in Jumbo Pharaoh and Manchurian golden quail breeds, and higher in A&M giant breeds. The high yolk CP content observed in the present study could potentially be explained by the jumbo brown quail's ability to utilize dietary N more effectively; therefore, the protein may accumulate more in the egg yolk. Consequently, factors such as the protein bioavailability of a specific diet and individual metabolism rate may determine how effectively quails can utilize nitrogen in their diet. Furthermore, genetic factors may also play a role in the ability of jumbo brown quail to produce eggs with a higher CP content.

Lipid oxidation is one of the most important indicators of egg quality. In this study, it was observed that the lipid oxidation marker MDA content of quail eggs stored on different days (0, 14, and 28 days) was not affected by genotype, storage time, and genotype*storage time interactions, and the yolk MDA value ranged between 0.75-0.83 mg/kg yolk. However, in studies conducted on quail eggs, no findings related to the oxidative stability of the egg yolk have been reported. Therefore, these findings were evaluated through similar studies on chicken eggs. Indeed, Goliomytis et al. (2018) reported that the egg yolk MDA value in Lohmann brown-classic laying hens varied between 3.85-5.58 ng/g yolk in eggs stored for different periods. Skřivan et al. (2016) measured the egg yolk MDA value as 0.38 mg/kg yolk on day 0 and 0.82 mg/kg yolk on day 28 in Lohmann brown laying hens. Selim and Hussein (2020) reported an egg yolk MDA value of approximately 45 ng/g yolk after a 7-day storage period in Lohman brown lite-laying hens. High MDA values may indicate the weakness of antioxidant defence mechanisms in cells or their low capacity to cope with oxidative stress. Therefore, the high MDA value in quail eggs suggests that egg yolk is more sensitive in terms of oxidative stability, and that antioxidant defense mechanisms may need to be strengthened. However, these differences between the two breeds may be attributed to diet, age, genetic factors, rearing conditions, environmental stress, temperature, humidity, and hormonal changes during egg formation. The yolk fatty acid profile, egg storage time, and conditions may also affect MDA values. In addition, the fact that the egg yolk MDA values did not change between the two breeds in the current study can be explained by the quails not being subjected to any environmental and physical treatment that would change their oxidative and redox status and being fed with the same conditions and diets. However, it has been reported that the lipid oxidation value for foods suitable for consumption is below 3 mg MDA/kg, and the upper limit is 7-8 mg MDA/kg (Cadun et al., 2005). Therefore, the MDA values obtained were within the appropriate reference range.

A good fatty acid profile is important for egg quality. Indeed, in the present study, it was determined that the fatty acid composition of quail egg yolk is primarily medium-to long-chain, i.e. 14-21 carbon atoms in length. In addition, linolelaidic acid, total PUFA, and total n-6 fatty acids were higher in the jumbo brown breeds, whereas gadoleic acid and cis 11,14-eicosadienoic acid were higher in the golden Italian breeds. Güçlü et al. (2008) found that the total SFA content of egg yolk was higher, but other total MUFA, PUFA, n-3 and n-6 values were similar to the findings of the present study. Cufadar

et al. (2021) found that quail yolk palmitic acid content was similar to the present findings, ΣSFA, ΣPUFA and Σn-3 values were higher, and ΣMUFA values were lower. In a study in which sunflower oil was added to the diets of laying quails as a fat source, it was observed that yolk ΣSFA and ΣPUFA values were higher, ΣMUFA values were lower, and Σn-3 values were similar to the present findings (Göçmen et al., 2021). On the other hand, it is recommended that the human diet as a whole should have a PUFA/SFA ratio above 0.45 and an n-6/n-3 ratio of 4:1 for a healthy life (HMSO, 1994). However, modern Western-type diets usually have a higher n-6/n-3 ratio (10-20:1 or more) (Simopoulos, 1998; 1999). In this context, in the present study, the yolk ΣPUFA/ΣSFA ratio was found to be higher in the Jumbo Brown breeds (0.50) than in the Golden Italian breeds (0.40). Özbilgin et al. (2021) found that the ΣPUFA/ΣSFA ratio of quail egg yolk was 0.58, which is higher than the findings of the present study. Thus, differences were observed between the results of egg yolk fatty acid profile of quail breeds in the present study and those of previous studies. The differences in yolk fatty acid composition may be attributed to the different utilization of dietary nutrients by the quail breeds. Genetic and microbiome factors may also be effective in metabolizing and storing fatty acids. However, the influence of genetics on fatty acid composition is significantly lower than that of diet (Sinclair, 2007). On the other hand, the relatively high levels of trans-octadecanoic acid in the current yolk fatty acid profile have been attributed to the high-concentrate diets fed to quails and the easily fermentable carbohydrate content.

5. Conclusion

Based on these results, the relatively small size of Jumbo Brown quail eggs compared to Golden Italian quail breeds and the low eggshell proportion may be considered disadvantageous for the consumer, table, or hatchery industries. However, the Jumbo Brown quail egg yolk CP, ΣPUFA, total Ω6 fatty acid content, and ΣPUFA/ΣSFA ratio have a more advantageous profile in terms of use in human diets. Moreover, it was observed that even at different storage periods in both quail breeds, yolk lipid oxidation did not exceed the acceptable threshold values. Therefore, quail eggs can be safely added to daily diets in human nutrition without overdoing, considering the amount of trans fatty acids. However, to fully understand the differences in egg quality observed between quail breeds, the diet, genetic profiles, and gut microbiome of quail breeds should be investigated in detail.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	E.Ö.G.	H.H.İ.
C	50	50
D	50	50
S	50	50
DCP	70	30
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	30	70

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

In this study, it was reported by Dicle University Health Sciences Research and Application Center Animal Experiments Local Ethics Committee (DÜHADEK) that local ethical committee approval was not required (protocol code: E- 38588763-041.02-519389 and date: June 22, 2023).

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AGRICULTURAL INVESTMENT ACCOUNTING: A CASE STUDY OF AN ENTERPRISE IN THE THRACE REGION

Mehmet Hüseyin YÜCE[1], Aysun ATAGAN ÇETİN[1]

[1]Trakya University Keşan Yusuf Çapraz School of Applied Sciences, 22850, Edirne, Türkiye

Abstract: This article provides an in-depth examination of the investment process within the agricultural sector, focusing on A Birlik, a significant agricultural enterprise in the Thrace region. The study explores how A Birlik makes investment decisions, the types of investments it undertakes, and the impact of these investments on its operations and sustainability. A critical component of this research is the role of agricultural investment accounting in enabling A Birlik to make informed financial decisions, accurately evaluate investment feasibility, and optimize resource allocation for long-term economic and environmental sustainability. The research also considers how geographical conditions, technological developments, market dynamics, and competition influence agribusiness investment decisions. The findings from this study aim to enrich the existing literature on agricultural investments and offer valuable insights for practitioners, policymakers, and stakeholders in the agricultural sector. The article underscores the need for continuous adaptation and innovation in business practices within the agricultural sector.

Keywords: Agricultural investments, Investment process, Financial analysis, Financial accounting

***Corresponding author:** Trakya University Keşan Yusuf Çapraz School of Applied Sciences, 22850, Edirne
E mail: aatagancetin@trakya.edu.tr (A. ATAGAN ÇETİN)
Mehmet Hüseyin YÜCE https://orcid.org/0009-0002-5385-7131
Aysun ATAGAN ÇETİN https://orcid.org/0000-0001-7588-1322

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1. Introduction

Investment decisions are crucial for both the investing organization and the broader economy. These decisions require a thorough evaluation of alternatives to optimize resources. A comprehensive feasibility study for an investment project should encompass economic, technical, financial, and social analyses, catering to all parties involved. Investors prioritize profitability and payback rate, lenders focus on financial ratios, local communities consider the regional benefits, and the state evaluates contributions to employment and other macro criteria. Therefore, an investment project should present objective, data-driven analyses to meet the expectations of all stakeholders. Alternative investments should be assessed using financial analysis methods, with the most suitable project being selected based on strategic sensitivities and micro and macro policies.

Investment decisions hold strategic importance for both the investing organization and the wider economy. These decisions necessitate a comprehensive evaluation of available alternatives to ensure optimal resource allocation. The process of making an informed investment decision is complex and multifaceted, involving a careful balance of risk and reward.

A well-conducted feasibility study for an investment project should encompass a broad range of analyses. These include economic, technical, financial, and social aspects, each of which provides a different perspective on the potential value and impact of the investment. This comprehensive approach ensures that the study caters to the interests and concerns of all parties involved in the investment.

Different stakeholders have different focuses when it comes to evaluating an investment. Investors, for instance, prioritize the profitability and payback rate of the investment, seeking a return on their capital. Lenders, on the other hand, focus on financial ratios to assess the risk associated with their loans. Local communities, where the investment is made, consider the regional benefits, such as job creation and economic development. Meanwhile, the state evaluates the investment's contributions to employment and other macroeconomic criteria, such as GDP growth and economic diversification.

Therefore, an investment project should present objective, data-driven analyses to meet the expectations of all these stakeholders. It is crucial that the results are not manipulated and accurately reflect the potential outcomes of the investment.

In the context of agricultural investments, alternative projects should be assessed using robust financial analysis methods. The most suitable project should then be selected based on strategic sensitivities, as well as micro and macro policies. This approach ensures that the chosen investment aligns with both the specific goals of the investing organization and the broader economic and policy environment.

1.1. Investment

An investment is an allocation of resources, such as time, money, effort, or other assets, with the expectation of generating future benefits or returns. These returns may come in the form of income, profit, or the achievement of specific objectives (Valencia et al., 2020).

Investments can be made in various forms, including financial assets like stocks and bonds, real estate, or even in the development of new products, processes, or technologies. In the context of businesses and organizations, investments often involve the allocation of resources towards projects or initiatives that are expected to generate financial returns or contribute to the achievement of strategic objectives (Kalinina et al,, 2019).

According to Mulska and Kloba (2021), investments serve as a pivotal catalyst in propelling economic growth and development. They have the potential to stimulate job creation, foster wealth generation, and enhance living standards. Furthermore, investments targeted towards projects focused on energy conservation can significantly contribute to environmental sustainability and promote the judicious utilization of resources.

1.2. Investment Types

Investment types are diverse and can be categorized based on various factors such as the asset class, risk level, and investment objective. Common types of investments include stocks, bonds, mutual funds, real estate, and commodities, each offering unique benefits and risks. For instance, stocks offer potentially high returns but carry a higher risk, while bonds are generally safer but offer lower returns. Mutual funds and exchange-traded funds provide diversification by investing in a variety of assets. Real estate and commodities like gold and oil offer tangible assets that can act as a hedge against inflation. Understanding these investment types is crucial for investors to make informed decisions that align with their financial goals and risk tolerance.

Here are some of the main types of investments (Table 1). The Table 1 categorizes different types of investments, each with its unique characteristics and risk-return profiles. It includes direct investments in physical properties through Real Estate Investments, equity ownership in companies via Stock Investments, lending to entities through Bond Investments, and indirect real estate investments through Real Estate Investment Trusts (REITs). Each investment type offers distinct opportunities for income generation and capital appreciation, while also presenting unique risks and management requirements.

1.3. Investment Process

The investment process is a systematic approach to investing that involves several key steps, each designed to help investors make informed and strategic decisions. It begins with setting clear financial goals, which could range from short-term objectives like saving for a vacation to long-term goals like retirement planning. The next step is assessing risk tolerance, which determines the level of risk an investor is comfortable with. This is followed by asset allocation, where investors decide how to distribute their investments across various asset classes to maximize returns and minimize risk (Bodie et al., 2014). According to Reilly and Brown (2011), the process continues with the selection of specific investments within those asset classes, based on thorough research and analysis. Finally, the investment process involves regular performance reviews to ensure that the investments are still aligned with the investor's goals and risk tolerance, and adjustments are made as necessary.

The investment process involves a series of steps that guide investors in making informed decisions about where to allocate their resources. Here's an overview of the process:

Table 1. Investment types

Real Estate Investments	This involves the purchase of physical property, such as residential homes, commercial buildings, or land. Real estate can provide income through rental returns and potential appreciation in property value over time. However, it also comes with risks, such as market fluctuations and property management challenges (Janoschka et al., 2020).
Stock Investments	Buying stocks, or shares, means purchasing a piece of ownership in a company. Stock investors make money when the company performs well and the stock's price increases. They may also receive dividends, which are a portion of the company's profits distributed to shareholders. The risk in stock investing comes from the potential for the company to perform poorly, causing the stock's price to fall (Bouri et al., 2020).
Bond Investments	Bonds are essentially loans that investors make to entities like governments or corporations. The bond issuer promises to pay the investor back the principal amount of the loan, along with regular interest payments, over a specified period. Bonds are generally considered lower risk than stocks, but they also typically offer lower returns (Zhang t al., 2016).
Real Estate Investment Trusts (REITs)	REITs are companies that own, operate, or finance income-generating real estate. Investors can buy shares in a REIT, similar to how they can buy shares in a company's stock. REITs provide a way for individual investors to earn a share of the income produced through real estate investment, without having to buy, manage, or finance any

properties themselves (Tiwari et al., 2020).

- Goal Setting: The first step in the investment process is to define your financial goals. These could be short-term (e.g., saving for a vacation), medium-term (e.g., saving for a down payment on a house), or long-term (e.g., saving for retirement). Your goals will guide your investment strategy and help you decide how much risk you're willing to take on (Asadzadeh et al., 2014).
- Risk Assessment: This involves evaluating your risk tolerance, which is the degree of variability in investment returns that an investor is willing to withstand. Risk tolerance can be influenced by factors such as age, income, financial goals, and personal comfort with risk (Li and Madanu, 2009).
- Asset Allocation: This step involves deciding how to distribute your investments among different asset classes, such as stocks, bonds, and real estate. The goal is to maximize returns and minimize risk by diversifying your portfolio (Trianni et al., 2017).
- Investment Selection: Once you've decided on your asset allocation, the next step is to choose specific investments within those asset classes. This could involve researching and analyzing individual stocks, bonds, or real estate properties (Cheng et al., 2021).
- Performance Review: This involves regularly reviewing your investments to ensure they're still aligned with your financial goals and risk tolerance. If necessary, you may need to rebalance your portfolio, which involves buying or selling assets to maintain your desired asset allocation (Li and Madanu, 2009).

1.4. Agricultural Investments

Agricultural investments encompass a broad range of capital allocations aimed at enhancing productivity, profitability, and sustainability in the agricultural sector. These investments can take various forms, including direct investments in farming activities, infrastructure development, technological advancements (AgTech), supply chain improvements, and financial services tailored for agricultural needs (Belhadi et al., 2021). For instance, direct investments might involve purchasing farmland or livestock, while infrastructure investments could focus on irrigation systems or storage facilities (Di Matteo and Schoneveld, 2016). AgTech investments might include precision farming technologies or biotech innovations, and supply chain investments could aim to improve efficiency from production to distribution (Vorley et al., 2012). Lastly, financial services like loans or insurance can provide farmers with the necessary resources to manage risks and invest in productivity-enhancing technologies. Each form of investment plays a crucial role in supporting the agricultural sector's growth and its ability to meet global food demand (Kadigi et al., 2017).

Agricultural investments refer to the allocation of capital in initiatives related to the agricultural sector. These investments can take various forms (Table 2).

Agricultural investments encompass a broad range of capital allocations aimed at enhancing productivity, profitability, and sustainability in the agricultural sector. These investments can take various forms, including direct investments in farming activities, infrastructure development, technological advancements (AgTech), supply chain improvements, and financial services tailored for agricultural needs. For instance, direct investments might involve purchasing farmland or livestock, while infrastructure investments could focus on irrigation systems or storage facilities (Vorley et al., 2012). AgTech investments might include precision farming technologies or biotech innovations, and supply chain investments could aim to improve efficiency from production to distribution.

Table 2. Agricultural investment types

Direct Investments in Agricultural Production	This involves investing directly in farming activities, such as crop cultivation, livestock rearing, or aquaculture. It may involve purchasing or leasing farmland, buying farming equipment, or investing in seed and fertilizer (Belhadi et al., 2021).
Investments in Agricultural Infrastructure	This includes investments in infrastructure that supports agricultural activities, such as irrigation systems, storage facilities, and processing plants. These investments can help increase agricultural productivity and reduce post-harvest losses (Matteo and Schoneveld, 2016).
Investments in Agricultural Technology (AgTech)	AgTech refers to the use of technology to enhance agricultural production. This can include investments in precision farming technologies, biotechnology, farm management software, and other technological innovations that can improve efficiency and productivity in the agricultural sector (Belhadi et al., 2021).
Investments in Agricultural Supply Chains	This involves investing in the various stages of the agricultural supply chain, from production to processing to distribution. These investments can help improve supply chain efficiency and ensure that agricultural products reach consumers in a timely and cost-effective manner (Vorley et al., 2012).
Investments in Agricultural Finance	This includes providing loans, insurance, and other financial services to farmers and agricultural businesses. These financial services can help farmers manage risks, invest in productivity-enhancing technologies, and ensure the financial sustainability of their

operations (Kadigi et al., 2017).

Lastly, financial services like loans or insurance can provide farmers with the necessary resources to manage risks and invest in productivity-enhancing technologies. Each form of investment plays a crucial role in supporting the agricultural sector's growth and its ability to meet global food demand (Kadigi et al., 2017).

1.5. Evaluating the Financial Returns of Investment Projects

The survival and growth of an enterprise depends on its ability to cope with its competitors, that is, its competitiveness. A healthy determination of the competitiveness of an enterprise is realized by measuring and analyzing the financial performance of the enterprise in question. In this context, investors are of vital importance for agricultural enterprises in the long term (Acar, 2003). Five methods are used to evaluate the financial returns of investment projects. These are giving below.

1.5.1. Net present value method (NPV)

The net present value of an investment project is the ratio of the cash inflow and cash outflow planned to be provided from the investment each year to the expected return on the investment (Gedik et al. 2005). Net present value and other financing are concepts that are valid in making decisions about capital investments and show the time value of money (Graham, 2019).

1.5.2. Internal rate of return method (IRR)

According to Shafiee et al. (2020), the Internal Rate of Return (IRR) is a crucial financial metric used in investment decision-making. It is particularly relevant in the context of projects with significant upfront costs and long-term returns, such as renewable energy projects. The IRR is the discount rate that makes the net present value (NPV) of all cash flows (both positive and negative) from a particular project equal to zero. In other words, it is the rate at which the present value of the project's expected benefits exactly equals the present value of its associated costs.

1.5.3. Benefit cost ratio

The Benefit-Cost Ratio (BCR) is a significant tool used in economic and financial analysis. It is the ratio of the present value of benefits relative to the present value of costs. The decision rule is the following: if the BCR is greater than 1 or the Net Present Value (NPV) is greater than 0, the project will be accepted. Conversely, if the BCR is less than 1 or the NPV is less than 0, the project will be rejected. This ratio is used to measure project worth and allows estimations of the degree of project risk exposure to be made (Ruegg and Marshall, 2013).

1.5.4. Payback period method

The Payback Period Method is a capital budgeting technique that determines the time it takes for an investment to generate cash flows equal to the original investment cost. It is a simple and widely used method for quick estimation of investment recovery. For instance, in the context of emission abatement in maritime shipping, the payback period can be used to assess the economic feasibility of different abatement options. The payback period of such investments can be significantly influenced by factors such as fuel prices. Lower prices could delay the payback period of investments, even up to two times in some cases (Zis et al., 2016).

1.5.5. Annual equivalent cash flow method

The Annual Equivalent Cash Flow Method, also known as the Equivalent Annual Value (VAE) method, is used for economic analyses, particularly in determining the economic feasibility and rotation age of projects. This method involves a comprehensive analysis of cash flow variables, including costs and revenues, over the expected lifetime of a project. The VAE is calculated using costs at the end of each year, revenues at the end of each year, the discount or interest rate, and the project's duration in years. The economic rotation age is defined as the age at which the highest VAE is attained. This method is particularly useful in scenarios where the costs and revenues of a project change over time, such as in the cultivation of nonnative candeia, a plant used for essential oils and fence posts (Silva et al., 2012).

2. Materials and Methods

The study conducts a case study on A Birlik, a cooperative union with an important role in the agricultural sector in the Thrace region. The study examines how A Birlik makes investment decisions, what types of investments it makes, and the consequences of these investments on its operations and sustainability. These include decisions to acquire assets, increase production capacity and improve storage facilities. The study also assesses how geographical conditions, technological developments, market dynamics and competition affect agribusiness investment decisions. The data collection method of the study was obtained from primary and secondary sources.

2.1. Primary Data

Interviews were conducted with key stakeholders, including A Union management and staff, to gather information on investment processes, decision-making criteria and challenges faced. Senior managers, middle managers and operational staff were interviewed.

2.2. Secondary Data

A Birlik's financial reports, company documents and investment plans were collected. In addition, national agricultural data and policies related to the Thrace region were collected from government sources and databases.

2.3. General Information on the Enterprise

A Birlik was established in 1966 with the merger of Edirne, Lüleburgaz and Babaeski agricultural sales cooperatives. Today, the Union is an organization consisting of 48 cooperatives and 36,342 producer members spread across 13 provinces, mainly in Thrace and Marmara regions. The Union started its industrial activities by purchasing an oil factory in 1976 in order to

utilize the sunflower products produced by its partners. Since 1980, Tekirdağ integrated facilities have been put into operation in units. As a result of this process, both plants have an annual production capacity of 300,000 tons of sunflower, 150,000 tons of refined oil, 30,000 tons of margarine, 315,000 tons of fodder, 75,000 tons of crude oil storage and 45,000 tons of sunflower collection. The Union has an annual sunflower processing capacity of 300,000 tons. In this sector, it has the highest sunflower processing capacity in the country. The Union is a leader in the vegetable oil production sector. In this context, it has a market share of 17-18% in refined oil and 7-8% in margarine. The Association carries out production with "TS-EN-ISO 9001 Quality Management System", "TS-EN-ISO 22000 Food Safety Management System" and "TS-EN-ISO 14001 Environmental Management TSE OHSAS 18001" certificates. Both enterprises of the Union have waste treatment units in accordance with European standards and production is carried out in an environmentally friendly manner.

In the images in Figure 1 and Figure 2, general views of the integrated plant and oil factories of A Birlik enterprise, which we examined within the scope of the study, are presented.

3. Results

In the application part of the study, various calculations were made to evaluate the financial return of the steel silo investment project of A Birlik.

Net present value of a birlik steel silo investment project was given in Equation 1.

$$NBD = \sum_{t=0}^{n} \frac{Bt}{(1+r)^t} - \sum_{t=0}^{n} \frac{Ct}{(1+r)^t} \quad (1)$$

here;

Bt= Cash inflow in year t - 250,000

Ct= Cash outflow in year t – 1,250,000

n= Economic life of the project (years) - 8 years

r= discount rate - 10%

In this case, the net present value calculation of our enterprise is as follows according to the years:

- 2015: 227,272.73 TL
- 2016: 260,330.58 TL
- 2017: 300,525.92 TL
- 2018: 307,356.05 TL
- 2019: 248,368.53 TL
- 2020: 338,684.36 TL
- 2021: 384,868.59 TL
- 2022: 373,250.90 TL

Net Present Value, in the net present value calculation made by using the data obtained by the enterprise, the net present value of the investment made by A Birlik was found to be 1,190,612.66 TL. The net present value expectation for the investment made by the enterprise is greater than zero. It is seen that the investment made is highly efficient.

Figure 1. Integrated facilities of a Birlik enterprise.

Figure 2. Karacabey oil factory

Net present value calculation of enterprise A for 8 years: TL 1,190,612.66 (Figure 3).

A Birlik's internal rate of return for 8 years: 27.82% (Figure 4).

- 2015: -1,250,000
- 2016: 250,000
- 2017: 315,000
- 2018: 400,000
- 2019: 450,000
- 2020: 600,000
- 2021: 750,000
- 2022: 800,000

The internal rate of return was found to be 27.82% as a result of the internal rate of return analysis using the data obtained from the enterprise. It is the project with the highest internal rate of return among the projects planned by the enterprise. While the profitability rate expected by the enterprise from the investment is 22%, the internal rate of return of 27.82% shows the feasibility of the project.

The benefit-cost ratio is a performance measure that expresses the ratio between the returns and costs of a project or investment (Figure 5). This ratio is used to assess the net benefits of a project or investment on society or business.

The payback period calculation made with the data obtained from the enterprise was found to be 5.72 years, which means that the enterprise recovered the money it spent for this project after 5.72 years (Figure 6).

Figure 3. Net present value calculation.

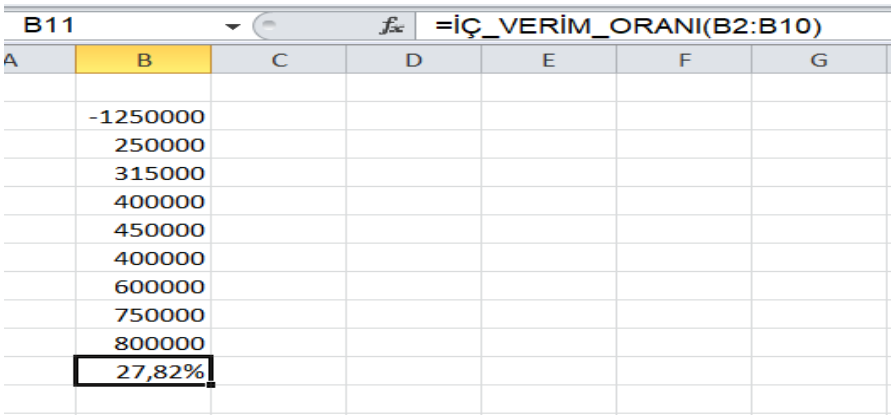

Figure 4. Internal rate of return of a Birlik steel silo investment project.

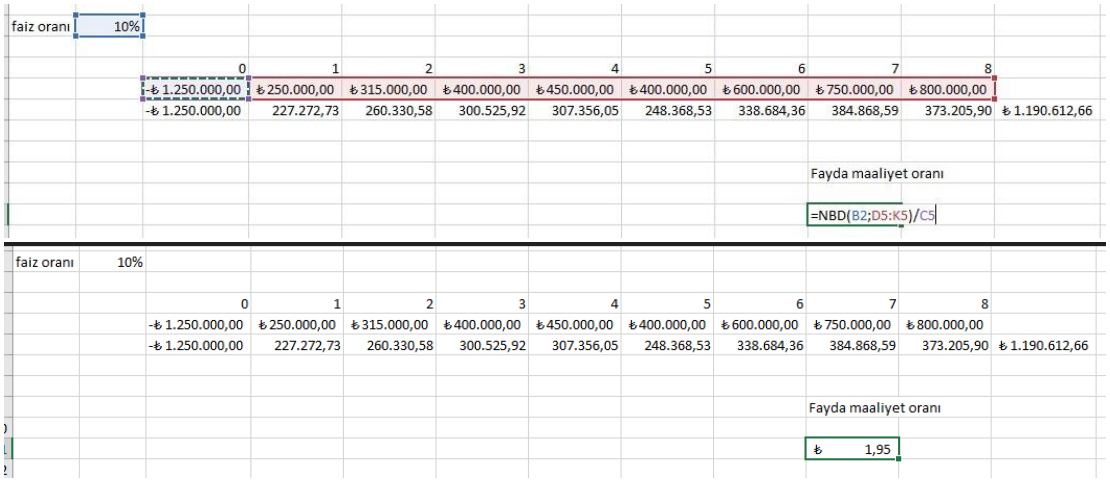

Figure 5. Benefit-cost ratio of a Birlik steel silo investment project.

11									
12									
13	faiz oranı	10%							
14	A.O.S.M.	18%	Dönem	NNA	İSK.ED.NNA	BAKİYE	GER.ÖD.SÜRE		
15			0	-1.250.000	-1.250.000,00	-1.250.000,00	0		
16			1	250.000	211.864,41	-1.038.135,59	1		
17			2	315.000	226.228,10	-811.907,50	2		
18			3	400.000	243.452,35	-568.455,15	3		
19			4	450.000	232.104,99	-336.350,15	4		
20			5	400.000	174.843,69	-161.506,47	5		
21			6	600.000	222.258,92	60.752,46	0,726659096	5,72 yıl	
22			7	750.000	235.443,77				
23			8	800.000	212.830,53				
24									

Figure 6. Payback period of a Birlik steel silo investment project.

3.1. Annual Equivalent Cash Flow of a Birlik Steel Silo Investment Project

The annual equivalent cash flow method (AECFM) enables the evaluation of a project or investment by converting its cash flows into their equivalent values over a certain period of time (usually annually). This method is widely used to analyze the economic profitability of the project or investment. The cash flows of the project or investment may be irregular, i.e. not the same amount of cash inflows or outflows every year. In Excel, special formulas and functions may need to be used to handle such irregular cash flows and calculate equivalent values accurately.

3.2. SWOT Analysis of a Birlik Steel Silo Investment Project

SWOT analysis is a tool used to evaluate investment decisions and identify potential risks and opportunities. The results of the analysis can enable the company to capitalize on opportunities using its strengths and help it develop strategies to deal with its weaknesses. However, SWOT analysis alone is not enough. Other financial analysis, market research and strategic planning should also be used to support investment decisions. SWOT analysis is an analysis method used to assess the strengths, weaknesses, opportunities and threats of a business.

A Birlik's SWOT analysis of the Steel Silo investment decision is given in Figure 7.

According to the Figure 7, this SWOT analysis offers a detailed overview of the factors that could influence the implementation and success of oil production in steel silos. It offers a foundation for strategic planning to optimize the strengths and opportunities while addressing weaknesses and mitigating threats. As with any strategy, these actions should be implemented and regularly reviewed to align with changing business conditions.

Strengths	Weaknesses
Steel silos are durable and long-lasting, providing a suitable storage solution for oil production. The silos maintain hygienic conditions, preserving the freshness and quality of the oils. Steel silos are more resistant to external factors such as fire and pests. The capacity of silos is usually high, which is advantageous for the production of large quantities of oil. Steel silos facilitate storage and unloading processes and can be integrated with automated systems.	The cost of steel silos can be high, accessibility can be an issue, especially for small-scale businesses. Installation and maintenance of silos may require some expertise, which can increase operating costs. There may be limitations to the area where the silos will be located, which may affect operational planning. Problems such as air leakage or corrosion may occur during the oil production process in the silos.
Opportunities	**Threats**
There is potential for growth in the oil production sector and steel silos can support this growth. The integration of steel silos with technological advances can make storage and unloading processes more efficient. Integration of silos with automated monitoring and control systems can improve operational efficiency. Steel silos that provide healthy and hygienic storage conditions can provide a competitive advantage in terms of quality and reliability.	Other storage methods or alternative technologies can reduce the competitiveness of steel silos. Other materials or storage methods used in oil production may be subject to environmental and regulatory restrictions. Market price fluctuations or changes in demand may affect demand for oil production. Technical failures or operational errors in silos can affect the production process and increase costs.

Figure 7. SWOT analysis of oil production in steel silos.

4. Discussion

In the current economic conditions, where international competition is increasing, customer needs and expectations are diversifying, and the understanding of the global market is developing, businesses need to adapt to technological developments more easily. Investing in new technologies will accelerate businesses' adaptation to the new world economy. Businesses that invest in technology will provide both a competitive advantage for the company and significant contributions to the country's economy as long as they analyze their customers' demands better and manage their investments correctly.

There are many factors that are effective in the institutionalization stage of investment businesses. Firstly, it is necessary to plan the production well and pay attention to what factors will affect the production. All functions of the business should be well assimilated. It should not be forgotten that the business has certain responsibilities, not only profit-oriented, in line with its goals and objectives. The investment project to be made, the place to be established, and the technology to be used are the most important decision points of the investment business. When all the elements necessary for the establishment of investment businesses are considered, a successful investment will be inevitable.

After all stages are completed, the establishment analysis of the business should be done correctly. From this point of view, understanding the concept of investment, reflecting the correct values of feasibility studies that are important for the operation of a good business, and evaluating these types of studies by experienced people who have knowledge in this field will provide a very beneficial process for the business.

In this study, the investment process and applications of A Birlik, an agricultural enterprise located in the Thrace region, were examined. The study has revealed that technology investments are of critical importance for modern agricultural enterprises. Technological developments allow businesses to better analyze customer demands, facilitate their operations, and manage their investments effectively.

5. Conclusion

The net present value (NPV) of the investment made by A Birlik was calculated using the data obtained from the enterprise. The NPV of the investment was found to be 1,190,612.66 TL, indicating that the investment is highly efficient. The expectation for the NPV of the investment made by the enterprise is to be greater than zero. The NPV was calculated for each year from 2015 to 2022, with values ranging from 227,272.73 TL in 2015 to 373,250.90 TL in 2022.

The internal rate of return (IRR) for A Birlik's 8-year investment was calculated to be 27.82%. The IRR was calculated using data obtained from the enterprise. The IRR is the highest among the projects planned by the enterprise. The enterprise's expected profitability rate from the investment is 22%, but the IRR being 27.82% indicates the feasibility of the project.

The benefit-cost ratio is a performance measure that expresses the ratio between the returns and costs of a project or investment. This ratio is used to evaluate the net benefits of a project or investment on society or the enterprise.

The payback period was calculated to be 5.72 years using data obtained from the enterprise, meaning that the enterprise recouped the money it spent on this project after 5.72 years.

The annual equivalent cash flow method (YENAY) allows for the evaluation of a project or investment's cash flows by converting them into equivalent values over a certain period (usually annually). This method is widely used to analyze the economic profitability of a project or investment. The cash flows of the project or investment may be irregular, meaning there may not be the same amount of cash inflow or outflow each year. Special formulas and functions may need to be used in Excel to handle such irregular cash flows and calculate equivalent values accurately.

In today's economic conditions, where international competition is increasing, customer needs and expectations are differentiated, and global market understanding has developed, businesses need to adapt to technological developments more easily. Investing in new technologies will accelerate the adaptation of businesses to the new world economy. As long as the enterprises investing in technology analyze the demands of their customers better and manage their investments correctly, both the company will provide competitive advantage and significant contributions will be made to the national economy.

There are many factors that are effective in the institutionalization phase of investment enterprises. First of all, it is necessary to plan production well and pay attention to the factors that will affect production. All functions of the business must be well assimilated. In line with the goals and objectives of the business, it should be remembered that it is not only profit-oriented but also has certain responsibilities. Elements such as the investment project, the place to be established and the technology to be used are the most important decision point of the investment enterprise. When all the elements necessary for the establishment of investment enterprises are taken into consideration, a successful investment will be inevitable.

In this study, the investment process and practices of A Birlik, an agricultural enterprise located in the Thrace region, were examined. The study revealed that technology investments are critical for modern agribusinesses. Technological developments can enable enterprises to better analyze customer demands, facilitate their operations and manage their investments

effectively. This provides competitive advantage and offers added value to the national economy.

In the case of A Birlik, the company's investment in processing and storage facilities has greatly contributed to its growth and its ability to meet the country's agricultural product needs. Its certifications in quality management and environmental standards emphasize the importance of adhering to high standards.

Accounting serves as a foundational element in agricultural investment strategies, particularly in metrics like NPV and IRR, which are key indicators of an investment's viability. Within this context, the role of accounting goes beyond merely balancing books; it provides the structured methodology through which agribusinesses like A Birlik can rigorously evaluate their financial performance and sustainability measures. By using standardized accounting practices, A Birlik was able to calculate an NPV of 1,190,612.66 TL and an IRR of 27.82%, thereby substantiating the feasibility and high efficiency of the investment. As the sector continues to evolve, particularly in relation to sustainability and technology, a comprehensive accounting framework remains critical for validating investment decisions, assessing risks, and achieving long-term economic and environmental objectives.

It is essential that agricultural enterprises adopt sustainable practices. A Birlik has waste treatment units that comply with European standards and shows that it is an enterprise that is aware of its environmental responsibilities. Investing in environmentally friendly technologies and practices is not only beneficial for the business, but also for society and ecology.

Consequently, investment in new technologies, comprehensive planning, a clear understanding of business functions and a commitment to sustainability are critical to the success of agribusinesses in today's globalized economy. This research contributes to the understanding of the investment process in the agricultural sector and emphasizes the need for continuous adaptation and innovation in business practices.

Recommendations based on the findings of the study are as follows:

- Agribusinesses should invest in continuous training and development programs for their employees to remain competitive.
- Collaborative relationships with stakeholders, including suppliers, government and society, are important for sustainable growth.
- Future research should investigate the long-term impact of technology investments on agricultural productivity and sustainability.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.H.Y.	A.A.Ç.
C	50	50
D	100	
S		100
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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