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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Phenological, Morphological and Pomological Characteristics of 'Samsun Güzeli' Pear Genotype on BA29 Rootstock

'Samsun Güzeli' Armut Genotipinin BA29 Anacı Üzerindeki Fenolojik, Morfolojik ve Pomolojik Özellikleri

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Abstract

Türkiye has many local pear cultivars; some of them do not have much production and propagation potential due to their low quality and undesirable vegetative and generative characteristics. However, those that are superior in terms of fruit quality and yield characteristics have found a place for themselves in both local and national markets. In this respect, the 'Samsun Güzeli' genotype, which is an important local cultivar, is an ideal cultivar with many features and has a high potential to spread its cultivation all over Türkiye and other countries. 'Samsun Güzeli' is an autumn genotype, can easily meet the high demand in local and international markets due to its attractive color, smooth fruit shape and high quality. Despite all these valuable features, this cultivar is still not well known in Türkiye. There is a great need to disseminate cultivation and research on this subject. This study mainly aimed to reveal the phenological, morphological and pomological characteristics of the 'Samsun Güzeli' local pear genotype grafted on BA29, a commercially traded rootstock in 2021-2022. The results showed that the 'Samsun Güzeli' pear genotype completed its phenological cycle in 255 days in 2021 and 228 days in 2022 in Samsun climatic conditions. Fruit set rate of the genotype was 12.05% and the average yield was 17306.23 kg ha^{-1}. It was also determined that the morphological characteristics averages were as the following; rootstock diameter 76.71 mm, trunk diameter 61.97 mm, trunk cross-sectional area 34.06 cm^2, tree height 253.12 cm, canopy volume 0.96 m^3, annual shoot length 37.19 cm and leaf area 11.74 cm^2. Averages values of pomological and chemical properties recorded as the following; fruit weight 114.60 g, fruit volume 109.90 ml, soluble solid content (SSC) 13.16%, acidity 0.33%, and pH 4.37. In the sensory evaluations, the highest scores were obtained by juiciness (6.32) in the year 2022 while scores of overall visual quality were lowest in both years. In conclusion, it can be said that the 'Samsun Güzeli' genotype performs adequately in terms of fruit yield and quality on the standard dwarf rootstock and conducting new studies might be beneficial to disseminate the genotype.

Keywords: Yield, Fruit quality, Vegetative growth, Characterization, Yield efficiency

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Öz

Çok sayıda yerel armut çeşidine sahip olan Türkiye'de bu armut çeşitlerinin bazıları düşük kaliteleri ve istenmeyen vejetatif ve generatif özellikleri nedeniyle çok fazla üretim ve yayılma potansiyeline sahip değillerdir. Ancak meyve kalitesi ve verim özellikleri bakımından üstün olanlar gerek yerel pazarlarda gerekse ülkesel pazarlarda kendilerine yer bulabilmişlerdir. Bu bakımdan önemli bir yerel çeşit olan 'Samsun Güzeli' genotipi birçok özelliği ile ideal bir yerel genotip olmakla birlikte yetiştiriciliği tüm Türkiye ve diğer ülkelerde yayılma potansiyeli yüksektir. Özellikle çekici rengi, düzgün meyve şekli ve güzlük bir çeşit olması dolayısıyla yerel ve uluslararası pazarlarda yüksek taleplere kolayca ulaşabilir. Sahip olduğu tüm bu değerli özelliklere rağmen bu genotip Türkiye'de hala iyi bilinmemektedir. Yetiştiriciliğinin yaygınlaştırılması ve bu konuda araştırmalar yapılmasına büyük ihtiyaç duyulmaktadır. Bu çalışmada 2021-2022 yıllarında BA29 anacı üzerine aşılı 'Samsun Güzeli' genotipinin fenolojik, morfolojik ve pomolojik özelliklerinin ortaya konulması asıl olarak amaçlanmıştır. Araştırma sonuçları 'Samsun Güzeli' armut genotipinin fenolojik döngüsünü Samsun iklim koşullarında 2021 yılında 255 gün, 2022 yılında 228 günde tamamladığını göstermiştir. Genotipin meyve tutum oranının %12.05 ve ortalama veriminin 17306.23 kg ha^{-1} olduğu saptanmıştır. Araştırmada genotipin morfolojik özelliklerinden ortalama anaç çapının 76.71 mm, gövde çapının 61.97 mm, gövde kesit alanının 34.06 cm^2, ağaç boyunun 253.12 cm, taç hacminin 0.96 m^3, yıllık sürgün uzunluğunun 37.19 cm ve yaprak alanının 11.74 cm^2 olduğu belirlenmiştir. Pomolojik ve kimyasal özelliklerinden, ortalama meyve ağırlığının 114.60 g, meyve hacminin 109.90 ml, suda çözünebilir kuru madde miktarının (SÇKM) %13.16, asitliğin %0.33 ve pH'nın 4.37 olduğu bulunmuştur. Duyusal olarak yapılan değerlendirmelerde en düşük puan her iki yılda da görsel kaliteden elde edilirken en yüksek duyusal puan sululuktan (6.32) 2022 yılında elde edilmiştir. Sonuç olarak, 'Samsun Güzeli' genotipinin meyve verim ve kalite bakımından standart bodur anacı üzerinde yeterli performans gösterdiği, genotipin yaygınlaştırılması için yeni çalışmaların yapılmasının uygun olacağı söylenebilir.

Anahtar Kelimeler: Verim, Meyve kalitesi, Vejetatif büyüme, Karakterizasyon, Verimlilik

1. Introduction

Pear is one of the most important temperate fruits and is adapted to wide range of climatic conditions (Bhat et al., 2021). World pear production was reported as 26.3 million tons in 2023, of which 19.2 million tons were produced by China, U.S.A, Argentina, and Türkiye that takes place in the 4th position (Anonymous, 2024a). Türkiye's pear production was reported as 551.086 tons in 2022 (Anonymous, 2024b). In order to get the optimum vegetative and generative developments from fruit trees, performing appropriate cultural practices, planting density and selection of proper rootstock/scion combination, according to ecological conditions are important strategies (Pasa et al., 2015). In modern orchards with high planting density, pear cultivars are grafted on quince rootstocks as they are less vigorous than both clonal and seedling pear rootstocks (Hancock and Lobos, 2008). The use of quince as rootstock for pear cultivars is recommended as they provide precocity and improved fruit quality, and they offer easier crop management such as pruning, spraying, and harvesting (Stern and Doron, 2009; Francescatto et al., 2014). With the use of quince rootstocks, it is easy to establish an orchard with around 2000 - 5000 trees ha^{-1} (Pasa et al., 2012; Jovanovic et al., 2023). This ensures the ideal yield performance of pear cultivars per area due to higher photosynthetic efficiency (Ladaniya et al., 2020). Based on previous studies, high-density orchards with quince clonal rootstocks provide more yield than conventional ones due to the maximum utilization of solar energy, nutrients and water (Ladaniya et al., 2021). European countries mostly use quince rootstocks to establish high-density planting pear orchards, as they have prominent features (Brewer and Palmer, 2011; Musacchi et al., 2021). With the use of quince rootstocks for pear cultivars, it is possible to increase quality as less fruit is produced per canopy volume, but the economic life of those pear orchards was reported to be shorter compared to ones established on pear rootstocks (Sansavini and Musacchi, 2002; Musacchi, 2008; Zhang et al., 2016; Musacchi et al., 2021). In Türkiye, where there are many local fruit varieties in different provinces, the pear is an important species with approximately 600 local varieties (Özçağıran et al., 2005; Gundogdu et al., 2021). Nevertheless, they don't have a high potential for expansion of cultivation due to their low qualities and other unwanted vegetative and generative features such as stone cells, rustiness, internal browning and thorniness. Fortunately, in case of many characteristics, the 'Samsun Güzeli' is an ideal local genotype with a high cultivation potential to be expand all around the Türkiye. Especially for its attractive color, pyriform shape and for being an autumn cultivar, 'Samsun Güzeli' can easily achieve high demand in the local and international markets. However, despite having all these valuable characteristics, 'Samsun Güzeli' is not a well-known genotype and there is a need for researches that explore its cultivation and contribute significantly to its expansion potential. This research focused on investigating the performance of 'Samsun Güzeli', a late ripening local pear genotype on BA29 quince rootstock in an orchard planted under high density, during two consecutive years.

2. Materials and Methods

2.1. Materials

The 'Samsun Güzeli' pear genotype that was grafted onto BA29 quince rootstock was used as material. The experimental pear orchard was established with grafted, one-year old saplings in 2018 at the Bafra Agriculture Research center of Ondokuz Mayis University (41° 33' 50" N; 35° 52' 21" E; altitude 20 m). The plants started to yield in 2020. Observations and measurements were performed in 2021 and 2022. The study area has a hot and humid climate in summers and a cool climate in winters, and precipitation mainly occurs in the late fall and early winter. According to long-term climatic data of Samsun/Bafra district, average maximum temperature is 26.2 °C, average minimum temperature is 3.3 °C, and average annual temperature is 14.1 °C in the study area (Anonymous, 2022).

2.2. Methods

Grafted plants were planted at a spacing of 3.5 m x 1.5 m (1910 tree ha^{-1}) and trained according to the modified leader system. The plants were supported with metal poles that allow a good anchoring to support the saplings against wind and tie up the branches to prevent breaking at the yielding age. Pear trees were irrigated regularly with pressure compensating drippers at 1.20 m intervals, with two driplines per row, one on each side of the tree row. Weed control, as well as pruning, was done regularly every year in the study area.

2.3. Phenological, morphological and pomological traits

In the study the BBCH scale defined for pome fruits by Meier et al. (1994) was used to describe and define phenological stages. Observed timing of phenological stages, such as beginning of bud break, before the flowering

stage, balloon stage, first flowering, full flowering, end of flowering, fruit set, fruit maturity (harvest) and leaf fall were presented in *Table 1*. The number of days from full flowering to harvest, the number of flowers per plant (pieces tree[-1]), the number of flowers turned to fruit per plant (pieces tree[-1]) and the fruit set ratio (%) were also determined according to previous studies (Maas, 2008; Pasa et al., 2015; Oliveira et al., 2016; Kurt et al., 2022a). Rootstock diameter (mm), trunk diameter (mm), trunk cross-sectional area (cm[2]), tree height (cm), canopy width (West-East - cm), canopy length (North-South - cm), canopy height (cm), canopy volume (m[3]), annual shoot length (cm), bud number in the annual shoot (pieces shoot[-1]), leaf length (cm), leaf width (cm), leaf petiole length (cm), leaf petiole thickness (mm), and leaf area (cm[2]) were determined according to previous studies as morphological observations (Ozturk and Ozturk, 2014; Kurt et al., 2022a). Pomological observations were made based on previously performed research (Massai et al., 2008; Akcay et al., 2009; Stern and Doron, 2009; Ozturk and Ozturk, 2014; Kucuker et al., 2015; Ozturk et al., 2022). Fruit weight (g) was measured in randomly harvested 30 fruits in each replication with 0.01 g sensitive digital balance (CAMRY L-500). Fruit width (mm), fruit length (mm), fruit height (mm), fruit stalk length (mm), fruit stalk thickness (mm), and fruit skin thickness (mm) were measured with a 0.01 mm digital caliper (Mitutoyo CD-20CPX). Fruit volume (ml) was calculated using a 1000 ml graduated cylinder. Fruit firmness (kg cm^{-2}) was evaluated with a hand penetrometer (EXTECH FHT 200- with 5/16 head) according to Ertürk et al. (2009). Total soluble solids (TSS, Brix) were measured with a digital refractometer (ATAGO, PAL-1), pH was measured with a digital pH meter (Milwaukee MW150 Max) and titratable acidity of fruit (expressed as % malic acid) was determined by titration using standard 0.1N NaOH solution (Kurt et al., 2022a). Fruit skin and flesh color indices: *L*, a*, b** (from white to black, green to red and blue to yellow chromaticity coordinate), chroma, and hue degree (h°) were measured from two different points on the equatorial part of the fruit with a colorimeter (Minolta, model CR-300, Tokyo, Japan) as described by (Erdem and Ozturk, 2012; Kurt et al., 2022a). The number of fruits (fruits tree[-1]), yield per tree (kg tree[-1]), yield per hectare (kg ha^{-1}), yield per trunk cross-sectional area (kg cm^{-2}), and yield per canopy volume (kg m^{-3}) were recorded to determine the yield performance of the 'Samsun Güzeli' pear genotype (Ozturk et al., 2022). A group of 12 experienced panelists evaluated the organoleptic properties of 'Samsun Güzeli' fruits. Samples were evaluated for juiciness, taste, aroma, and visual qualities (overall appearances; fruit shape and skin color density). Panelists scored these attributes on 7-point hedonic scale to indicate the degree of like or dislike with the lowest score (1) corresponding to do not like at all while the highest score (7) corresponding to extremely like (Pasquariello et al., 2013).

2.4. Data analysis

One-way analysis of variance and descriptive statistics of obtained data were done with IBM SPSS 21.0 program (SPSS Inc. Chicago, ABD. Correlation analysis of data was done with XLSTAT 2022 statistical package.

3. Results and Discussion

3.1. Phenological characteristics

Description of the phenological stages and phenological codes were recorded using BBCH growth stage identification keys for mono and dicotyledonous plants (Hack et al., 1992). The observed dates of phenological growth stages of the 'Samsun Güzeli' pear genotype grafted on BA29 rootstock were presented in *Table 1*.

The results showed that the 'Samsun Güzeli' genotype completed its phenological cycle, reaching the leaf fall stage (code 97) in 255 days in 2021, and in 228 days in 2022 in Samsun climatic conditions.

Vegetative cycle of 'Samsun Güzeli' genotype started on 10 March in 2021 and 10 April in 2022. In the year 2021, flower buds were visible (code 55) on 12 March. Balloon stage (code 57) and first flowering (code 60) occurred on 15 March and 30 March, respectively, while full flowering (code 65) took place on 04 April. The end of flowering (code 69) occurred on 20 April. The fruit set (code 72) occurred on 26 April. The fruit maturity (code 89) eventuated on 25 September.

In the year 2022, flower buds were visible (code 55) on 14 April. Balloon stage (code 57) and first flowering (code 60) occurred on 17 April and 19 April, respectively, while full flowering (code 65) took place on 23 April. The end of flowering (code 69) occurred on 05 May. The fruit set (code 72) occurred on 11 May. The fruit maturity (code 89) on 10 October.

The number of days from full flowering to harvest was recorded as 174 days in 2021 and 171 days in 2022. The number of flowers per tree was recorded as 521 in 2021 and 719 in 2022, while the number of flowers turned to fruit observed as 67.00 pieces tree[-1] in 2021 and 86.67 pieces tree[-1] in 2022. The fruit set ratio was recorded as 12.85% in 2021, while recorded as 12.85% in 2022 (*Table 1*).

Table 1. Observed dates of phenological stages of 'Samsun Güzeli' pear genotype on BA29 rootstock, in the years 2021-2022.

	Phenological growth stages (PGS) codes according to BBCH scale	Description	2021	2022
Phenological characteristics	07	Beginning of bud break (BB)	10 March	10 April
	55	Flower buds visible (FBV)	12 March	14 April
	57	Balloon stage (BS)	15 March	17 April
	60	First flowering (First flower open) (FiFO)	30 March	19 April
	65	Full flowering (at least 50% of flowers open, first petal falling) (FuF)	04 April	23 April
	69	End of flowering (all petals fallen) (EoF)	20 April	5 May
	72	Fruit set (fruit size up to 20 mm) (FS)	26 April	11 May
	89	Fruit maturity (Fruit ripe for consumption) (FM)	25 September	10 October
	97	Leaf fall (all leaves fallen) (LF)	20 November	24 November
		Days from full flowering to harvest (DFFTH)	174	171
		Number of flowers (per tree-[1]) (NF)	521	719
		Number of flowers turned to fruit (pieces tree-[1]) (NFTTF)	67.00	86.67
		Fruit set ratio (%) (FSR)	12.85	12.05

The studies conducted on phenology of fruit cultivars is crucial when introducing them to new environments, especially those that differ from their native environments (Oliveira et al., 2016). The phenological differences between cultivars were reported to be related to the genetic differences and climatic conditions in that fruit trees are grown (Osmanoğlu et al., 2013; Ozturk et al., 2016; Mertoğlu and Evrenosoğlu, 2017). In hot and dry weather, all fruit trees' flowers open quickly, while in cool and rainy weather, flowering continues on the same tree for 2 to 10 days (Özçağıran et al., 2005). In our research, we recorded the flowering period of 'Samsun Güzeli' genotype was for about 20 days.

3.2. Morphological characteristics

Records of morphological and leaf traits of the 'Samsun Güzeli', grafted on BA29 rootstock are presented in *Table 2* and it was demonstrated that the differences regarding morphological and leaf characteristics between two consecutive research years were statistically non-significant. The mean values of morphological characteristics recorded as follows; rootstock diameter 76.71 mm, trunk diameter 61.97 mm, trunk cross-sectional area 34.06 cm^2, tree height 253.12 cm, canopy width 188.28 cm, canopy length 150.85 cm, canopy height, 223.28 cm, canopy volume 0.96 m^3, annual shoot length 37.19 cm, number of buds in an annual shoot 16.72, leaf length 5.22 cm, leaf width - 3.25 cm, leaf petiole length 3.55 cm, leaf petiole thickness - 0.39 mm and leaf area 11.74 cm^2.

It has been reported that rootstock diameter significantly varies in different rootstocks (Giacobbo et al., 2010). Cetinbas et al. (2018) stated that both the rootstock effect and the cultivar effect were significant with regard to rootstock diameter. The stem diameter of the cultivars on vigorous rootstocks was observed to be superior to those on the rootstocks with weaker growth performance (Sugar and Basile, 2011). The trunk cross-sectional area reported to be varied based on the production years, cultivars, and rootstocks (Jovanovic et al., 2022). Tree height

Table 2. Morphological and leaf characteristics of 'Samsun Güzeli' pear genotype on BA29 rootstock.

	Characteristics	2021	2022	Mean	P-value*	CV%	Std. D. of Mean
Morphological	Rootstock diameter (mm)	74.90	78.51	76.71	0.104	3.56	2.734
	Trunk diameter (mm)	56.31	67.63	61.97	0.113	14.06	8.713
	Trunk cross-sectional area (cm^2)	32.66	35.45	34.06	0.109	6.25	2.128
	Tree height (cm)	237.41	268.83	253.12	0.129	9.85	24.927
	Canopy width (West East) (cm)	169.72	206.83	188.28	0.132	15.73	29.614
	Canopy length (North-South) (cm)	135.29	166.42	150.85	0.130	16.40	24.735
	Canopy height (cm)	210.65	235.92	223.28	0.201	10.21	22.804
	Canopy volume (m^3)	0.92	1.01	0.96	0.157	7.61	0.073
	Annual shoot length (cm)	34.91	39.46	37.19	0.433	16.81	6.252
	Number of buds in an annual shoot	15.44	18.01	16.72	0.164	13.00	2.174
Leaf	Leaf length (cm)	5.17	5.27	5.22	0.300	2.19	0.114
	Leaf width (cm)	3.07	3.43	3.25	0.290	11.64	0.378
	Leaf petiole length (cm)	3.42	3.67	3.55	0.355	8.12	0.288
	Leaf petiole thickness (mm)	0.38	0.41	0.39	0.204	5.62	0.022
	Leaf area (cm^2)	11.22	12.27	11.74	0.190	7.97	0.936

*: There were no statistically significant differences at *P*=0.05

was affected by the rootstocks and cultivars as reported by (Lepsis and Drudze, 2011). In evaluations of 'Seleta' pear cultivar performance on quince rootstocks and *Pyrus calleryana* pear seedlings, Giacobbo et al. (2018) found that all quince rootstocks reduced the tree height by 60% compared to *Pyrus calleryana* rootstock. Giacabbo et al. (2010) reported that rootstocks significantly affect the canopy volume of cultivars. We found that the canopy volume of 'Samsun Güzeli' was slightly similar to 'Santa Maria', reported as 0.26 - 1.02 m³ by Engin (2011). Annual shoot length differs depending on rootstocks and cultivars; for example 'Abate Fetel' cultivar had 82.0 cm shoot length on seedling and 4.6 cm (the lowest) shoot length on BA29 (Castro and Rodriguez, 2002). Kurt et al. (2022b) reported that the leaf width of some standard pear cultivars grafted on different quince clone rootstocks varied between 23.98-28.81 mm and the leaf length varied between 35.56-49.20 mm. They determined the width and length of leaves as follows, respectively: 24.89 mm and 42.28 mm in 'Deveci'; 24.25 mm and 35.56 mm in 'Williams'; 28.81 mm and 49.20 mm in 'Santa Maria', and 23.98 mm and 48.46 mm in 'Abate Fetel'. Coban and Ozturk (2020) reported that the leaf length varied between 6.67 and 6.88 cm in terms of rootstock averages and between 6.42 and 7.23 in terms of cultivar averages. Results presented by Coban and Ozturk (2020) are also in line with Serttaş and Ozturk (2020), showing these differences are related to the genetic structures of rootstocks and cultivars. Serttaş and Öztürk (2020) recorded the highest leaf width (3.75 cm and 3.44 cm, respectively) in 'Deveci' and 'Santa Maria' and the lowest (3.40 cm and 3.34 cm, respectively) in 'Abate Fetel' and 'Williams' cultivars. We recorded the petiole length of 'Samsun Güzeli' as 37.20 mm, while Ozturk and Ozturk (2014) reported that of in 'Deveci' as 44.3 mm on BA29 and as 33.5 mm on pear seedling rootstocks. Similarly, Coban and Ozturk (2020) found that rootstocks and cultivars had a significant effect on petiole length. As in case of the petiole length, same conclusion on petiole thickness reached by Ozturk and Ozturk (2014) that petiole thickness of 'Deveci' cultivar varied (0.58mm to 0.76 mm) according to rootstocks and cultivars. Contrary to our findings, Coban and Ozturk (2020) obtained significantly higher values of petiole thickness in the rootstocks (0.97 - 1.27 mm) and in the cultivars (1.06 - 1.16 mm). Leaf area, which is generally used in fruit physiology studies, is an important criterion in determining respiration, transpiration, photosynthesis, light interception, water and nutrient use, flowering, fruit set, yield and quality in plants. And also leaf area is an important factor that determines the canopy volume efficiency of trees and fruit quality (Zhang et al., 2016). Leaf area of 'Samsun Güzeli' pear genotype varied from 11.22 cm^2 to 12.27 cm^2 in this study. Kurt et al. (2022b) reported that the leaf area of some standard pear cultivars varied between 6.24 and 10.64 cm^2. They observed that the leaf area was 7.63 cm^2 in 'Deveci', 6.24 cm^2 in 'Williams', 10.64 cm^2 in 'Santa Maria', and 8.68 cm^2 in 'Abate Fetel'. The determined leaf area in the study is partially similar to the leaf area determined by Kurt et al. (2022b) in some standard pear cultivars. These variations in reports may be attributed mainly to genetic differences.

3.3. Pomological and chemical characteristics

In two consecutive research years of 2021 and 2022, we found insignificant differences in case of pomological, chemical and color characteristics of the 'Samsun Güzeli' pear genotype grafted on BA29 rootstock (*Table 3*). The mean values of pomological, chemical, fruit skin and flesh color observations recorded as the following; fruit weight 114.60 g, fruit width 53.85 mm, fruit length 65.27 mm, fruit height 54.28 mm, fruit stalk length 22.66 mm, fruit stalk thickness 5.75 mm, fruit volume 109.90 ml, fruit skin thickness 0.26 mm, flesh firmness 3.39 kg cm^{-2}, total soluble solids 13.16%, acidity 0.33% and pH 4.37. Fruit skin L* 65.05, a* -3.92, b* 41.55, chroma 32.48, h^o 81.80, fruit flesh color L* 69.68, a* -0.75, b* 14.30, chroma 14.30 and h^o 91.85 (*Table 3*).

The size of the pear fruit is a critical marketing factor (Stern and Doron, 2009). The fruit weight, width, length and height of 'Samsun Güzeli' pear genotype on BA29 rootstock were 123.41 g, 51.87 mm, 62.55 mm and 51.05 mm, respectively, for 2021 and 105.78 g, 55.83 mm, 67.99 mm and 57.51 mm, respectively, for 2022. It should be pointed out that fruit weight of 'Samsun Güzeli' pear genotype was lower than other cultivars. The fruit weight of 'Santa Maria' on BA29 rootstock varied between 147.5 g and 169.4 g as reported by Erdem and Ozturk (2012) and from 140.0 to 156.2 g as reported by Kucuker et al. (2015). Ozturk et al. (2009) found the fruit weight 190.36 g in the 'Santa Maria' cultivar. The results determined in the study were partially similar to previous studies. These differences may be partly due to rootstock and cultivar effect as it pointed out by Kucuker and Aglar (2021).

Table 3. Pomological. chemical and color characteristics of 'Samsun Güzeli' pear genotype on BA29 rootstock.

Characteristics		2021	2022	Mean	P-value*	CV%	Std. D. of Mean
Pomological	Fruit weight (g)	123.41	105.78	114.60	0.138	12.41	14.224
	Fruit width (mm)	51.87	55.83	53.85	0.120	5.74	3.088
	Fruit length (mm)	62.55	67.99	65.27	0.198	7.47	4.874
	Fruit height (mm)	51.05	57.51	54.28	0.139	9.62	5.222
	Fruit stalk length (mm)	23.04	22.28	22.66	0.683	8.54	1.934
	Fruit stalk thickness (mm)	5.48	6.02	5.75	0.241	8.94	0.514
	Fruit volume (ml)	108.72	111.07	109.90	0.746	6.84	7.514
	Fruit skin thickness (mm)	0.28	0.24	0.26	0.291	15.20	0.040
	Flesh firmness (kg cm^{-2})	4.55	2.23	3.39	0.192	60.84	2.061
Chemical	Total soluble solids (%)	13.02	13.30	13.16	0.277	2.19	0.288
	Titratable acidity (%)	0.35	0.31	0.33	0.253	11.22	0.037
	pH	4.18	4.56	4.37	0.112	6.71	0.293
Fruit skin color	L*	64.39	65.71	65.05	0.511	3.28	2.134
	a*	-4.15	-3.69	-3.92	0.832	57.30	2.247
	b*	41.91	41.18	41.55	0.688	4.56	1.894
	Chroma	31.81	33.15	32.48	0.351	4.86	1.579
	h^o	76.91	86.70	81.80	0.229	11.34	9.279
Fruit flesh color	L*	71.72	67.63	69.68	0.370	7.12	4.962
	a*	-0.83	-0.67	-0.75	0.474	30.30	0.227
	b*	15.68	14.90	15.29	0.528	8.60	1.315
	Chroma	13.84	14.76	14.30	0.563	11.70	1.674
	h^o	90.85	92.84	91.85	0.291	2.29	2.107

*: There were no statistically significant differences at *P*=0.05

With regard to fruit stalk thickness the combination of Samsun Güzeli' pear genotype on BA29 quince rootstock resulted in higher values, i.e., 5.48 mm (2021) and 6.02 mm (2022) than the other similar studies. Fruit stalk thickness was recorded as 3.81 mm in 'Deveci'/QA combination and 4.12 mm in the 'Williams'/QA combination by Akcay et al. (2009). Uysal et al. (2016) reported that it varied from 3.94 to 4.75 mm in 'Deveci'/BA29 combination. And it was between 4.6 mm and 5 mm in 'Abate Fetel'/QA combination (Ozturk et al. (2016). Fruit stalk length was measured as 24.73 mm in 'Deveci'/QA and 29.43 mm in 'Williams'/QA

combinations by Akcay et al. (2009); as 31.54 and 32.56 mm in 'Deveci'/BA29 combinations by Uysal et al. (2016); 11.1 and 14.2 mm in 'Abate Fetel'/QA combinations by Ozturk et al. (2016).

Fruit firmness was reported to show variations according to rootstocks, growing years, and management practices in the pear orchards (du Plooy and Van Huyssteen, 2000; Urbina et al., 2003). Lepaja et al. (2014) found the fruit firmness of 'Santa Maria' 4.96 kg cm^{-2}. Ikinci et al. (2014) found that flesh firmness was highest in BA29 rootstock than in some other rootstocks. In our study the fruit firmness value was lower (2.33 kg cm^{-2}) than above mentioned cultivars which again may be the effect of cultivar and/or cultivar/rootstock combination.

The total soluble solids of pear fruits had a positive correlation with maturity, while acidity showed a negative relation (Kawamura, 2000). The rootstocks and research years significantly affected the TSS and acidity of the 'Shamiveh' pear cultivar (Askari-Khorosgani et al., 2019). Titratable acidity was reported between 0.39 and 0.47% in relation to rootstock and between 0.39and 0.42% in relation to year (Ozturk, 2021). It has been reported that the average pH of 'Santa Maria' pear cultivar was between 3.98 and 4 on BA29 rootstock by Erdem and Ozturk (2012) and between 3.98 and 4.06 on BA29 rootstock (Kucuker et al., 2015). The Crown structure and leaf area of the tree were mentioned to have a significant effect on fruit color (Ozturk, 2021), as the trees with lower crown parts can achieve more sunlight (Özçağıran et al., 2005).

3.4. Yield performance

Yield performance of the 'Samsun Güzeli' pear genotype grafted on BA29 rootstock are given in *Table 4*. Data obtained from 2-year study did not differ significantly with regard to yield performances of Samsun Güzeli'. Yield averages were recorded as 47.11 and 82.23 for number of fruits per tree; as 5.86 and 8.68 kg for yield per tree; as 13949.67 and 20662.80 kg for yield per hectare, 0.17 and 0.26 kg cm^{-2} for yield per trunk cross-sectional area and 5.90 and 8.70 kg m^{-3} for yield per canopy volume, respectively for the first and second year of the study.

Significant effect of rootstocks, cultivars, and their interactions on yield and fruit numbers mentioned by Maas (2008), Cabrera et al. (2015), Pasa et al. (2015), Kucuker and Aglar (2021) and Gill et al., (2011). Cabrera et al. (2015) reported that rootstocks significantly affected yield and 'Williams'/Farold 40 combination provided 190 ton ha^{-1} yield. Pasa et al. (2015) explained that the number of trees per area can affect the number of fruits per plant, yield, and yield efficiency in the 'Santa Maria' pear cultivar. Kucuker and Aglar (2021) reported that the yield of 'Santa Maria'/QA combination was between 3.80 and 7.60 kg tree^{-1}. Yield efficiency of 'Santa Maria'/QA combination ranged from 2.22 kg cm^{-2} to 2.97 kg cm^{-2} (Kucuker and Aglar, 2021), while ranged from 0.43 to 1.17 kg m^{-3} in 'Patharnakh', a cultivar of *Pyrus pashia* (Gill et al., 2011).

Table 4. Yield performance of 'Samsun Güzeli' pear genotype on BA29 rootstock.

Yield Performance	2021	2022	Mean	Sig.*	CV%	Std. D. of Mean
Number of fruits (per tree)	47.11	82.23	64.67	0.231	51.57	33.352
Yield per tree (kg)	5.86	8.68	7.27	0.370	47.13	3.427
Yield per hectare (kg)	13949.67	20662.80	17306.23	0.251	38.18	6.607
Yield per trunk cross-sectional area (kg cm^{-2})	0.17	0.26	0.22	0.341	51.67	0.111
Yield per canopy volume (kg m^{-3})	5.90	8.70	7.30	0.393	48.81	3.562

*: There were no statistically significant differences at *P*=0.05

3.5. Sensory evaluation

Sensory evaluations of the 'Samsun Güzeli' pear genotype grafted on BA29 rootstock were given in *Figure 1*. As is evident on the figure, there were no statistically significant differences between the years in terms of sensory attributes of fruit, based on 1 to 7 rating scale. Among the sensory attributes, however, the highest mean of liking score (6.32) was received from juiciness in 2022 and lowest score (3.57) from visual quality, in the same year.

Sensory evaluation has been defined as a scientific discipline used to evoke, measure, analyze, and interpret those reactions to the fruits as discern through the senses of sight, smell, taste, touch, and hearing (Pasquariello et al., 2013). Sensory evaluation of 'Samsun Güzeli' was performed to define the sensory attributes in relation to the

consumer preference, like juiciness, taste, aroma, and visual quality all of which subsequently have determining effect on eating quality of pears fruits (Zerbini, 2002).

Figure 1. Sensory characteristics of 'Samsun Güzeli' pear genotype on BA29 rootstock.

Figure 2. 'Samsun Güzeli' pear genotype

The juiciness of pear fruit may be affected by the pectic enzymes (Pasquariello et al., 2013). Pectic enzymes are a group of enzymes responsible for pectin degradation in fruits. Although data generated from panelists may be vary and relationship between observed and sensory responses are also likely to be vary, it may be speculated that the favorable results can be indication of good maturity quality of this cultivar. Predieri et al. (2005) pointed out that aroma together with the taste, is major determinant of quality, and that these aromatic volatiles define the distinctive pear fruit flavor. When considering taste, sweetness, sourness, and aroma of pear fruit are important characteristics since these characters are associated with the composition of soluble sugars, organic acids, and

volatile compounds (Zerbini, 2002; Zhang et al., 2008). Pre-harvest factors, genetic differences, maturity at harvest, storage conditions, and fruit physiology are among the factors reported to affect the aroma of pear fruits (Rapparini and Predieri, 2002). The shape of pear fruits can range from round to elongated (Pasquariello et al., 2013). The external quality (visual appearance) of pears is closely related to shape, skin smoothness, color and general attractiveness and affects consumer demand (Özçağıran et al., 2005). As can be seen in *Figure 2*, the 'Samsun Güzeli' pear genotype has an attractive golden yellow-red color and a pyriform shape at maturity, and also has high consumer demand for this reason.

3.6. Correlation analysis

Additionally, a correlation-based technique (Pearson coefficient) was used to characterize the 'Samsun Güzeli' pear attributes in-depth and to assess relationships between dependent variables. The correlation patterns between the various features assessed in the study are represented in (*Figure 3*). The numerous parameters that were employed showed some positive and negative associations. It is found that number of flowers is highly significantly and positively correlated with fruit weight and fruit length.

Figure 3. The figure shows the Pearson correlation coefficients between selected characteristics. The color and size of the circles reflect the strength of the correlation.

NFlr= Number of Flowers, NFTF= Number of Flowers Turned to Fruit, ASL= Annual Shoot Length, LA= Leaf Area, FWe= Fruit Weight, FWi= Fruit Width, FL= Fruit Length, FV= Fruit Volume, NFrt= Number of Fruits, YT= Yield per Tree, YH= Yield per Hectare, YTCSA= Yield per Trunk Cross Sectional Area, YCV= Yield per Canopy Volume, J= Juiciness, T= Taste, A= Aroma, VQ= Visual Quality.

The number of flowers turned to fruit showed a strongly positive correlation with the number of fruit, yield per tree, yield per trunk cross-sectional area, and yield per canopy volume, on the other hand showed a strongly negative correlation with fruit width and fruit length. Annual shoot length had a strongly positive correlation with

leaf area, fruit volume, and yield per hectare. Both the number of fruit per tree and yield per tree showed a strongly negative association for fruit width and fruit length. A strongly positive correlation was found between the number of fruit and yield per tree with the yield per trunk cross-sectional area and yield per canopy volume. Fruit volume was found to be negatively strongly correlated with the yield per tree, number of fruits, yield per trunk cross-sectional area, yield per canopy volume and taste. Fruit aroma and taste characteristics had a strong negative correlation with annual shoot length and leaf area.

4. Conclusions

Vegetative cycle of 'Samsun Güzeli' pear genotype started with flower bud break on 10 March and lasted until leaf fall on 20 November in 2021, and on 10 April and lasted until leaf fall on 24 November in 2022. The first flowering in the mentioned year occurred on 19 April, while full flowering took place on 23 April. The number of flowers per tree was recorded as 719 in 2022 as 521 in 2021, while the number of flowers turned to fruit was observed as 86.67 per tree in the 2022. The average values of morphological characteristics recorded as the following; rootstock diameter 76.71 mm, trunk diameter 61.97 mm, trunk cross-sectional area 34.06 cm^2, tree height 253.12 cm, canopy width, length and height 188.28 cm, 150.85 cm, 223.28 cm, respectively, canopy volume 0.96 m^3, annual shoot length 37.19 cm, number of buds in an annual shoot 16.72 per shoot, leaf length and width 5.22 cm and 3.25 cm, respectively, leaf petiole length and thickness 3.55 cm, 0.39 mm, respectively and leaf area 11.74 cm^2. It may be speculated that results obtained for yield response of 'Samsun Güzeli' genotype on BA29 (7.27 kg tree^{-1}) could be used as a good basis for further research on this genotype.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflict of interest

The authors declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authorship Contribution Statement

Concept: Öztürk, A., Faizi, Z.A.; Design: Öztürk, A., Faizi, Z.A.; Data Collection or Processing: Öztürk, A., Faizi, Z.A.; Statistical Analyses: Faizi, Z.A.; Literature Search: Öztürk, A., Faizi, Z.A.; Writing, Review and Editing: Öztürk, A., Faizi, Z.A.

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ARAŞTIRMA MAKALESİ   RESEARCH ARTICLE

Genetic Diversity and Relationship of Native *Phalaenopsis* Orchids: A Case Study of Indonesian Archipelago

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Abstract

The native *Phalaenopsis* is valuable germplasm for future orchid breeding programs and for its conservation because it provides many beneficial traits or genes. This study aimed to determine and analyze the molecular diversity and phylogeny of Indonesian native *Phalaenopsis* by a DNA barcoding (*mat*K) marker. A total of 19 samples of *Phalaenopsis* orchids were used in this study. All leaf samples of orchid were extracted and purified using a commercial DNA isolation kit from Geneaid Biotech Ltd., Taiwan (GP100). The DNAs were then amplified by specific *mat*K primers: Forward (5'-CGTACAGTACTTTTGTGTTTACGAG-3') and Reverse (5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'). The DNA targets or products (*mat*K) were purified and sequenced by the Sanger-bidirectional method at 1st Base Ltd., Malaysia. Before further analysis, the *mat*K sequences of *Phalaenopsis* were edited, reconstructed, and aligned with the assistance of Clustal W in the MEGA 11 software. Its genetic diversity was determined using the nucleotide diversity index (π%), and the phylogenetic analysis was performed using the maximum likelihood (ML) method with a statistical bootstrap. The phylogenetic relationship was also assessed using principal component analysis (PCA). Based on this marker, the native *Phalaenopsis* has a high genetic diversity (π% = 1.70). In addition, the phylogenetic analysis revealed that this germplasm was separated into seven clades, where *P. pantherina* has the closest relation to *P. cornu-cervi* and *P. gigantea*. Conversely, the highest genetic distance was to *P. amabilis* from South Kalimantan and to *P. celebensis* from Sulawesi, at a coefficient divergence of 0.084. Our findings provide an essential foundation for supporting future orchid breeding practices, including conservation, on local and global scales.

Keywords: Breeding program, Genetic diversity, Moth orchid, Ornamental plant, Phylogenetic relationship.

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1. Introduction

Phalaenopsis, commonly known as a moth or moon orchid, is the most famous orchid genus in the world, with a relatively high number of species (Hsu et al., 2018). Globally, this orchid comprises about 66 native species and is distributed mainly in the Asiatic regions (Hinsley et al., 2018), such as India, Sri Lanka, Taiwan, Philippines, and Indonesia (Deng et al., 2015). In Indonesia, more than 20 species of *Phalaenopsis* are present in seven large islands, including Sumatra, Java, Borneo (Kalimantan), Celebes (Sulawesi), Timor (Nusa Tenggara), Moluccas (Maluku), and Papua (Rahayu et al., 2015). In this case, Borneo is the third largest island with a high diversity of orchids worldwide (Siregar, 2008). Besi et al. (2021) estimated that 2500-3000 species (equivalent to 10% of the world's orchids) are present on this island.

In general, native orchids are unique germplasm. They can grow in their habitat without human assistance. Besides, they genetically stored many valuable genes or traits for conservation and breeding programs (Li et al., 2021). However, existing of these orchids in their customary habitat is challenged, even threatened by natural disturbance and human intervention (Mursyidin et al., 2021a). Ecologically, orchids grow in specific habitats, so they are often present in small populations and a narrow-pattern distribution. Deforestation, habitat degradation, overexploitation, and illegal trading are human factors that contribute to reducing this orchid population in nature (Zahara and Win, 2019). Consequently, the preservation, breeding, and analysis of the genetic diversity of *Phalaenopsis* orchids are of great importance.

Conventional methods utilizing morphological markers have been used for years to analyze the genetic diversity of orchids (Kwon et al., 2017). However, these methods are nevertheless time-consuming, and morphological markers are affected by environmental conditions (Nadeem et al., 2018). The genetic diversity of *Phalaenopsis* has also been studied using several molecular markers, e.g., Random Amplified Polymorphic DNA (RAPD) (Niknejad et al., 2009) and Simple Sequence Repeat (SSR) (Tsai et al., 2015). Again, these markers also have drawbacks, such as being highly subjective and producing less precise analytical results (Lee et al., 2017).

Nowadays, sequencing-based DNA markers, such as DNA barcoding or chloroplast DNA markers, are commonly used in determining the genetic diversity and relationship of orchids (Jheng et al., 2012). While these markers have a few disadvantages, including relatively high costs, they are faster and more accurate in determining the genetic diversity of germplasm (Singh et al., 2017). Among markers present, the Consortium for the Barcode of Life has recommended *maturase* K (*mat*K) as one of the DNA barcoding markers for these objectives, i.e., determining the genetic diversity and phylogenetic relationship of germplasm (CBOL Plant Working Group, 2009).

Conceptually, *mat*K is a functional gene in the chloroplast genome (cpDNA) that shows a high mutation rate and, therefore, is known as a fast-evolving gene (Barthet et al., 2020). Kar et al. (2015) reported that this gene has a substitution rate three times higher than the *rbc*L, another similar gene in cpDNA. This gene also displays varying numbers and sizes of indel (insertions-deletions) mutation. As a result, it provides a high phylogenetic signal for resolving genetic relationships among plants at all taxonomic units (Kar et al., 2015). In other words, *mat*K provides many excessive variability sequences that can be aligned to determine evolutionary relationships of germplasm from species to higher taxonomic levels (Kar et al., 2015).

The objectives of our study are to determine and analyze the molecular diversity and phylogeny of the Indonesian native *Phalaenopsis* using the *mat*K marker. Our study may provide results to support future orchid breeding programs and its conservation, locally and globally, particularly for *Phalaenopsis*.

2. Materials and Methods

2.1. Plant materials

We have collected and used 19 samples of orchids from several parts of Indonesia (*Figure 1*). Samples were categorized into 15 *Phalaenopsis* species and two *Paraphalaenopsis* (used as an outgroup). Detailed information on the collected samples were presented in *Table 1*.

Figure 1. Map of Indonesia showing locations of 19 native Phalaenopsis samples collected for this study.

Table 1. Sampling location (province origin), common name, and length of matK sequence of native Phalaenopsis used in this study.

Sampling location (Province Origin)	Species	Common name	Length of *matK* (bp)
South Kalimantan	*P. amabilis*	Moon or moth orchids	861
	P. cornu-cervi	Deer antlered *Phalaenopsis*	918
	P. deliciosa	Delicate *Phalaenopsis*	815
	P. difformis	Dark brown *Phalaenopsis*	977
	P. modesta	Modest *Phalaenopsis*	826
	P. pantherina	Panther-like *Phalaenopsis*	803
	P. sumatrana	Sumatran *Phalaenopsis*	891
	P. zebrina	Zebra-like *Phalaenopsis*	832
East Kalimantan	*P. bellina*	Beautiful *Phalaenopsis*	911
	P. gigantea	Giant *Phalaenopsis*	931
	P. lamelligera	Deer antlered *Phalaenopsis*	876
	P. modesta	Modest *Phalaenopsis*	878
	Para. labukensis	Mouse tail orchid	839
West Kalimantan	*P. bellina*	Beautiful *Phalaenopsis*	835
	P. corningiana	Corning's *Phalaenopsis*	811
	Para. serpentilingua	Mouse tail orchid	819
Mentawai, Sumatra	*P. violacea*	Violet moth orchid	795[a]
Sulawesi	*P. celebensis*	Celebes *Phalaenopsis*	1045[b]
Maluku	*P. amboinensis*	Amboin Island *Phalaenopsis*	898

Remarks: [a] the shortest; [b] the longest

2.2. DNA isolation and purification

All samples of orchid leaves were extracted and purified using a DNA isolation kit, commercially from Geneaid Biotech Ltd., Taiwan (GP100). In this stage, 50 g of leaf samples were crushed by mortar and prepared according to the manufacturer's instructions until pure DNA was obtained.

2.3. DNA quantification, amplification, and sequencing

The concentration and purity of the extracted DNA samples were determined using a UV-VIS spectrophotometry method. By specific primers of *mat*K (Le et al., 2020), i.e., Forward (5'-CGTACAGTACTTTTGTGTTACGAG-3') and Reverse (5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'), the DNAs were amplified as following these conditions (Mursyidin et al., 2022): Initial denaturation was carried out at 94ºC for 5 min and denaturation at 94ºC for 30 sec. This was followed by 30 sec of alignment at the annealing temperature (48ºC); 45 sec of alignment at 72ºC for extension, and 7 min of alignment at 72ºC for final extension (72ºC). The reaction was repeated for 35 cycles and employed in a total volume of 25 µL, i.e., DNA template (2 µL; 20 ng), *mat*K-F/R primers (1 µL; 0.2 µmol), and 22 µL of PCR mix (MyTaq HS Red Mix, Bioline, UK). The separation of the DNA target (product) was conducted with electrophoresis of agarose gel (2%) in a 1X TBE buffer solution and GelRed DNA stain (Biotium Inc., USA) and visualized with a UV transilluminator. Finally, the purification and sequencing of DNA products was done by the Sanger-bidirectional method at 1st Base Ltd., Malaysia.

2.4. Data analysis

The analysis was initiated by manual reconstruction and editing of the *mat*K consensus sequence of *Phalaenopsis*, with the assistance of ClustalW in the MEGA 11 software (Tamura et al., 2021). After completion, all sequences were aligned using similar programs (Tamura et al., 2021) and in MultAlin (Mitchell, 1993). The purpose of multiple alignments was to observe the conserved region and some mutational events, such as substitution (transition-transversion) and indels (insertion-deletion) therein. Several variables were also analyzed, including GC content, polymorphic region (variable site; parsimony-informative; singleton site), transition/transversion ratio (Ti/Tv), including genetic diversity (π%). The latest (genetic diversity) was determined using the Nei and Li (1979) criteria: low (0.1 - 0.4); moderate (0.5 - 0.7), and height (0.8 - 2.0). The final analysis was the phylogenetic tree reconstruction carried out using the maximum likelihood (ML) method with a statistical bootstrap, a Kimura-2 parameter of substitution model, and a Nearest-Neighbor-Interchange (NNI) interference approach (Kimura, 1980; Tamura et al., 2021). The phylogenetic relationship was also confirmed using principal component analysis or PCA (Bahar et al., 2019; Mursyidin and Khairullah, 2020).

3. Results and Discussion

Determination of genetic diversity and the phylogenetic relationship of native orchids is beneficial in supporting future orchid plant conservation and breeding programs. According to Yusop et al. (2022), the native orchid is a germplasm with high genetic diversity for use in these activities, mainly as the parental cross. This study's native orchids of *Phalaenopsis* showed high genetic diversity (π%, 1.70) based on *mat*K markers (*Table 2*). The high genetic diversity is strongly correlated with the mutations that occur in it, i.e., substitutions (transitions and transversions) and indels (insertion-deletions) (*Figure 2*).

Table 2. Molecular characteristics of matK of Indonesian native Phalaenopsis

Parameter	*mat*K
Sequence length observed (bp)	795-1045
GC content (%)	31.42
Nucleotide diversity (π%)	1.70
Singleton site/SNP (bp)	126
Transition/transversion bias value (R)	0.70
Polymorphic sites (bp)	175

According to Suriani et al. (2021), variations in the order of gene sequence in cpDNA, including the *mat*K gene, are due to a single nucleotide mutation (SNP) present in the region. In this study, SNP recorded 126 incidents (*Table 2*). The number is almost the same as the report of Chen and Shiau, (2015) on the *mat*K sequence of *Anoectochilus*.

Molecularly, *mat*K is one of the chloroplast genes involved in photosynthesis. According to Barthet et al. (2020), this gene exhibits a relatively high mutation, which is beneficial for genetic diversity and relationship studies. Kar et al. (2015) reported that *mat*K has a substitution rate three times higher than other chloroplast genes,

especially *rbc*L, so often called a rapidly evolving gene. In contrast, indels are occasionally found in the gene, demonstrating a conservative pattern of nucleotide replacement (Clegg, 1993). However, indels in some plant families raise many questions about whether the gene can maintain its structure and function in the cell, especially in protein expression (Kar et al., 2015).

Figure 2. The multiple alignments of matK sequences, showing a barcoding motif of Indonesian native Phalaenopsis. Red marks = a conserved region; green highlight = substitutions; purple highlight = insertions-deletions (INDELs)

According to Chen and Shiau (2015), this mutation causes a difference in the length of the *mat*K sequence. In this study, the partial region of *mat*K of native *Phalaenopsis* ranged from 795 to 1045 bp in length (*Table 2*). Conversely, the complete gene was 1500 bp (Thakar et al., 2016) to 1536 bp in length (Mustafa et al., 2018). Partially, those gene has a different size in some plants, such as *Ficus* (Li et al., 2012), *Lycopersicum* (Căprar et al., 2017), and *Oryza sativa* (Mursyidin et al. 2021b), for about 830 bp–857 bp.

In this case, the high *mat*K sequence variation provides a high phylogenetic signal for resolving evolutionary relationships among plant germplasm at all taxonomic levels (Kar et al., 2015). In this context, the high genetic diversity of the native *Phalaenopsis* became a valuable source for the preservation and breeding program of orchid plants. According to Teixeira and Huber (2021), these genetic variations are beneficial in the preservation program, especially in promoting population survival and guaranteeing the adaptive potential of natural populations in the face of rapid environmental change. For plant breeding, genetic diversity is imperative, particularly in developing new cultivars with desired traits (Govindaraj et al., 2015), including parental selection (Aesomnuk et al., 2021) or selecting parents that are genetically distinct (Wu et al., 2021). In other words, determining resources with this high variation will become beneficial for widening the genetic base or gene pool of germplasm (Aesomnuk et al., 2021).

Conceptually, the different levels of genetic diversity are correlated with several factors, such as the breeding system, geographic range and variation, seed dormancy and dispersal mechanism, and natural selection (Huang et al., 2016). Life span and other life-history traits, such as the history of populations, are also affected by the genetic diversity level. Furthermore, environmental factors are often responsible for observed diversity patterns at small spatial scales (Huang et al., 2016; Ozer et al., 2021). However, on a practical level, the improvement of orchid genetic diversity must be continued through hybridization, introgression, mutation, and genetic engineering (Allier et al., 2020).

The phylogenetic analysis, using ML, showed that the native *Phalaenopsis* separates into seven groups or clades (*Figure 3*). Clades II and VI were the largest with 5 and 4 species, respectively. As in the ML, the PCA reveals seven groups (*Figure 4*), albeit with different numbers and compositions of species members. The closest relationship is shown between P. pantherina and P. cornu-cervi from South Kalimantan, as for *P. pantherina* with *P. gigantea* from East Kalimantan (*Figure 5*). Morphologically, although these three orchids have different flower shapes, they have relatively the same primary flower color and are decorated with line or strip motifs on the petals (Lafarge, 2015). In contrast, the farthest relationship by *P. amabilis* from South Kalimantan with *P. celebensis* from Sulawesi, with a divergence coefficient of 0.084 (*Figure 5*). According to Lafarge (2015), morphologically, they have almost the same shape and floral motifs, i.e., flowers with a white-base color and a moth-like shape.

Figure 3. Phylogenetic tree of maximum likelihood (ML), showing a separation of Indonesian native Phalaenopsis into seven clades. The tree was evaluated by bootstrap, indicated by values on internal nodes

Figure 4. The PCA (principal component analysis) groupes Indonesian native Phalaenopsis into seven clades.

Figure 5. A heatmap, showing the genetic divergence among Indonesian native Phalaenopsis. OTUs = operational taxonomic units

Based on the *rbc*L and *trn*L-F markers, Mursyidin et al. (2021a) reported the close relatedness between *P. amabilis* and *P. celebensis* with a similarity coefficient of 93.90%. On the other hand, based on ITS (internal transcribed spacers) markers, Tsai et al. (2010) reported the close relatedness between *P. amabilis* and *P. sanderiana*; between *P. cornu-cervi* and *P. borneensis*. According to Fatimah and Sukma (2011), the close relatedness of *P. amabilis* to *P. fuscata* is shown by microsatellite markers. Based on the RAPD marker, *P. amabilis* is closely related to *P. hieroglyphica*, whereas *P. cornu-cervi* with *P. mannii* and *P. pantherina* (Niknejad et al., 2009).

According to Acquaah (2015), crossing parents with distant relationships may generate descendants with high diversity. In contrast, crossing elders with close relationships tend to produce descendants with narrow genetic diversity and tend to inbreed (CBOL Plant Working Group, 2009). Briefly, this information is useable in the orchid's breeding and preservation efforts. Furthermore, for the preservation program, our results can be utilized in inferring species and their evolutionary history, including gene flow, genetic differentiation, and species delimitation.

4. Conclusions

Based on the *mat*K region, the native *Phalaenopsis* from Indonesia has a high genetic diversity ($\pi\%=1.70$). The phylogenetic analysis revealed that this germplasm is grouped into seven main clades, where *P. pantherina* has the closest relation to *P. cornu-cervi* and *P. gigantea*. In contrast, the farthest relationship was found to be between *P. amabilis* from South Kalimantan and *P. celebensis* from Sulawesi, with a divergence coefficient of 0.084. Our results provide an essential foundation for supporting future orchid breeding and conservation practices on local and global scales.

Acknowledgment

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: DHM; Design: DHM.; Data Collection or Processing: MRF; Statistical Analyses: DHM, MRF; Literature Search: MRF; Writing, Review, and Editing: DHM; Read and approved the final manuscript: DHM, MRF.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Classification of Open and Closed Pistachio Shells Using Machine Vision Approach

Makina Görme Yaklaşımı Kullanılarak Açık ve Kapalı Antep Fıstığı Kabuklarının Sınıflandırılması

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Abstract

Pistachio nuts are a type of nut that is widely consumed around the world due to their high nutritional value and pleasant taste. Pistachios are usually sold in their shells, either open or closed. However, closed-shell pistachios are not well received by consumers, resulting in a lower commercial value. It is essential to be able to distinguish between open and closed pistachio shells in order to ensure quality control during production processes and processing. This can be done manually or by using mechanical devices. Manual inspection and categorization of pistachio nuts have traditionally been done by workers, but this process is inefficient in terms of time and money. Mechanical separation of open and closed-shell pistachio can damage the kernels of open-shell nuts due to the needle mechanism used in the sorting process. This study aims to classify pistachio nuts using a machine vision-based system and evaluate its applicability in terms of classification accuracy. The system is evaluated on the Antep pistachio species, which can be distinguished from other pistachio varieties, such as Siirt and Urfa pistachios, based on their shape, size, and taste properties. The machine vision system in this study classifies pistachio nuts into closed and open shell classes in a completely automated manner. In this study, 1,000 Antep pistachio nuts images were obtained and examined, including 500 open and 500 closed nuts. The images were pre-processed and prepared for feature extraction. From the images, a total of 14 color features were extracted. Although the single feature was used, promising classification accuracy rates of 95.6%, 94.8%, and 93.6% from the Random Forest, Support Vector Machine (SVM), and Logistic Regression were achieved, respectively. The performances of classifiers were compared to each other. Almost similar performances were detected. These results demonstrate that the Random Forest classifier is the most effective algorithm for classifying open and closed Antep pistachio nuts.

Keywords: Pistachio, Image processing, Color feature, Logistic regression, Random forest, Support vector machine

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Öz

Antep fıstığı, besin değeri yüksek ve hoş tadı nedeniyle dünya çapında yaygın olarak tüketilen bir fıstık türüdür. Genellikle Antep fıstığı açık veya kapalı kabuklu olarak satılmaktadır. Ancak, kapalı kabuklu Antep fıstığı tüketiciler tarafından tercih edilmemekte ve bu da fıstığın ticari değerinin düşmesine neden olmaktadır. Üretim süreçleri ve işleme sırasında kalite kontrolünü sağlamak için açık ve kapalı uçlu Antep fıstığı kabuklarını ayırt edebilmek esastır. Bu manuel olarak veya mekanik cihazlar kullanılarak yapılabilir. Manuel sınıflandırma işlemi işçiler tarafından yapılmakta olup bu şekilde yapılan ayırma işlemi zaman ve maliyet açısından verimsiz sayılmaktadır. Mekanik sınıflandırma işleminde ise fıstığının mekanik olarak ayrılması, ayıklama işleminde kullanılan iğne mekanizması nedeniyle açık kabuklu somunların çekirdeklerine zarar verebilmektedir. Bu çalışma, Antep fıstığı tanelerinin makina görme tabanlı bir sistem kullanılarak sınıflandırılmasını ve sınıflandırma doğruluğu açısından uygulanabilirliğinin değerlendirilmesini amaçlamaktadır. Bu çalışmada kullanılan sistem, Siirt ve Urfa fıstıklarından şekil, boyut ve tat özellikleri bakımından farklı olan Antep fıstığı çeşidi için yapılan değerlendirmeleri içermektedir. Bu çalışmadaki makina görme sistemi, Antep fıstığı tanelerini tamamen otomatik bir şekilde kapalı ve açık uçlu kabuk sınıflarına ayırabilmektedir. Bu çalışmada 500 açık ve 500 kapalı uçlu tane olmak üzere 1.000 adet Antep fıstığı tane görüntüsü elde edilmiş ve incelenmiştir. Görüntüler işlenerek özellik çıkarma için hazırlanmıştır. Görüntülerden toplam 14 renk özelliği çıkarılmıştır. Tek özellik kullanılmasına rağmen, Rastgele Orman, Destek Vektör Makinesi ve Lojistik Regresyon modellerinden sırasıyla % 95.6,% 94.8 ve% 93.6'lık umut verici sınıflandırma doğruluk oranları elde edilmiştir. Sınıflandırıcıların performansları birbirleriyle karşılaştırılmıştır. Sınıflandırıcılar arasında yaklaşık benzer performanslar elde edilmiştir. Bu sonuçlar, Rastgele Orman sınıflandırıcısının, açık ve kapalı kabuklu uçlu olarak Antep fıstığı tanelerini sınıflandırmak için en etkili algoritma olduğunu göstermektedir.

Anahtar Kelimeler: Fıstık, Görüntü işleme, Renk özelliği, Lojistik regresyon, Rastgele orman, Destek vektör makinesi

1. Introduction

Pistachio (*Pistacia vera* L.) is one of the most popular tree nuts in the world, second only to the hazelnut in terms of fat. It is also rich in minerals and vitamins as it contains phosphorus, potassium, calcium, magnesium, and iron and contains vitamins such as E, C, B1, and B2 (Aktaş, Kızıldeniz, & Ünal, 2022). Pistachio nuts (*Pistacia vera* L.) are economically important for Turkey, Iran, China, the United States, and Southern European countries. Pistachio nuts are widely known as green gold due to their health-promoting nutrients and economic importance (Hosseinpour-Zarnaq, Omid, Taheri-Garavand, Nasiri, & Mahmoudi, 2022). Turkey is one of the main pistachio nut-producing countries in the world. In Turkey, there are eight main domestic pistachio vera varieties such as Uzun, Kırrmızı, Halebi, Siirt, Beyazben, Sultani, Değirmi, and Keten Gömleği. Also, there are five foreign varieties named Ohadi, Bilgen, Vahidi, Sefidi, and Mümtaz (Ak & Acar, 2001). Generally, pistachio nuts are grown in 56 provinces of Turkey. With its fluctuating production structure, Turkey ranks fourth behind Iran, the USA, and China, and with its annually increasing exports, Turkey ranks fourth behind the USA, Iran, and Germany (FAOSTAT, 2022). Iran's non-oil exports heavily rely on pistachio production, which has played a crucial role in the country's economy. Until 2010, Iran had produced over half of the world's pistachios and had supplied over 70% of global pistachio exports (Yaghoubi & Niknami, 2022). Harvested pistachio nuts contain many empty, undeveloped, closed shells due to factors such as unsuitable climate, incomplete pollination, lack of nutrition, and disease. However, pistachio nuts with closed shells have low consumer acceptance, leading to lower added commercial value. In the production and processing of pistachio nuts, it is very important to be able to classify opened and closed pistachio nuts accurately. This can be done manually and with mechanical devices. Distinguishing open and closed pistachio nuts can be achieved through both manual and mechanical methods. However, manual sorting is time-consuming and can be influenced by various factors, such as the operator's age, fatigue, visual acuity, and room conditions) (Omid, 2011). Mechanical devices to separate closed-shell pistachios from open-shell nuts can cause damage to the nuts of open-shell due to the needle mechanism used in the sorting process. In order to address these challenges, researchers have been working to develop new, non-destructive sorting methods in recent decades, including acoustic signal analysis and machine vision, to sort nuts quickly and accurately without causing any damage. Mechanical sorting is one of the popular techniques for sorting closed and open pistachio shells. This sorting method is done using a device called a "Pinpicker." Nevertheless, this technique damages open pistachio nuts, leading to a reduction in quality (C. Pearson, A. Doster, & J. Michailides, 2001). Pearson created a system for sorting open and closed-shell pistachio nuts in real time using acoustic signals. The system recorded audio signals, analyzed three static features in the time domain, and used linear discriminant analysis to differentiate the nuts. The acoustic sorter was found to have higher accuracy in classifying the nuts compared to a commercially available mechanical sorting system (Hosseinpour-Zarnaq et al., 2022). Decision tree and fuzzy logic classifiers were used to sort two Iranian pistachio nuts, Akbari (Ak) and Kaleghouchi (Ka). 240 Ak and 120 Ka pistachios fell down a chute onto a stainless steel plate, and the acoustic signal of these hits was recorded with a microphone and stored on a computer via a sound card. In this study, statistical parameters were extracted from these time-domain signals using the J48 algorithm. Feature selection and classification were performed using 200 Ak and 100 Ka as the training set and 40 Ak and 20 Ka as the test set. The results of the study showed that the proposed system achieved a classification accuracy of 93.3% for the training set and 96.67% for the test set (Jalali & Mahmoudi, 2013).

In recent years, there have been several studies on the classification of open and closed pistachio nuts using machine learning algorithms. Improved k-NN Classifier used in a study carried out by (Ozkan, Koklu, & Saraçoğlu, 2021) to classify open and closed pistachio nuts. In this study, shape and morphological features were extracted from images. These features were then used as inputs to train and test the machine learning algorithm. The results of the study showed that the proposed technique achieved a classification accuracy of 94.18%. Ghezelbash et al. (2013) developed a system that adopts a combination of two flat mirrors and one low-cost camera to obtain appropriate 3-dimensional images from pistachios which are processed to detect closed-shell nuts. The experimental results for the three varieties of pistachio nuts showed an average removal accuracy of 92.7 and 86.7% for open – and closed pistachio nuts, respectively. Omid (2011) proposed an expert system based on an acoustic emission signal and a fuzzy logic classifier for sorting open and closed pistachios nuts, and the overall accuracy of the sorting system for testing data sets was 95.56% (Hosseinpour-Zarnaq et al., 2022). Rahimzadeh and Attar (2022) proposed a computer vision system aimed to distinguish between open and closed pistachios of

various varieties. Their approach involved employing Convolutional Neural Network (CNN)-based models, such as ResNet50, ResNet152, and VGG16, to extract relevant features from pistachio images and execute classification tasks. The resulting average classification accuracies attained by these models were 85.28%, 85.19%, and 83.32%, respectively (Rahimzadeh & Attar, 2022). The audio signal processing technique is one of the most popular and non-destructive methods used to separate closed pistachios from open pistachios. However, this method also has disadvantages. Represented the negative effect of ambient noise, and the moisture content of nuts affects the impact of sound (Farhadi, Abbaspour-Gilandeh, Mahmoudi, & Mari Maja, 2020). J48 Decision Tree, Naïve Bayes, and Multi-Layer Perceptron (MLP) were used in a study conducted by (Ataş & Doğan, 2015) to classify open and closed pistachio nuts. In this study, the J48 decision tree was utilized as a main classifier. The classification performance of J48 was also compared to other classifiers, including Naïve Bayes and Multi-Layer Perceptron (MLP). The results of the study showed that the proposed system using the J48 decision tree achieved a simple and interpretable classifier along with a satisfactory classification accuracy performance of 94.5%. Traditional machine learning algorithms were utilized to assess the effectiveness of color features in categorizing cashew kernels as either white or scorched. The outcomes indicated that the color features were effective in distinguishing between the two categories. Furthermore, all traditional machine learning methods tested yielded promising results in this classification task (BAİTU, GADALLA, & ÖZTEKİN). Hosseinpour-Zarnaq et al. (2022), developed a one-dimensional convolutional neural network (CNN) model for sorting (open-closed) pistachio nuts using acoustic emission signals. A total of 1600 pistachios from two pistachio varieties (Akbari and Ahmad Aghaei) were used. The overall accuracy of the CNN classifier was 98.75% (Hosseinpour-Zarnaq et al., 2022). In another study conducted by Singh et al. (2022), a pre-trained dataset consisting of a total of 2148 images was employed. Among these images, 1232 were of the Kirmizi type, and 916 were of the Siirt type. The study employed three distinct convolutional neural network models to classify these images. The models were trained using the transfer learning method, utilizing AlexNet as well as pre-trained VGG16 and VGG19 models. The classification results from this study indicated the following success rates for the respective models: AlexNet achieved a success rate of 94.42%, VGG16 achieved a notably high 98.84% success rate, and VGG19 achieved a slightly lower success rate of 98.14% (Singh et al., 2022). Lisda et al (2023) conducted a study on classifying two pistachio nuts varieties using the Inception-V3 and ResNet50 models. The study utilized a dataset comprising 2148 photos, with 916 images of the Siirt type and 1232 images of the Kirmizi type. The classification results revealed that the Inception-V3 model achieved an impressive accuracy of 96%, while the ResNet50 model achieved an accuracy of 86% (Lisda, Kusrini, & Ariatmanto, 2023).

This study aims to evaluate the performance of various machine learning algorithms, including Logistic Regression, Support Vector Machines, and Random Forest for classifying open and closed pistachio nuts. The algorithms were written using the Python programming language and run in Google Colab environment.

2. Materials and Methods

2.1. Image acquisition

This study was carried out at the Faculty of Agriculture's laboratory in the Department of Agricultural Machinery and Technologies Engineering at Ondokuz Mayis University in Samsun. One thousand samples of open and closed pistachio nuts were obtained from the Ministry of Agriculture and Forest Pistachio Research Institute in Gaziantep. The nuts were examined and stored at room temperature. The imaging system was equipped with a high-resolution Guppy camera model Pro F-032, which had a CCD progressive Sony ICX424 sensor, 4.9 mm x 3.7 mm, consisting of 656 horizontal by 492 vertical cells. The images were captured at a resolution of 80x80 pixels. In this experiment, a black surface was used as a background. Because good lighting is crucial for getting high-quality images, two 8-watt fluorescent lamps were used as the light source in the image acquisition chamber. The images of both open and closed pistachio nuts were taken under the same conditions (camera, position, and background). The image capture system used in this study (*Figure 1*) consisted of a camera connected to a computer equipped with an image capture card.

Figure 1. Image capturing system

2.2. Image pre-processing and segmentation

After the images were captured, the segmentation process was done to extract the object of interest from the background. In this study, the Canny edge detection approach was applied to segment the images. The canny edge detection algorithm was developed by John F. Canny (1986) and is widely used in image processing due to its good balance of speed and accuracy. It is a multi-stage algorithm that involves detecting edges through the use of image gradients, suppressing non-maximums, hysteresis thresholding, and edge tracking. Various image segmentation methods were tested to find out the one that effectively separated the foreground from the background. After testing different image segmentation methods, Canny edge detection was found to give the best performance in segmenting the foreground from the background. An example of raw and segmented images is shown in (*Figure 2*).

Figure 2. Raw image segmentation

2.3. Feature extraction and selection

Extracting appropriate features from an image is crucial for an accurate classification process. In order to enhance the accuracy of pistachio classification, this study focused on extracting color features from both RGB and CIELAB color spaces. Therefore, Pistachio images in RGB and CIELAB (L*a*b*) color spaces were split into R (red), G (green), B (blue), L (l), A (a), and B (b). Moments features representing mean, variance, and range were captured from the split color. The motivation behind using color features from both RGB and CIELAB color spaces lies in their ability to capture different aspects of color information, providing a more comprehensive representation of the data. In this study, 18 color features were extracted, and after evaluating the effectiveness of all features in predicting the category of the pistachio nuts, only 14 features were found to have a significant effect, and the other four irrelevant features were left out. Utilizing irrelevant features could detrimentally impact algorithm performance. Therefore, to enhance the efficiency of a classification model and decrease the running time, it is highly recommended to use only the important features and get rid of irrelevant features. This procedure is commonly referred to as feature selection. In this particular study, feature selection was executed in Python through the Boruta library, which employs a wrapper approach to identify relevant features by constructing an XGBoost classifier. The list of accepted features and those rejected are shown in (*Table 1*).

Table 1. List of accepted and rejected features.

No.	Feature	Status	No.	Feature	Status
1.	Red Mean	Accepted	10.	L _ Mean	Accepted
2.	Green Mean	Accepted	11.	A_ Mean	Accepted
3.	Blue Mean	Accepted	12.	B _ Mean	Accepted
4.	Red Variance	Accepted	13.	L_ Variance	Accepted
5.	Green Variance	Accepted	14.	A_ Variance	Rejected
6.	Blue Variance	Accepted	15.	B_ Variance	Rejected
7.	Red Range	Accepted	16.	L _ Range	Accepted
8.	Green Range	Accepted	17.	A _ Range	Rejected
9.	Blue Range	Accepted	18.	B _ Range	Rejected

2.4. Performance evaluation

The performance of the classification can be assessed by counting the number of cases that were accurately identified as being part of the class (true positives), the number of cases correctly recognized as not being part of the class (true negatives), the number of cases mistakenly categorized as belonging to the class (false positives), and the number of cases that were not properly identified as part of the class (false negatives) (Cinar & Koklu, 2022).

Calculation formulas for success criteria, such as accuracy, error rate, recall, specificity, and precision, were calculated using the confusion matrix for binary classification performance measurements, and their equations are given in *(Table 2)* (Hossin & Sulaiman, 2015).

Table 2. Performance measurements and calculation equations for binary classification.

No.	Performance Metrics	Explanation	Equation
1.	Accuracy	It measures the ratio of true prediction on all samples included in the assessment.	$\frac{tp+tn}{tp+fp+tn+fn} \times 100$ (Eq.1).
2.	Error Rate	It measures the ratio of false prediction on all samples included in the assessment.	$\frac{fp+fn}{tp+fp+tn+fn} \times 100$ (Eq.2).
3.	Recall	It is used to measure the ratio of correctly classified positive values	$\frac{tp}{tp+fn} \times 100$ (Eq.3).
4.	Specificity	It measures the ratio of correctly classified negative values.	$\frac{tn}{tn+fp} \times 100$ (Eq.4).
5.	Precision	It measures the ratio of accurately classified positive samples to estimated total positive samples.	$\frac{tp}{tp+fp} \times 100$ (Eq.5).

3.   Results and Discussion

In this study, a total of 1,000 Antep Pistachio nuts images, including 500 open and 500 closed nuts, were used as a dataset. 75% of this dataset was used for training the model, and the rest 25% was used for testing the model. In this experiment, three traditional ML algorithms, Logistic Regression, Support Vector Machine (SVM), and Random Forest models, were tested. The correct compilation and training of the algorithms were done. The algorithm's performance was tested using a set of test data to evaluate the classification accuracy. Classifiers'

performances were compared to each other using the confusion matrix. It is a table of the predicted classes and the actual classes that were observed. The confusion matrix allows seeing where the model is making correct predictions, where it is making incorrect predictions, and how accurate the model is overall. It also allows for identifying which classes are being confused with each other. This information can be used to improve the model by adjusting parameters or changing features. Confusion matrices are very important because they provide an easy way to visualize and understand how well a classification model is performing. The used classifiers and the confusion matrix for each model are presented below.

3.1. Logistic Regression classifier

Logistic regression is a powerful classification technique that can be used to classify opened and closed pistachio nuts. It is a supervised learning algorithm that uses a linear model to predict the probability of an outcome. In this study, the outcome was whether a pistachio nut is open or closed. The logistic regression classifier was able to achieve an accuracy of 93.6% in the test dataset. This indicates that the model accurately classified open and closed pistachio nuts with high accuracy. The achieved accuracy of 93.6% in the test dataset is quite impressive. It indicates that the model was able to identify the different classes of nuts accurately. The confusion matrix for the logistic regression classifier is shown in *(Figure3)*.

Figure 3. Logistic Regression confusion matrix

3.2. Support Vector Machine classifier

In this experiment, an SVM classifier was used to classify opened and closed pistachio nuts. The dataset was split into training and test datasets, with the training dataset used to train the model and the test dataset used to evaluate its performance. The model achieved an accuracy of 94.8% on the test dataset, indicating that it was able to accurately classify opened and closed pistachio nuts with high accuracy. The confusion matrix of the Support Vector Machine classifier is shown in *(Figure 4)*.

Figure 4. Support Vector Machine confusion matrix

3.3. Random Forest

Using a random forest classifier to classify open and closed pistachio nuts is an effective approach for this task. In this study, a random forest made of 100 trees was used. The dataset was split into training and test datasets. The training dataset was used to train the model, and the test dataset was used to test its performance. The achieved accuracy of 95.6% in the test dataset is an excellent result and indicates that the model was performing well. The confusion matrix of the random forest is shown in *(Figure5)*.

Figure 5. Random Forest confusion matrix

3.4. Performance measurements

Performance measurement in a confusion matrix is important because it provides a comprehensive overview of the performance of a model. It allows us to evaluate the accuracy of the model, as well as identify areas where it may be underperforming. It also helps us to identify potential sources of error, such as misclassification or false positives and negatives. By understanding these metrics, we can adjust our models to improve their performance and ensure that they are providing accurate results. Performance measurement values of all algorithms used in classification are given in *(Table3)*.

Table 3. Performance measurements values of all algorithms used in classification

Performance Metrics	Logistic Regression	Support Vector Machine	Random Forest
Accuracy (%)	93.6	94.8	95.6
Error (%)	6.4	5.2	4.4
Specificity (%)	92.2	95.2	95.2
Precision (%)	92	94.4	96
Recall (%)	95	94.4	95.2

From the performance measurement values given in *(Table 3),* it can be seen that the classification accuracy for all algorithms is above 90%. It seems that the best classification accuracy belongs to the random forest algorithm, with 95.6%. The lowest classification accuracy belongs to the logistic regression algorithm, with 93.6%. Classifiers' performances were compared to each other. Almost similar performances were detected.

many research efforts have been carried out to enhance the accuracy of classifying open and closed pistachio nuts and different types of pistachio varieties through the application of machine learning and deep learning algorithms. Presented explicitly in *(Table 4)* are studies focused on the classification of two aspects: open-closed pistachio nuts and the differentiation of distinct pistachio varieties. *(Table 4)* also gives the number of images in the data set, the varieties that were classified, the models used, and their accuracy rates.

Table 4. Comparison of accuracy rates of classifying open-closed pistachio nuts and different types of pistachio varieties using traditional machine learning and deep learning models

Author/ authors	Model architecture	Images numbers	Varieties	Accuracy
Jalali and Mahmoudi, (2013)	J48 Decision tree, Fuzzy logic	360	Akbari and Kaleghouchi	96.67%
Hosseinpour-Zarnaq et al., (2022)	CNN model	1600	Akbari and Ahmad Aghaei (open or closed)	98.75%
Singh, et al. (2022)	AlexNet, VGG16 and VGG19	2.148	Kırmızıı and Siirt	94.42%, 98.84%, and 98.14%.
Lisda et al (2023)	Inception-V3 and ResNet50	2.148	Kırmızı and Siirt	96% and 86%
Rahimzadeh and Attar (2022)	ResNet50, ResNet152, and VGG16	3.927	different pistachio types (open-mouth or closed-mouth)	85.28%, 85.19%, and 83.32%
Ozkan et al. (2021)	Improved k-NN	2.148	Kırmızı and Siirt (open or closed)	94.18 %
Ataş and Doğan (2015)	J48 Decision Tree, Naïve Bayes, and Multi-Layer Perceptron (MLP)	200	different pistachio types (open – closed)	95.5%, 94.5% and 94.5%
Our proposed machine learning models	Logistic Regression, Support Vector Machine, and Random Forest	1.000	Antep Pistachio	93.6%, 94.8%, and 95.6%

From the previous studies, it has been observed that the highest classification accuracy for distinguishing different varieties of pistachios using deep learning algorithms was attained by Singh et al. (2022), who accomplished an impressive accuracy rate of 98.84%. On the other hand, the highest accuracy of classification of open-closed pistachio nuts using deep learning model was achieved by Hosseinpour-Zarnaq et al. (2022), which achieved an impressive accuracy of 98.75 using CNN model. Also, we have noticed that the highest accuracy of classification of open-closed pistachio nuts using a traditional machine learning model was achieved in a study conducted by Jalali and Mahmoudi (2013), which they were able to achieve a classification accuracy of 96.67%% using J48 Decision Tree model, which is close to the result that we have achieved with the Random Forest model (95.6%). These results indicate that the image segmentation method employed in our research and the extraction of color features from the images effectively captured the distinctive patterns, resulting in improving the performance of the classification.

4.   Conclusion

In this study, the classification of closed and open-shell pistachio nuts was conducted using machine-learning algorithms based on color features. A total of 1,000 Antep pistachio nut images, including 500 open and 500 closed nuts, were obtained and examined. The images were pre-processed and prepared for feature extraction. From the images, a total of 14 color features were extracted. Three models were applied and studied; logistic regression, SVM, and random forest. The single-color feature was used. For classification, models were created, and the classification process was performed. Accuracy rates of 95.6%, 94.8%, and 93.6% from the random forest, SVM, and logistic regression, were achieved, respectively. Also, the classifiers` performances were compared to each other almost similar performances were detected. The result of this study showed that the random forest model has the highest accuracy among the studied models. Considering false negative issues, the random forest model was found more applicable than other models and suggested to be used due to its higher accuracy.

This study proved that only color features can be used for the classification of closed and open-shell pistachio nuts with high accuracy. This research can be useful for the pistachio industry to improve the quality of products, reduce labor costs, and speed up the classification process. Future studies should focus on the use of additional features such as texture, shape, size, and morphological p to improve the accuracy of classification.

Acknowledgment

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Khaled Adil Dawood Idress; Writing, Designing, Data Collection, and Processing; Omsalma Alsadig Adam Gadalla; Literature Search:, Review and Editing: Yeşim Benal Öztekin.

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ARAŞTIRMA MAKALESİ

RESEARCH ARTICLE

Relationship Between Heterosis and Genetic Distance Determined by SSR Markers in Oriental Tobacco

Oryantal Tütünde SSR Markörleriyle Belirlenen Heterosis ile Genetik Mesafe Arasındaki İlişki

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Abstract

Hybrid cultivars could be beneficial to achieve high leaf yields while maintaining good quality properties in oriental tobacco. Identification of parents constitutes a major part of a hybrid breeding program. The aim of the present study was to determine the relationship between the genetic distance of parents determined by simple sequence repeats (SSR) markers and heterosis levels in hybrids produced from those parents for leaf yield and quality properties in oriental tobacco. Twenty-one hybrids produced by half diallel crossing among seven tobacco genotypes used in oriental tobacco production in Turkey were grown along with their parents in three locations in 2012 and 2013. Twenty-nine SSR markers were used to determine genetic distances among seven tobacco genotypes. A total of 80 alleles were produced by all twenty-nine markers. Average number of observed alleles per polymorphic marker was 2.96. Twenty-seven of 80 alleles were observed in only one of the seven parents. The polymorphic information content of markers varied from 0.215 to 0.810 (average 0.480). Mid-parent heterosis levels ranged from 18.03 to 42.00% for leaf number, between -19.75 and 38.06% for leaf width, between -17.51 and 36.25% for leaf length, between -34.38 and 76.12% for leaf yield, between -78.30 and 154.01% for sugar content and between -45.40 and 143.29% for nicotine content. Heterosis levels were correlated with genetic distances between parents for leaf number in Erbaa 2012 and Tokat 2012 locations, for leaf width in Erbaa 2013 location, for leaf length in Erbaa 2013 location, and for leaf yield in Tokat 2012 location only. SSR markers were very effective to determine genetic distance of oriental tobacco, and only two markers could distinguish all seven genotypes used in the study. The findings indicated that genetic distance determined by SSR markers used in the present study is not sufficient to predict hybrid performance in oriental tobacco.

Keywords: *Nicotiana tabacum* L., Genetic diversity, Hybrid, Microsatellite markers, Yield

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Öz

Hibrit çeşitler, oryantal tütünde iyi kalite özelliklerini korurken yüksek yaprak verimi elde etmek için faydalı olabilir. Hibrit çeşitleri oluşturacak ebeveynlerin belirlenmesi hibrit çeşit ıslah programlarında önemli bir yer tutmaktadır. Bu çalışmanın amacı, basit dizi tekrarları (simple sequence repeats, SSR) markörleri ile belirlenen ebeveynlerin genetik uzaklığı ile oryantal tütünde yaprak verimi ve kalite özellikleri için bu ebeveynlerden üretilen hibritlerdeki heterozis seviyeleri arasındaki ilişkiyi belirlemektir. Türkiye'de yoğun kullanılan yedi tütün genotipinden ve bu ebeveynlerden yarım diallel melezlemeyle ile elde edilen 21 hibrit ebeveynleriyle ile üç bölgede 2012 ve 2013 yıllarında yetiştirilmiştir. Yedi tütün genotipi arasındaki genetik uzaklıklarını belirlemek için yirmi dokuz SSR markörü kullanılmıştır. Yirmi dokuz markör toplamda 80 allel üretmiştir. Polimorfik markör başına ortalama gözlenen allel sayısı 2.96 olarak belirlenmiştir. Seksen allelin 27'si sadece bir ebeveynde gözlemlenmiştir. Markörlerin polimorfik bilgi içeriği 0.215 - 0.810 (ortalama 0.480) arasında değişmiştir. Ebeveyn ortalamasına göre hesaplanan heterozis değerleri yaprak sayısı için %18.03 - %42.00, yaprak eni için %-19.75 - %38.06, yaprak boyu için %-17.51 - %36.25, yaprak verimi için %-34.38 - %76.12, şeker oranı için %78.30 - 154.01 için ve nikotin içeriği için %-45.40 - %143.29 arasında değişmiştir. Genetik uzaklık ile heterozis arasındaki korelasyon, yaprak sayısında Erbaa 2012 ve Tokat 2012, yaprak eninde Erbaa 2013, yaprak boyunda Erbaa 2013 ve yaprak veriminde Tokat 2012 lokasyonlarında önemli bulunurken, diğer lokasyonlarda önemli bulunmamıştır. Çalışmada kullanılan yedi genotipi yalnızca iki markörün ayırt edebilmesinden dolayı, SSR markörlerinin oryantal tütünün genetik uzaklıkları belirlemek için çok etkili olduğu söylenebilir. Bu araştırmada kullanılan SSR markörleriyle belirlenen genetik uzaklık değeri doğrudan hibrit performansı tahmin etmek için yeterli olmamıştır.

Anahtar Kelimeler: *Nicotiana tabacum* L., Genetik çeşitlilik, Hibrit, Mikrosatellit markör, Verim

1. Introduction

Heterosis is a phenomenon producing higher performance in all crops irrespective of their pollination biology. It is commonly used in the improvement of cross-pollinating crops. Recently, it also has been a focus of attention in the breeding of self-fertilizing crops (Adhikari et al., 2020; Farooq et al., 2024). In classical hybrid breeding programs, the determination of parent combinations with the best performance is a tedious and costly procedure that can take years. It was assumed that the best way of exploiting heterosis was through the use of genetically diverse parents (Tomkowiak et al., 2020) and that molecular markers could be a useful tool to estimate heterosis levels (Marcón et al., 2019)

Tobacco is one of the most important non-food crops. The quality of cigarettes is mainly determined by the quality of tobaccos used in their blends (Xiao et al., 2007). Oriental tobaccos are used as regulators in cigarette blends throughout the world for their balanced nitrogen/carbohydrate contents and high aroma levels (Kinay and Kurt, 2022). Unlike their qualities, yields of oriental tobaccos are low. The use of hybrid cultivars could provide a way to increase leaf yields without lowering their quality (Kinay et al., 2020).

SSR markers have been used to reveal genetic diversity in different tobacco types. Through using SSR markers, He et al. (2020), determined genetic diversity levels in Virginia tobaccos, Davalieva et al. (2010), in burley tobaccos, and Kurt et al. (2022; 2023), in oriental tobaccos. He et al. (2020), found that 91 SSR markers produced 304 alleles on 33 flue-cured tobacco genotypes. Yongliang et al. (2020), studied 33 tobacco cultivars with 22 SSR markers and found a total of 81 alleles. In another study by Darvishzadeh et al. (2014), on the other hand, a polymorphism ratio of 100% was found in 13 SSR markers studied on 70 oriental tobacco genotypes. Examining 29 markers in 319 tobacco genotypes collected from different regions of Turkey (Saygili et al., 2022), observed polymorphism for 86% of the markers and an average of four alleles per marker. Therefore, SSR markers could efficiently reveal high genetic diversity levels found in cultivated tobacco.

It has been proposed that genetic distance determined by DNA markers could be used to estimate heterosis. Heterosis was estimated by genetic distance based on DNA markers in cotton (Geng et al., 2021), corn (Palaniyappan et al., 2023), cabbage (Dong et al., 2021) and sunflower (Buti et al., 2013), However, there are also reports that heterosis was not associated with genetic diversity of parents in rapeseed (Tian et al., 2017), wheat (Liu et al., 1999), soybean (Cerna et al., 1997), corn (Dermail et al., 2020) and rice (Kwon et al., 2002). Two studies conducted on wheat, on the other hand, found that the genetic distance was correlated with heterosis, but was not enough to determine the best parental combinations (Corbellini et al., 2002; Dreisigacker et al., 2005). The level of correlation between genetic distance calculated by molecular markers and heterosis could vary depending upon marker type (Solomon et al., 2012) and genetic material (Kwon et al., 2002).

Hybrid breeding has been the topic of different studies. Along with new developments that allow a better understanding of the heterosis phenomenon, there have been recent efforts to facilitate hybrid breeding in self-pollinating crops. Determining the best parental combinations constitutes a major part of hybrid breeding programs. Conventionally, parents of hybrids are determined based on top-crosses and diallel crosses which take many years to produce and evaluate in field trials. A possible relationship between genetic distance and heterosis could make it easier to identify the best parental combinations for superior hybrid cultivars. The present study was carried out to examine the possible relationship between genetic distance determined by SSR markers and heterosis levels for yield and quality traits in hybrids produced between seven oriental tobacco genotypes commonly grown in Tokat-Central, Tokat-Erbaa, and Samsun-Bafra regions of Turkey.

2. Materials and Methods

2.1. Plant material

Seven tobacco cultivars/lines in traditional oriental tobacco areas in Northern Turkey and 21 F_1 hybrids produced by half diallel crosses among them were used. Detailed plant characteristics of genotypes are available in (Peksüslü et al., 2012) and (Kinay et al., 2019). Parents and hybrid combinations used in the study were given in *Table 1*. Hybrid seeds were produced on greenhouse-grown plants based on methods described by Wernsman and Matzinger (Wernsman and Matzinger, 1980). Hybrid seeds were planted in viols and seedlings were produced in a greenhouse.

Table 1. Parents and hybrids used in the study

No	Parents/Hybrids	No	Parents/Hybrids
1	Xanthi-2A	15	Nail x Taşova
2	Nail	16	Nail x Katerini
3	Gümüshacıköy	17	Nail x Canik
4	Tasova	18	Nail x Erbaa
5	Katerini	19	Gümüshacıköy x Taşova
6	Canik	20	Gümüshacıköy x Katerini
7	Erbaa	21	Gümüshacıköy x Canik
8	Xanthi-2A x Nail	22	Gümüshacıköy x Erbaa
9	Xanthi-2A x Gümüshacıköy	23	Taşova x Katerini
10	Xanthi-2A x Taşova	24	Taşova x Canik
11	Xanthi-2A x Katerini	25	Taşova x Erbaa
12	Xanthi-2A x Canik	26	Katerini x Canik
13	Xanthi-2A x Erbaa	27	Katerini x Erbaa
14	Nail x Gümüshacıköy	28	Canik x Erbaa

2.2. Field trials

Field trials were conducted in Tokat, Erbaa, and Bafra locations in the 2012 and 2013 growing periods. Climatic data of Tokat, Erbaa, and Bafra locations in experimental years were given in *Table 2*.

Table 2. Climatic datas of field trial years and long term

Months	Years	Average temperature (°C)			Average relative humidity (%)			Total precipitation (mm)		
		Tokat	Erbaa	Bafra	Tokat	Erbaa	Bafra	Tokat	Erbaa	Bafra
May	1975-2011	18.5	17.8	15.4	53.8	60.7	78.6	73.5	54.2	46.3
	2012	17.6	18.1	17.4	62.6	68.5	89.0	114.7	63.4	28.6
	2013	19.4	19.7	18.5	53.1	66.0	84.1	32.3	25.9	28.8
June	1975-2011	21.1	21.8	20.1	49.8	58.1	74.2	36.2	42.1	44.9
	2012	21.4	22.0	22.4	56.5	67.9	79.7	36.3	25.4	69.8
	2013	20.8	21.2	21.5	52.4	63.4	81.1	36.1	21.1	43.6
July	1975-2011	22.7	23.5	22.8	49.1	55.3	72.5	16.2	17.5	29.8
	2012	23.6	24.3	24.2	54.6	64.2	81.5	30.7	19.6	116.8
	2013	21.9	23.0	23.3	51.0	62.0	81.4	1.6	10.1	48.0
August	1975-2011	23.1	23.3	22.7	47.4	56.0	73.9	1.0	8.6	44.4
	2012	23.4	23.8	23.2	51.8	58.3	82.8	1.5	5.4	209.8
	2013	22.8	23.5	23.8	49.3	63.9	87.8	0.4	30.7	66.8
September	1975-2011	20.1	20.1	19.2	54.1	58.3	76.3	8.7	14.2	58.5
	2012	20.3	20.6	20.5	50.9	62.1	85.8	5.1	15.3	78.2
	2013	19.8	18.7	18.9	47.8	69.6	82.8	12.3	16.5	37.2

*Turkish State Meteorological Service

Soil samples were taken from the upper 30 cm of experimental soils. Five samples were taken in each location. Soil samples were analyzed in Tokat Gaziosmanpasa University (TOGU) Faculty of Agriculture Soil Science laboratories. Soil analysis results were given in *Table 3*. Field trials were carried out in Erbaa county of Tokat province (latitude 40º60′ N, longitude 36º62′ E, elevation 580 m), Bafra county of Samsun province (latitude 41º03′ N, longitude 35º04′ E, elevation 162 m), and central of Tokat province (latitude 39º51′ N, longitude 35º27′ E, elevation 623 m).

Table 3. Properties of experimental locations and soils in Tokat, Erbaa and Bafra

	2012			2013		
	Tokat	**Erbaa**	**Bafra**	**Tokat**	**Erbaa**	**Bafra**
Altitude (m)	623	580	162	623	580	162
Latitude	39º51′	40º60′	41º03	39º51′	40º60′	41º03
Longitude	35º27	36º62	35º04	35º27	36º62	35º04
Texture	Clayed loam	Sandy clayed loam	Clayed	Clayed	Clayed	Clayed
pH	8.01 Slightly alkaline	7.87 Slightly alkaline	8.05 Slightly alkaline	7.10 Neutral	6.98 Neutral	8.34 Moderately alkaline
EC (dS/m)	0.29	0.23	0.57	0.80	1.10	0.48
$CaCO_3$ (%)	14.33 Rich	10.66 Rich	20.87 Excess	3.93 Moderate	3.71 Moderate	4.6 Moderate
Organic matter (%)	6.24 High	4.41 High	2.03 Moderate	3.86 Good	1.82 Insufficient	3.8 Good
P_2O_5 (kg/ha)	31.4 Insufficient	35.5 Insufficient	23.8 Very insufficient	73.5 Insufficient	93.8 Sufficient	175.0 Sufficient
K_2O (kg/ha)	589.4 Sufficient	429.8 Sufficient	502.4 Sufficient	309.9 Sufficient	491.4 Sufficient	471.9 Sufficient

Plant stands were established via seedling transplanting to fields. Planting dates of seedlings in field conditions for each trial were given in *Table 4*. Field trials were carried out in Randomized Complete Blocks Design with three replications. Each plot consisted of four rows of 4 m long. A 1 m space was left between plots and 1.5 m between blocks. Based on local tobacco growing practices, planting density was 45 x 12 cm in Tokat and Erbaa while 50 x 12 cm in Bafra. Experimental soils were fertilized with 60 kg/ha nitrogen, 40 kg/ha phosphorus (P_2O_5), and 60 kg/ha potassium (K_2O) (Kinay and Kurt, 2022). All fertilizers were given to soil-prepared plots just before planting. During the growing period, maintenance procedures such as hoeing and disease/pest management practices were carried out. Field trials were conducted under rainfed conditions (no irrigation). Leaves that reached harvest maturity were harvested on dates given in *Table 4*.

During the vegetation period, leaf width, length, and number measurements were made on ten plants per plot (Saygili et al., 2021). Leaves were put on strings manually in the Tokat and Erbaa locations, while a machine was used for this purpose in the Bafra location. Stringed leaves were wilted for one or two days and taken to drying areas. Dried leaves were weighed and leaf yields were determined on a 17% moisture basis. Samples were taken from the second main hands for reducing sugar and nicotine assays (Kinay and Kurt, 2022), which were carried out in the Analysis Laboratory of Field Crops Department of Tokat Gaziosmanpasa University Faculty of Agriculture.

Table 4. Planting and harvest dates of field trials

	Planting dates		Harvest dates					
Locations			2012			2013		
	2012	**2013**	**First**	**Second**	**Third**	**First**	**Second**	**Third**
Erbaa	12 May	06 May	25 June	14 July	04 Aug.	26 June	14 July	15 Aug.
Bafra	22 May	11 May	12 July	27 July	13 Aug.	27 June	16 July	13 Aug.
Tokat	16 May	08 May	28 June	17 July	02 Aug.	28 June	12 July	07 Aug.

2.3. DNA extraction and polymerase chain reaction

DNA marker analyses were conducted in the Agricultural Biotechnology Laboratories of TOGU, Faculty of Agriculture. Genomic DNA was isolated from plants with 3-5 leaves using Turkuaz DNA preparation kit (Keskin et al., 2014). The quality and quantity of isolated DNAs was measured using 1% agarose gels and

spectrophotometer (Thermo, Biomate 3). DNA concentration was adjusted to 50 ng/µl. PCR was carried out in 40 µl volumes. PCR reaction contained 1X PCR buffer, 250 nM of each primer, 0.2 mM of each nucleotide, 1.5 mM MgCl$_2$, 50–100 ng genomic DNA, and 0.5 units Taq-DNA polymerase (Biobasic). A typical PCR procedure was as follows: 5 minutes at 94 °C, 40 cycles of 30 seconds at 94 °C, 30 seconds at 50-60 °C (depending on the primer annealing temperature), 30 seconds at 72 °C, and 5 minutes at 72 °C. Amplicons of SSR markers were run on a 3% Metaphor Agarose gel (Lonza cat no: 50180) using 1xTBE buffer in 90 V for at least two hours or more depending on amplicon size and differences among alleles. Amplicons were visualized on a gel imaging system (Vilber Lourmat CN-08) through ethidium bromide (10%) added to the gel (Bahar et al., 2019).

2.4. SSR markers

A total of 29 SSR markers were studied. Primers with PT prefix used in the study were selected among primers reported by Moon et al. (2009), Bindler et al. (2007), or Bindler et al. (2011), while the ones with TM and TME prefixes were selected from Tong et al. (2012) based on high Polymorphism Information Content (PIC) values (≥0.7). PIC is a measure of the informativeness of the DNA markers for genetic characterization (Serrote et al., 2020). Higher PIC value means the better utilizing the discriminatory power of a markers.

2.5. Data analysis

Bands were analyzed using BioCapt v.11.02 software. Dendrogram was prepared using the UPGMA algorithm in POPGENE v.1.32 (Yeh et al., 2000). Genetic distance between the lines was calculated according to Nei (1972). SSR marker polymorphism rates were determined using PIC values, which were calculated according to the following formula: PIC = $1 - \sum P^2_i$ where P$_i$ is the frequency of ith allele (Anderson et al., 1993). Alleles with frequencies of less than 5% were defined as rare alleles (He et al., 2020). Since only seven genotypes were used in the present study, alleles found in only one genotype were considered as rare allele. Heterosis was calculated as mid-parent heterosis using the following formulae: MPH = [Vh - (Vp+Vm)/2] / (Vp+Vm)/2*100, where Vh: hybrid's value for a trait, Vp: paternal value, Vm: maternal value (Lamkey and Edwards, 1997).

3. Results and Discussion

Twenty-nine SSR markers were used to determine genetic distances between seven tobacco genotypes. Twenty-six of the markers were polymorphic and three were monomorphic (*Table 5*). Thus, the polymorphism rate was 90% (26/29). A polymorphic PT30274 marker profile was given in *Figure 1a*. Number of alleles produced by each marker was given in *Table 5*. One marker produced six alleles, while two markers produced five alleles, three markers four alleles, nine markers three alleles, eleven markers two alleles, and three markers one allele (*Table 5*). Thus, totally 80 alleles were produced by all twenty-nine markers. Average number of alleles per marker was 2.76 (mean for polymorphic markers were 2.96). Thirty percent of 80 alleles (27) were observed in only one of seven parents. PIC values of polymorphic markers varied from 0.215 to 0.810 (average 0.480).

A dendrogram was prepared based on SSR marker data. Three main groups appeared in dendrogram. Erbaa genotype was clearly different from others and constituted a group alone, while Katerini and Tasova genotypes were close to each other and formed the second group. The other four genotypes grouped together, making the third group (*Figure 1b*). Genetic distances of genotypes relative to each other were determined based on Nei's coefficient (Nei, 1972). The highest genetic distance (1.639) was observed between Katerini and Erbaa genotypes, followed by Erbaa and Nail (1.476) and Erbaa and Gümüshaciköy (1.252). The lowest value was obtained between Tasova and Katerini (0.637). Erbaa genotype had the highest genetic distances from other genotypes, which meant that it carries alleles different from other genotypes grown in the region.

Twenty-six of the 29 SSR markers studied were informative. Even just two SSR markers (PT20172 and PT30265 or PT30274) yielded enough information to distinguish all parents studied. The polymorphism rate of 90% observed in the present study is higher than the 80% reported by Davalieva et al. (2010) for 10 tobacco cultivars including Virginia, burley, and oriental tobacco types using 30 SSR markers. In previous studies polymorphism rate values from 80 to 100% (Gholizadeh et al., 2012; Kurt et al., 2023; Saygili et al., 2021). On tobacco, reported average allele numbers of SSR markers ranged from 2.69 to 3.68 (Gholizadeh et al., 2012; He et al., 2020). The average allele number of polymorphic markers in this study (2.9) was similar to or slightly lower than what was reported in previous studies. Considering the genotype numbers in other studies, the allele numbers determined in only seven genotypes in the present study showed that genetic diversity is high among the genotypes.

Preferred SSR markers proved to be useful in determining the existing diversity. PIC value, which expresses the discriminatory power of SSR markers (Serrote et al., 2020), is an important criterion for selecting markers to be used for genetic diversity analyses. The markers used in the present study were selected among the markers with high PIC values. This is why the PIC values were high despite the small number of genotypes in the present study. In the studies dealing with genetic variation in tobacco, PIC values ranged from 0.39 to 0.781 (Davalieva et al., 2010; Saygili et al., 2021; Tong et al., 2012). Thus, a high level of genetic variation was observed in the present study. In addition, the power of SSR markers as a tool to distinguish tobacco genotypes was also confirmed.

Table 5. Information about SSR markers used

SSR	Annealing Temperature (°C)	Repeat motifs	Observed allele number	Rare allele number	PIC	Polymorphism
PT20172	55	CTT	6	5	0.810	Polymorphic
PT20242	55	AGG	3	1	0.530	Polymorphic
PT30034	55	TAA	4	2	0.641	Polymorphic
PT30099	55	CGA	4	3	0.502	Polymorphic
PT30114	55	TA	3	1	0.530	Polymorphic
PT30132	55	TA	2	1	0.215	Polymorphic
PT30137	55	TAA	2	0	0.370	Polymorphic
PT30159	55	TA	2	1	0.215	Polymorphic
PT30265	55	TA	5	3	0.740	Polymorphic
PT30274	55	GAA	5	3	0.740	Polymorphic
PT30350	55	TAA	3	0	0.580	Polymorphic
PT30364	58	TAA	3	0	0.580	Polymorphic
PT30375	55	TAA	4	1	0.685	Polymorphic
PT30392	55	TAA	3	1	0.502	Polymorphic
PT30449	55	TA	3	0	0.580	Polymorphic
PT40005	55	GAA	2	1	0.215	Polymorphic
PT40015	55	GA	2	0	0.325	Polymorphic
PT50182	55	TA	2	1	0.215	Polymorphic
PT53303	55	GA	1	0	0.000	Monomorphic
PT61056	55	TA	3	1	0.502	Polymorphic
TM10013	60	ATA	2	0	0.370	Polymorphic
TM10181	60	AGA	3	0	0.534	Polymorphic
TM10211	60	ACA	2	0	0.370	Polymorphic
TM10654	60	TA	2	1	0.530	Polymorphic
TM10821	60	TA	2	0	0.370	Polymorphic
TM10976	57	AAT	3	1	0.502	Polymorphic
TM11110	60	AAC	2	0	0.370	Polymorphic
TM11359	60	ACA	1	0	0.000	Monomorphic
TME0293	60	TCA	1	0	0.000	Monomorphic

The number of leaves, leaf width and length, dried leaf yield, sugar, and nicotine contents of dried leaves were determined in 21 hybrids developed in a half-diallel crossing program among seven parents. Percent heterosis values calculated based on mid-parent heterosis were given in *Table 6-11*. Mid-parent heterosis levels calculated in each location varied based on different agronomic traits studied. Heterosis levels of crosses varied from -18.03 to 42.00% (average -1.50%) for leaf number (*Table 6*), from -19.75 to 38.06% (average 8.43%) for leaf width (*Table 7*), from -17.51 to 36.25% (average 5.65%) for leaf length (*Table 8*), from -34.38 to 76.12% (average 20.46%) for dried leaf yield (*Table 9*), from -78.30 to 154.01% (average -6.02%) for sugar content (*Table 10*) and from -45.40 to 143.29% (average 3.82%) for nicotine content (*Table 11*). These results meant that heterosis values varied considerably by environment.

(a)   (b)

Figure 1. (a) The SSR marker profile of genotypes using PT30274 marker. The first lane shows molecular weight marker (50 bp). Length of bands in Taşova is 212 bp, Gümüşhacıköy and Nail 215 bp, Erbaa 239 bp, Canik and Katerini 218 bp, Xanthi-2A 209 bp.; (b) Dendrogram showing genetic distances among parents based on 29 SSR markers.

Table 6. Mid-parent heterosis levels of different hybrids (%) in six growing environments for leaf number

Hybrid	Genetic distance	Leaf number						
		E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	0.974	-1.91	-11.7	-0.45	-11.24	-7.27	-5.76	-6.39
Xanthi-2A X Gümüşhacıköy	0.792	-2.34	10.89	-2.19	8.69	0.31	1.53	2.82
Xanthi-2A X Taşova	1.061	2.82	-1.11	-6.23	-1.06	0.95	-3.36	-1.33
Xanthi-2A X Katerini	0.956	-0.79	-5.35	0.86	-4.04	-3.98	-9.09	-3.73
Xanthi-2A X Canik	0.773	-2.5	3.59	6.84	6.11	-4.81	-2.5	1.12
Xanthi-2A X Erbaa	0.997	10.68	14.41	26.55	-3.48	13.79	2.33	10.7
Nail X Gümüşhacıköy	0.657	-11.9	-5.62	8.83	-7.64	-14.84	-2.14	-5.55
Nail X Taşova	0.879	-5.99	-7.31	5.81	-14.33	-12.25	-5.48	-6.59
Nail X Katerini	1.08	0.3	-6.13	1.79	-17.73	-7.94	-11.4	-6.85
Nail X Canik	0.639	-10.14	-6.65	2.53	-10.32	-8.97	-1.51	-5.84
Nail X Erbaa	1.476	1.84	-7.12	10.7	-10.43	10.2	-2.43	0.46
Gümüşhacıköy X Taşova	1.331	0.59	-6.9	-1.76	5.14	-3.71	1.96	-0.78
Gümüşhacıköy X Katerini	1.08	-13.63	1.63	-10.84	-7.66	-9.11	-7.24	-7.81
Gümüşhacıköy X Canik	1.08	-7.58	-0.17	-8.25	13.89	-10.65	4.8	-1.33
Gümüşhacıköy X Erbaa	1.252	6.7	9.18	10.65	1.99	10.44	3.05	7
Taşova X Katerini	0.619	-10.02	-3.61	-13.54	-12.72	-5.59	-13.84	-9.89
Taşova X Canik	0.956	-7.89	0.39	-6.64	6.87	-4.04	-8.63	-3.32
Taşova X Erbaa	1.169	5.25	7.14	16.45	3.39	19	5.1	9.39
Katerini X Canik	1.179	-8.4	-0.29	-10.34	-14.04	-11.69	-9.84	-9.1
Katerini X Erbaa	1.639	-1.51	0.96	-7.51	-18.03	4.74	-5.28	-4.44
Canik X Erbaa	1.051	14.26	3.68	25.36	4.68	16.3	-4.96	9.89
Average	1.03	-2.01	-0.48	2.32	-3.9	-1.39	-3.56	-1.5

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Table 7. Mid-parent heterosis levels of different hybrids (%) in six growing environments for leaf width

Hybrid	Leaf width						
	E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	-1.32	-1.62	28.94	19.02	6.48	15.22	11.12
Xanthi-2A X Gümüşhacıköy	9.42	-2.21	15.9	17.84	6.22	15.18	10.39
Xanthi-2A X Taşova	-2.51	-7.27	-18	-3.57	2.19	-10.36	-6.58
Xanthi-2A X Katerini	20.73	0.18	25.56	14.57	7.34	9.93	13.05
Xanthi-2A X Canik	10.44	-7.35	32.35	3.62	6.15	6.77	8.67
Xanthi-2A X Erbaa	8.02	2.39	8.61	5.04	0.4	5.01	4.91
Nail X Gümüşhacıköy	2.97	-2.22	22.4	11.77	11.83	-0.69	7.68
Nail X Taşova	-10.45	-1.36	2.56	7.24	3.94	7.95	1.65
Nail X Katerini	12.26	0	38.06	3.01	12.69	19.53	14.26
Nail X Canik	2.38	-6.37	22.79	1.4	10.79	19.04	8.34
Nail X Erbaa	1.96	11.66	19.93	17.67	7.67	18.1	12.83
Gümüşhacıköy X Taşova	-3.3	4.54	14.07	14.36	3.51	12.13	7.55
Gümüşhacıköy X Katerini	17.71	1.93	19.35	6.45	8.04	8.43	10.32
Gümüşhacıköy X Canik	17	1.58	9.41	2.43	5.41	3.71	6.59
Gümüşhacıköy X Erbaa	14.71	10.88	28.81	23.78	5.53	16.87	16.76
Taşova X Katerini	-0.28	-0.03	7.67	7.7	12.03	13.98	6.85
Taşova X Canik	-2.37	-9.42	-14.1	8.07	7.83	4.67	-0.89
Taşova X Erbaa	1.68	5.05	0.34	8.51	4.43	-5.35	2.44
Katerini X Canik	19.37	10.75	27.16	13.21	19.13	29.14	19.79
Katerini X Erbaa	20.85	6.13	24.14	0.36	7.53	15.38	12.4
Canik X Erbaa	22.62	-0.95	19.79	2.4	4.44	5.38	8.95
Average	7.71	0.77	15.99	8.81	7.31	10	8.43

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Table 8. Mid-parent heterosis levels of different hybrids (%) in six growing environments for leaf length

Hybrid	Leaf length						
	E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	-1.16	-6.14	23.48	8.84	4.48	6.8	6.05
Xanthi-2A X Gümüşhacıköy	11.55	-5.83	10.78	7.65	7.97	9.24	6.89
Xanthi-2A X Taşova	-2.01	-8.7	-17.5	0.19	-2.46	-10.28	-6.8
Xanthi-2A X Katerini	20.08	-0.95	26.62	10.3	7.17	8.19	11.9
Xanthi-2A X Canik	6.41	-4.82	-2.7	0.54	3.34	6.57	1.56
Xanthi-2A X Erbaa	13.93	0.77	11.15	1.03	1.78	-1.05	4.6
Nail X Gümüşhacıköy	4.16	-5.94	20.63	8.42	10.44	-4.43	5.55
Nail X Taşova	-7.57	-5.91	2.5	4.21	3.07	6.93	0.54
Nail X Katerini	10.44	-2.99	36.25	1.12	12.21	14.63	11.94
Nail X Canik	-1.05	-6.09	18.22	-0.09	7.57	15.24	5.63
Nail X Erbaa	-2.66	0.63	14.55	7.22	6.15	10.05	5.99
Gümüşhacıköy X Taşova	-5.8	-0.41	12.47	9.71	2.21	5.56	3.96
Gümüşhacıköy X Katerini	15.96	-0.91	13.83	9.56	7.34	9.99	9.3
Gümüşhacıköy X Canik	6.71	-2.22	1.64	-0.66	3.19	1.35	1.67
Gümüşhacıköy X Erbaa	15.6	7.87	25	21.24	8	11.44	14.86
Taşova X Katerini	-2.26	-2.55	10.08	4.11	6.37	14.31	5.01
Taşova X Canik	-5.15	-7.86	-13	4.45	2.69	3.77	-2.51
Taşova X Erbaa	5.13	0.22	2.55	6.66	3.74	-9.27	1.5
Katerini X Canik	8.87	2.63	19.12	3.01	12.88	23.11	11.6
Katerini X Erbaa	24.18	2.4	20.63	4.76	9.07	16.55	12.93
Canik X Erbaa	16.71	-1.06	14.98	-1.14	4.16	5.49	6.53
Average	6.29	-2.28	11.97	5.29	5.78	6.87	5.65

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Leaf width and length are other traits with relatively high heterosis levels (8.43 and 5.65%, respectively). Nicotine content and leaf number had relatively low levels of heterosis, while the sugar content of dried leaves had negative heterosis (-6.02%). Since oriental tobaccos are mainly used to improve the quality of tobacco blends, quality is of primary importance in oriental tobaccos. Sugar content is an important quality trait in tobacco. Higher sugar content means higher quality. Despite relatively stable sugar and nicotine contents in oriental tobacco hybrids, dried leaf yields increased by about 20% in hybrids. These findings clearly showed the importance of heterosis to increase tobacco yields without deteriorating the quality.

Heterosis levels of hybrids for different traits varied considerably based on locations and years. Such a variation implies that hybrids could have high performance under specific conditions. Of the different traits studied, the highest heterosis level was obtained for dried leaf yield (20.46%). Similarly, other studies found that leaf yields had the highest heterosis levels of all traits studied (Butorac et al., 2004). Besides, the highest heterosis levels were obtained for yield per area in other crops such as wheat (Gimenez et al., 2021), rapeseed (Ali et al., 1995; Rao et al., 2023), corn (Dermail et al., 2020), and sunflower (Buti et al., 2013). These results indicate that heterosis could produce higher yields in all crops whether they are self or cross-pollinating.

Table 9. Mid-parent heterosis levels of different hybrids (%) in six growing environments for dried leaf yield

Hybrid	Dried leaf yield						
	E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	-2.69	10.47	36.85	-3.95	17.34	14.08	12.02
Xanthi-2A X Gümüşhacıköy	-0.93	20.49	76.12	23.75	33.76	41.33	32.42
Xanthi-2A X Taşova	-0.95	1.49	-10.3	15.63	3.32	-9.16	0
Xanthi-2A X Katerini	25.8	14.98	12.31	25.79	22.88	23.38	20.86
Xanthi-2A X Canik	5.73	9.96	-5.17	7.84	3.6	19.39	6.89
Xanthi-2A X Erbaa	-0.64	4.59	41.09	16.23	46.62	12.91	20.13
Nail X Gümüşhacıköy	16.68	6.19	50.57	3.4	28.76	21.61	21.2
Nail X Taşova	-9.56	19.14	10.49	9.73	11.63	20.64	10.35
Nail X Katerini	3.45	3.86	53.87	21.52	19.97	28.07	21.79
Nail X Canik	-3.55	20.99	14.65	26.45	52.28	42.49	25.55
Nail X Erbaa	1.87	10.95	42.21	9.93	59.23	34.85	26.5
Gümüşhacıköy X Taşova	7.06	20.6	43.63	32.15	37.71	41.63	30.47
Gümüşhacıköy X Katerini	21.04	14.04	43.56	8.57	48.18	23.02	26.4
Gümüşhacıköy X Canik	29.52	32.32	1.53	20.83	54.21	36.28	29.12
Gümüşhacıköy X Erbaa	17.58	27.84	26.88	18.58	69.22	38.37	33.08
Taşova X Katerini	2.33	2.79	-18.1	62.98	8.3	33.2	15.25
Taşova X Canik	-0.42	1.94	-34.4	17.42	31.98	21.88	6.4
Taşova X Erbaa	30.33	-0.72	22.15	-5.6	41.86	-14.79	12.21
Katerini X Canik	13.47	5.13	15.84	50.92	17.04	24.81	21.2
Katerini X Erbaa	8.9	14.61	32.79	19.78	60.37	24.33	26.8
Canik X Erbaa	31.33	15.72	53.79	14.86	63.07	7.21	30.99
Average	9.35	12.26	24.3	18.9	34.82	23.12	20.46

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Correlation coefficients between genetic distances and heterosis levels in different locations were given in *Table 12*. Significant positive correlations were found between genetic diversity levels of parents and heterosis of hybrids for leaf number in the Erbaa 2012 (P<0.05) and the Tokat 2012 (P<0.05) locations. Heterosis for leaf width was positively and highly significantly (P<0.01) correlated with the genetic diversity level of parents in the Erbaa 2013 location. Heterosis level for leaf length had a highly significant positive correlation (P<0.01) with genetic diversity only in the Erbaa 2013 location. Dried leaf yields were positively correlated with genetic diversity only in the Tokat 2012 location (P<0.05). Sugar and nicotine contents of dried leaves, on the other hand, had no significant correlations with genetic diversity in any of the locations.

Table 10. Mid-parent heterosis levels of different hybrids (%) in six growing environments for sugar content in dried leaves

Hybrid	Sugar content in dried leaves						
	E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	18.74	36.73	-51.2	-10.4	9.04	4.65	1.26
Xanthi-2A X Gümüşhacıköy	10.99	58.18	-33.2	31.34	59.16	-9.32	19.53
Xanthi-2A X Taşova	11.41	-24.28	-7.71	30.89	71.28	-7.05	12.42
Xanthi-2A X Katerini	23.26	30.45	-32.3	5.97	-0.45	-7.21	3.28
Xanthi-2A X Canik	11.25	-6.07	154	-14.23	18.41	-0.21	27.19
Xanthi-2A X Erbaa	14.09	10.23	31.36	-13.67	17.49	-18.66	6.81
Nail X Gümüşhacıköy	-16.2	5.07	-77	-2.13	-25.5	-57.47	-28.87
Nail X Taşova	-2.65	25.34	-37.5	-1	-26.01	25.94	-2.65
Nail X Katerini	-19.61	-31.08	-46.7	-24.97	-69.59	20.47	-28.58
Nail X Canik	-1.13	2.82	-6.74	-40.53	-70.61	3.94	-18.71
Nail X Erbaa	22.7	5.45	-9.47	-5.77	-78.3	2.96	-10.4
Gümüşhacıköy X Taşova	9.61	18.26	-37.6	12.08	36.64	-25.41	2.26
Gümüşhacıköy X Katerini	3.33	-7.56	-61.6	1.6	-27.26	-47.53	-23.17
Gümüşhacıköy X Canik	9.96	18.19	-17.9	-37.17	-44.04	27.89	-7.18
Gümüşhacıköy X Erbaa	3.83	-18.37	-36.8	-30.19	-26.65	-2.64	-18.47
Taşova X Katerini	-4.97	-1.95	-40.7	6.53	-28.28	-36.06	-17.56
Taşova X Canik	69.62	-18.02	11.17	-15.71	-16.78	-55.3	-4.17
Taşova X Erbaa	45.82	-1.88	-30.6	66.03	12.55	-24.02	11.32
Katerini X Canik	-0.61	-16.93	-52.2	-33.21	-16.34	-29.76	-24.84
Katerini X Erbaa	10.32	-5.4	-64.6	-32.73	-20.51	-16.6	-21.59
Canik X Erbaa	26.7	-7.97	-7.64	17.87	-18.28	-36.21	-4.25
Average	11.74	3.39	-21.7	-4.26	-11.62	-13.7	-6.02

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Molecular markers have been commonly searched as a possible tool to estimate hybrid performance in various crops (Dermail et al., 2020; Dong et al., 2021; Geng et al., 2021; Palaniyappan et al., 2023). In the present study, genetic distance was significantly associated with heterosis levels for leaf number in the Erbaa 2012 and Tokat 2012 locations, for leaf length in the Erbaa 2013 location, and for leaf yield in the Tokat 2012 location, while no significant correlations were found between genetic distance and heterosis levels for other traits in other locations. There are studies in literature concluding that heterosis levels could be estimated using DNA markers in cotton (Palaniyappan et al., 2023), corn (Palaniyappan et al., 2023) and cabbage (Dong et al., 2021). However, there are also reports that diversity levels determined by DNA markers were not associated with heterosis levels in rapeseed (Tian et al., 2017), wheat (Liu et al., 1999), soybean (Cerna et al., 1997), corn (Dermail et al., 2020) and rice (Kwon et al., 2002). In addition, two other studies found that genetic distance between wheat cultivars was correlated with heterosis, but was not enough to determine best parental combinations (Darvishzadeh et al., 2014; Ali et al., 1995). As suggested by Kwon et al. (2002), these conflicting results may indicate that genetic diversity and heterosis associations vary based on genetic material used. Some authors found that genetic diversity and heterosis associations could also be affected by type of DNA markers employed (Solomon et al., 2012). The present study adds to the complexity of marker - heterosis associations by showing that associations could change based on environment.

Table 11. Mid-parent heterosis levels of different hybrids (%) in six growing environments for nicotine content in dried leaves

Hybrid	Nicotine content in dried leaves						
	E12	E13	B12	B13	T12	T13	Ave.
Xanthi-2A X Nail	-18.98	20.62	5.9	0.32	11.91	-7.35	2.07
Xanthi-2A X Gümüşhacıköy	-11.86	-7.68	-24.8	11.12	-19.29	12.92	-6.6
Xanthi-2A X Taşova	2.83	-38.51	-3.2	-22.11	1.7	-16.39	-12.61
Xanthi-2A X Katerini	-13.87	4.05	-2.55	12.76	13.98	7.64	3.67
Xanthi-2A X Canik	-3.56	-2.83	-2.54	11.03	-15.93	14.89	0.18
Xanthi-2A X Erbaa	20.52	31.4	1.14	42.88	54.8	31.36	30.35
Nail X Gümüşhacıköy	27.92	-40.04	13.32	-31.09	22.71	-18	-4.2
Nail X Taşova	-7.36	-3.69	-20.4	9.94	-18.65	7.42	-5.46
Nail X Katerini	-11.7	12.19	13.51	-9.04	22.52	-4.74	3.79
Nail X Canik	-12.01	8.41	-15.4	-13.79	-1.62	-9.72	-7.35
Nail X Erbaa	3.91	-26.23	-8.08	-17.38	143.29	-7.07	14.74
Gümüşhacıköy X Taşova	-19.77	-0.15	22.58	-17.96	-31.94	-21.79	-11.51
Gümüşhacıköy X Katerini	-31.62	56.12	-30.6	0.36	18.66	46.81	9.96
Gümüşhacıköy X Canik	-24.73	-33.06	-8.22	-0.21	-28.03	-29.36	-20.6
Gümüşhacıköy X Erbaa	-17.26	-19.75	-44.9	-1.46	16.84	-10.51	-12.83
Taşova X Katerini	-6.11	-11.95	26.73	9.91	12.67	17.8	8.18
Taşova X Canik	-10.73	-11.88	22.56	35.56	-45.4	15.98	1.02
Taşova X Erbaa	-32.41	80.4	1.64	34.18	37.23	50.88	28.65
Katerini X Canik	-21.81	25.57	38.82	16.17	21.55	16.16	16.08
Katerini X Erbaa	-10.3	13.47	14.37	22	67.01	36.78	23.89
Canik X Erbaa	-2.55	51.13	3.09	14.89	44.71	1.99	18.88
Average	-9.59	5.12	0.15	5.15	15.65	6.46	3.82

*Growing environments: E12: Erbaa 2012, E13: Erbaa 2013, B12: Bafra 2012, B13: Bafra 2013, T12: Tokat 2012, T13: Tokat 2013

Table 12. Correlation coefficients between genetic distance and mid-parent heterosis levels for different traits in tobacco hybrids

Location	Correlation coefficient					
	Leaf number	Leaf width	Leaf length	Dried leaf yield	Sugar content in dried leaves	Nicotine content in dried leaves
Erbaa 2012	0.52*	0.29	0.31	0.21	0.22	-0.28
Erbaa 2013	0.17	0.68**	0.67**	0.13	-0.26	0.22
Bafra 2012	0.13	0.07	0.16	0.23	-0.15	-0.02
Bafra 2013	-0.02	0.12	0.25	-0.12	-0.10	0.13
Tokat 2012	0.50*	-0.25	-0.03	0.51*	-0.03	0.42
Tokat 2013	0.24	0.16	0.09	-0.06	0.14	0.09

*,**: Significant at 5% and 1% level of probability, respectively.

4. Conclusions

It was revealed that yields of oriental tobacco could be improved in hybrids without deteriorating their superior quality. SSR markers were very effective to determine genetic diversity of oriental tobacco, and only two markers (PT20172 and PT30265 or PT30274) could distinguish all seven genotypes used in the study. In other words, these the alleles identified by these two marker sets (PT20172 and PT30265 or PT20172 and PT30274) were able to indicate that all genotypes studied were genetically different. Heterosis levels were correlated with genetic distances calculated by SSR markers of parents for some traits in some environments. However, SSR markers cannot be used as reliable tools to estimate heterosis levels in oriental tobacco hybrids.

Acknowledgment

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Kinay, A., Saygılı, İ., Kandemir, N.; Design: Kinay, A., Saygılı, İ., Kandemir, N.; Data Collection or Processing: Kinay, A., Saygılı, İ.; Statistical Analyses: Saygılı, İ.; Literature Search: Kinay, A., Saygılı, İ., Kandemir, N.; Writing, Review and Editing: Kinay, A., Saygılı, İ., Kandemir, N.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Assessment of Different Cucurbit Genotypes for Resistance to Zucchini Yellow Mosaic Virus (ZYMV)

Farklı Kabakgil Genotiplerinin Kabak Sarı Mozaik Virüsü (Zucchini Yellow Mosaic Virus-ZYMV)'ne Karşı Duyarlılıklarının Belirlenmesi

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Abstract

Members of the *Cucurbitaceae* family, which includes species with quite different characteristics, have been used for food, medicine, and ornamental purposes for a long time. However, most plant diseases and pests cause yield and quality losses in cucurbits, and one of the most important of these diseases is zucchini yellow mosaic virus (ZYMV), which one of the most common potyviruses worldwide and causes serious yield losses in cucurbit production worldwide. Zucchini Yellow Mosaic Virus shows symptoms such as yellowing, mottling, curling, deformation, mosaic, shortening and thickening of the internodes, and may also cause loss of yield and quality. As widely known, there is no effective chemical control of viral diseases, and the use of resistant or tolerant varieties is the most effective solution. In this study, 92 watermelon genotypes, 14 zucchinis (pumpkin seeds) and 29 ornamental pumpkins collected from different parts of Türkiye were tested against ZYMV. Symptoms of ZYMV in different watermelon genotypes and pumpkins were observed for 21 days. Genotypes showing systemic infection after inoculation were evaluated on a scale of 0-5. Also, RT-PCR studies were carried out on selecting nine symptomless control plants, seven ZYMV-sensitive genotypes showing 5-scale value, one genotype with 1-scale value considered tolerant, and one genotype belongs to *C. lanatus* var. *citroides*. According to the results, it was determined that some watermelon and ornamental pumpkin genotypes could be considered as tolerant. Watermelon, which was having accession number PI560016, was found resistant to Turkish local strain of ZYMV. Although different susceptibility levels were detected between watermelon genotypes, all pumpkin genotypes were discovered to be susceptible to the Turkish local strain of ZYMV.

Keywords: Cucurbitaceae, Watermelon, Pumpkin, ZYMV, RT-PCR

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Öz

Oldukça farklı özelliklere sahip türlerin yer aldığı *Cucurbitaceae* familyasının üyeleri gıda, ilaç ve süs amaçlı kullanılmaktadır. Ancak çoğu bitki hastalığı ve zararlısı kabakgillerde verim ve kalite kayıplarına neden olmaktadır. Kabakgil üretiminde ciddi verim kayıplarına neden olan bu hastalıkların en önemlilerinden biri dünya çapında en yaygın görülen potyvirüslerden biri olan kabak sarı mozaik virüsüdür (ZYMV). Kabak sarı mozaik virüsü, sararma, beneklenme, kıvrılma, deformasyon, mozaikleşme, boğum aralarının kısalması ve kalınlaşması gibi belirtiler göstermekle birlikte verim ve kalite kaybına da neden olabilmektedir. Bilindiği gibi viral hastalıklara karşı etkili bir kimyasal mücadele mevcut olmayıp, dirençli veya toleranslı çeşitlerin kullanılması en etkili çözümdür. Bu çalışmada Türkiye'nin farklı yerlerinden toplanan 92 karpuz, 14 çekirdeklik kabak ve 29 süs kabağı genotipi, ZYMV'ye karşı test edilmiştir. Farklı karpuz genotipleri ve kabaklarda ZYMV belirtileri 21 gün boyunca gözlemlenmiştir. İnokulasyon sonrası sistemik enfeksiyon gösteren genotipler 0-5 hastalık skalasına göre değerlendirilmiştir. Ayrıca RT-PCR testiyle, dokuz simptomsuz kontrol bitkisi, 5 skala değerine giren ZYMV'ye duyarlı yedi genotip, toleranslı kabul edilen 1 skala değerine giren bir genotip ve *C. lanatus* var. *sitroidler* testlenmiştir. Sonuçlara göre bazı karpuz ve süs kabağı genotiplerinin toleranslı olarak değerlendirilebileceği belirlendi. PI560016 erişim numarasına sahip karpuzun Türkiye'deki yerel ZYMV türüne karşı dirençli olduğu belirlendi. Karpuz genotipleri arasında farklı duyarlılık düzeyleri tespit edilmesine rağmen tüm kabak genotiplerinin Türkiye'nin yerel ZYMV türüne karşı duyarlı olduğu tespit edilmiştir.

Anahtar Kelimeler: Cucurbitaceae, Karpuz, Balkabağı, ZYMV, RT-PCR

1. Introduction

The *Cucurbitaceae* family, commonly identified as cucurbits (cucumber, melon, watermelon, pumpkins, gourds, and squashes), consists of several economically and nutritionally important vegetable crops that are cultivated worldwide (Yanmaz and Düzeltir, 2003; Gáspár et al., 2022). Virus diseases are one of the important factors limiting production, regardless of where cucurbits are grown. At least 59 viruses belonging to major plant virus groups have been reported to infect cucurbits worldwide and cause significant losses (Lecoq and Desbiez, 2012). Diseases of pumpkin plants are caused by various viruses, such as papaya ringspot virus (PRSV), watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) which belongs to the Potyvirus genus (Simmons et al., 2013). Virus infections in cucurbit cultivation areas cause serious economic losses in many parts of the world. ZYMV is one of the main viruses that damage cucurbit crops in Turkey, which is an important cucurbit producer (Yılmaz et al., 1992)

ZYMV is also one of the most common potyvirus worldwide and has been reported to cause yield losses of up to 100% (Moradi et al., 2019). ZYMV is a member of the *Potyvirus* genus of the *Potyviridae* family. The virus is filamentous and flexible in shape, with a 750x13 nm dimension in size, and the genome consists of single-stranded RNA (Balint et al., 1990). ZYMV was first reported in 1973 in Italy (Lisa et al., 1981) and France (Lecoq et al., 1981). After being infected with ZYMV, zucchini plants exhibit symptoms such as a mosaic pattern and yellowing on their leaves. In more severe cases of infection, the leaves may show narrowing and a filamentous appearance. The photosynthetic pigment of leaves (Chl a, Chl b, and carotenoids) is significantly reduced during ZYMV infection (Radwan et al., 2007). In addition, a decrease of approximately 48% in the total pigment content was determined under ZYMV infection, while studies reported that leaf protein, carbohydrate, and proline content increased in infected plants (Radwan et al. 2007). In addition, serious deformations, deterioration in fruit shape and color changes are observed in fruits harvested from diseased plants, which makes them unmarketable. The yield losses can vary between 50-100% because of the virus infection depending on the severity of the infection and the conditions (Blua and Perring, 1992; Massumi et al., 2011).

There are 26 different races of the agent according to its biological, serological, molecular, and epidemiological characteristics. The most common races are ZYMV-MZ (Malaysia), ZYMV-TW (Taiwan), ZYMV-FL (Florida), ZYMV-CT (Connecticut), ZYMV-CH (China), and ZYMV-WK (France) (Wang et al., 1992; Sidek et al., 1999). While the spread of ZYMV in long distance occurs through the transfer of infected seeds from one region to another, while short distance spread by aphids (*Myzus persicae*) in short distances (Gal-On, 2007; Simmons et al., 2011). Plant diseases caused by viruses are among the most important factors limiting the production of watermelons, cucumber, melon, pumpkins, and ornamental pumpkins. The control of ZYMV largely depends on the suppression and control of the insect vector (aphid) population using insecticides; however, this practice may sometimes be ineffective, especially under high aphid population pressure (Shrestha et al., 2021). The development and usage of virus-resistant Cucurbita crops is the most effective strategy to reduce yield losses caused by ZYMV and is therefore a major target for pumpkin growers (Shrestha et al., 2021). Different watermelon, zucchini and ornamental pumpkin, genotypes of Turkiye were collected by our study group, self-pollinated severally to increase homozygosity of genetic structure, and these populations were characterized in terms of some morphological, molecular, and biochemical features (Dalda-Şekerci et al., 2017; Dalda-Şekerci et al., 2020; Morilipınar et al., 2021). However, no screening study for viral diseases has been conducted in these populations. The aim of this study was to determine the susceptibility level of ornamental pumpkin (29 genotypes), watermelon (92 genotypes) and seed pumpkin (14 genotypes) from Turkiye, collected from the main Cucurbit crops growing region of Turkiye, to ZYMV isolate. Identifying potential sources of disease resistance of these genotypes is very important for future breeding studies.

2. Materials and Methods

2.1. Plant Materials

In the study, pumpkin (*Cucurbita pepo*-14, seed pumpkin), ornamental pumpkin (*Cucurbita pepo* var. *ovifera*- 29) and watermelon (*Citrullus lanatus* var. *lanatus*- 92) plants collected from different regions of Turkiye were used as plant material. These genotypes used in the study have been selected from a population whose morphological, genetic, and biochemical characteristics have been determined, and have superior agronomic characteristics previous studies (Solmaz and Sarı, 2009; Solmaz et al., 2016; Dalda-Şekerci et al.,

2017; Coskun, 2019; Dalda-Şekerci et al., 2020; Morilipınar et al., 2021). In addition, one fusarium (F2) resistant accession (*Citrullus lanatus* var. *citroides*) (Wechter et al., 2016), six nematode-resistant watermelon accessions (N1, N3, N4, N5, N6, N7, F2) (*Citrullus amarus*) (Thies et al., 2016) and five ZYMV resistant *C. lanatus* var. *citroides* (Guner et al. 2019) obtained from the Gene Bank were used as the outgroup (Seeds of PI 595203 were obtained from Provvidenti, Cornell University, New York). One ZYMV resistant and one ZYMV susceptible *Lagenaria siceraria* (LS-R and LS-S (Ünlü et al., 2020), and ZYMV susceptible watermelon cultivars Sugar baby and Dixie Lee were used as a positive control. Seedlings were grown under greenhouse conditions in May and when the seedlings reached the 2-3 leaf stage. The experiment was designed with 5 replications of each genotype, they were transplanted into 15 cm diameter plastic pots in peat perlite mixture. The seedlings were kept at +22-24 °C temperature, 60-70% relative humidity and 16/8 (light/dark) hour light period (Agrios, 2005; Fidan et al., 2012; Ünlü et al., 2020).

2.2. Transmission of the Virus by Biological Methods

Virus isolate was obtained from the Akdeniz University Plant Virology Laboratory. In mechanical inoculation, a ZYMV isolate was used (NCBI accession number JF317296.1). The ZYMV isolate used in the study has been tested for all harmful viral diseases in cucurbits, and it has been determined to be a single infection (Helvaci et al., 2019; Nacar et al., 2021). Extractions were prepared on ice in a sterile mortar (to prevent inactivation) and the mixture was filtered with filter paper and smeared on the leaves of the plants with a sponge pad. Inoculations were carried out manually. When the plants were at the 2-3 true leaf stage, all leaves including the cotyledon leaves were inoculated. In addition, a second inoculation was made at the 5-6 leaf stage. Symptoms were observed and recorded daily.

2.3. Symptom Observations

After virus inoculation in genotypes of pumpkin (*Cucurbita pepo*-14), ornamental pumpkin (*Cucurbita pepo* var. *ovifera*- 29) and watermelon (*Citrullus lanatus*-92) plants used in the study, the symptoms of the plants were observed for 21 days, and the symptom types were recorded. Genotypes showing systemic infection after inoculation were evaluated on a scale of 0-5 (*Figures 1* and *2*). As a result of the evaluation, those with a value of 1 or higher were recorded as susceptible (S), and genotypes with a scale value of 0 that did not show systemic infection were recorded as resistant (R). The following 0-5 scale was used to determine the severity of virus symptoms (Aliyu et al., 2013).

0 = Plants without any symptom development

1 = 1 - 20% (very light); Plants showing very slight discoloration in leaf veins.

2 = 21 - 40% (light); Moderate mosaic plants along with slight discoloration in leaf veins.

3 = 41% - 60% (severe); Plants showing moderate to severe mosaic and yellowing in leaves.

4 = 61-80% (very severe); Severe mosaic symptoms de deformation in the leaves and stunted in the plant.

5 = 81-100% (almost dead); Severe mosaic in leaves, speckling, shortening of plant height, shoestring symptom, and deformation of leaves**.**

2.4. Nucleic Acid Isolation

A limited number of samples were randomly selected for nucleic acid isolation from inoculated plants. Sampling was carried out by selecting 9 symptomless control plants, 7 ZYMV-sensitive and 5-scale symptomatic genotypes, 1 1-scale genotype (56) considered tolerant, six genotypes (PI244019, PI560016, PI595200, PI595201, PI 595203, USVL252) obtained from the Gene Bank (Guner et al., 2019) and controls (negative and positive). Nucleic acid isolation was performed using the modified "Dellaporta Nucleic Acid Extraction" method reported by Presting et al. (1995) and carried out as follows. Leaf samples were crushed in 1.2 ml extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 500 mM NaCl, 10 mM 2-mercaptoethanol). 70 µl of 10% SDS was added and left at 65ºC for 10 minutes. After 200 µl of 5 M potassium acetate was added to the tubes, it was kept on ice for 10-15 minutes, and 600 µl of cold 96% ethanol was added to the pellet and washed. The dried pellet was mixed by adding 400 µl of sterile distilled water. It was incubated at 37°C for 15 minutes.

Figure 1. Zucchini Yellow Mosaic Virus symptoms and 0-5 scale in watermelon genotypes

Figure 2. Zucchini Yellow Mosaic Virus symptoms and 0-5 scale in pumpkin genotypes.

2.5. cDNA Synthesis and RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) Analyzes

cDNA was synthesized using total RNAs as templates. After the mixture was prepared with 1 µl of random hexamer primer, 10 µl of RNA and 1 µl of 10mM dNTP, it was incubated at 65°C for 5 minutes and then kept on ice for 5 minutes. 1 µl of M-MLV reverse transcriptase (Moloney Murine Leukaemia virus reverse transcriptase, 200 units/µl, Invitrogen), 4 µl of 5x Reverse Transcriptase buffer (Invitrogen), 2 µl of 0.1M DTT and 1 µl of

RNAse free water were added. After incubation at 25°C for 10 minutes, at 37°C for 50 minutes, and at 70°C for 15 minutes, they were kept on ice. The synthesized cDNAs were stored at -20°C. PCR was performed using cDNAs as templates. RT-PCR analyzes were performed with primers [ZYMVF, (5'–3' ATGCTCCAATCAGGCACYC) ZYMVR, (5'–3' GTGTGCCGTTCAGTGTCTTC)] (Papayiannis et al., 2005).

The content of the 15 µl mixture used in the reaction; 4 µl of cDNA and 2 µl of 10X Taq Buffer, 2 µl of 25 mM MgCl2, 2 µl of 5 mM dNTP, 0.5 µl of Taq polymerase, Forward primer 1µL, Reverse primer 1 µL were prepared and amplified in a Thermal Cycler device. In the amplification program used for RT-PCR analysis, initial denaturation at 95°C for 5mins, 30 sec denaturation at 95°C, 45 sec annealing at 52°C, 1 min extension at 72°C"repeated for 35 cycles and the final extension was performed at 72 °C for 7 minutes (Fidan et al., 2012; Ünlü et al., 2020). RT-PCR products were analyzed in 2% agarose gel electrophoresis. Afterward, the gel was stained with ethidium bromide and visualized in a UV imaging system.

3. Results and Discussion

3.1. Symptom Observation Results

Symptoms of ZYMV in different watermelon genotypes and pumpkins were observed for 21 days. The symptom types depending on the severity of the infection were recorded. Genotypes showing systemic infection after inoculation were evaluated according to the 0-5 scale, and two separate symptom scales were created for watermelon (*Figure 1*), pumpkin and ornamental pumpkin genotypes (*Figure 2*).

All watermelon and pumpkin genotypes were found to be infected with Turkish local isolate of ZYMV, except PI 560016 (*C. lanatus* var. *citroides*), which was reported to be resistant to strain ZYMV-FL by Guner et al. (2019) (*Table 1* and *Figure 4*). All pumpkin genotypes, 93% of ornamental pumpkin genotypes, and 90% of watermelon genotypes scored between 3-5 scale values and were determined to be highly susceptible to ZYMV (*Tables 1* and *2*). Nine watermelon and 2 ornamental pumpkin genotypes with 1-2 scale values were recorded as tolerant. None of the pumpkin and ornamental pumpkin genotypes used in the study were resistant to ZYMV after evaluation of the symptoms. While symptoms such as mosaic formation and chlorosis were observed on the leaves of the genotypes with a scale value of 1-2 (*Figures 1* and *2*), it has been observed that the 3-5 scale genotypes have a narrowing and filamentous appearance in the leaf blades. (*Figures 1* and *2*). According to the results, it was determined that some watermelon and ornamental pumpkin genotypes could be considered as tolerant. By continuing selfing and virus testing, high tolerance individuals can be developed from these genotypes that have not achieved homozygosity. It was observed that 7 of the genotypes obtained from the USDA Gene Bank fusarium and nematode tolerant genotypes showed severe symptoms (*Table 2*). The five watermelon (*C. lanatus* var. *citroides*) genotypes obtained from the gene bank and reported to be resistant to ZYMV (Guner et al. 2019), only PI560016 was found resistant to Turkish ZYMV isolate. Similarly, some PIs reported as resistant to ZYMV-FL by Provvidenti (1991) were found susceptible by Guner et al. (2019). The reason for this was stated that possible resistances may be strain-specific or temperature-dependent. In addition, the fact that most of the tested genotypes are not sufficiently purified may cause different results in the tests. For this reason, resistant genotypes can be found among the genotypes identified as susceptible in this study. It is also possible that genotypes identified as resistant sometimes have susceptible individuals. Therefore, researchers and breeders should continue to self-pollination and select the most resistant individuals to develop resistant inbred lines. Viruses usually change form and mutate very quickly and cause different symptoms in genotypes. Similarly, results from a study conducted by Helvaci et al. (2019) stated that a single melon and pumpkin genotype was found to be resistant to ZYMV in their test study with including 38 pumpkins, 19 melon and 8 watermelon genotypes. In recent studies on the resistance of different local cucurbit genotypes to ZYMV, it has been determined that a significant number of local genotypes were sensitive to ZYMV (Ünlü et al., 2020; Helvaci et al., 2019). As with other given studies, although the majority of genotypes tested are susceptible to ZYMV, resistant genotypes can also be developed by introducing resistance sources in the cucurbit populations.

Table 1 Zucchini Yellow Mosaic Virus symptom observation scores in watermelon (0-5 scale)

Genotype no	Scale value	Genotype no	Scale value	Genotype no	Scale value	Genotype no	Scale value
PI24401*	4	41	5	111	5	206	3
PI482259*	5	44	5	112	5	213	2
PI482319*	4	42	5	114	5	223	5
PI189225*	5	45	4	119	5	224	4
PI482324*	5	46	5	122	5	225	2
PI482303*	5	47	5	125	2	229	5
USVL252**	5	48	2	136	4	234	3
PI244019***	5	50	4	137	3	241	5
PI560016***	0	52	5	138	5	247	5
PI595200***	4	53	4	141	5	252	5
PI595201***	3	56	1	149	3	285	5
PI595203***	4	59	4	151	5	298	2
3	4	58	5	161	4	303	3
5	4	62	5	165	5	305	4
6	5	63	5	168	4	322	4
9	5	68	5	171	5	333	5
11	5	70	4	174	4	341	3
13	5	71	5	183	2	342	2
18	3	75	5	184	3	347	5
22	4	77	4	187	4	350	5
23	2	78	5	190	5	354	5
28	3	85	5	192	3	356	5
35	5	86	4	194	4	384	3
36	4	89	4	195	5	LS-R	0
37	5	90	3	199	5	LS-S	4
38	5	91	4	200	4	Sugar baby	5
40	4	96	5	203	5	Dixie Lee	5

*Nematode resistance *Citrullus amarus* accessions (Thies et al. 2016), **Fusarium oxysporum* f.sp. *niveum*-2 resistance *Citrullus lanatus* var. *citroides* accession (Wechter et al. 2016), *** ZYMV resistance *Citrullus lanatus* var. *citroides* accessions (Guner et al. 2019).

Table 2 Zucchini Yellow Mosaic Virus symptom observation scores in pumpkin genotypes (0.5 scale)

C. pepo		C. pepo var. ovifera			
Genotype no	Scale value	Genotype no	Scale value	genotype no	Scale value
12	5	1	5	27	5
15	5	3	4	29	4
17	5	4	5	30	3
19	5	5	4	31	5
23	5	6	5	32	5
24	5	17	4	33	5
27	5	18	5	36	5
29	5	19	2	37	5
33	5	21	5	39	5
39	5	22	3	40	5
44	4	23	2	41	5
45	5	24	5	42	5
46	5	25	4	43	4
68	5	26	5	44	4
				45	4

3.2. *PCR Analysis Results*

The RT-PCR method, which is one of the molecular methods used extensively in current research in the field of plant virology, was used for the detection of ZYMV in infected and negative (symptomless) control plants.

RT-PCR studies were carried out on selecting nine symptomless control plants, seven ZYMV-sensitive genotypes showing 5-scale value, one genotype (56) with 1-scale value considered tolerant, and one genotype (F2) belongs to *C. lanatus* var. *citroides* (*Figure 3*).

In RT-PCR studies, the expected band sizes were obtained after the usage of primers specific to the ZYMV coat protein in the amplification of gene fragments (*Figure 3*). Five *C. lanatus* var. *citroides* accessions reported to be resistant to ZYMV-FL by Guner et al. (2019), positive and negative control plants (watermelon cultivar and bottle gourd accessions) were also tested for the Turkish local strain of ZYMVY. The five accessions reported to be resistant to ZYMV, only PI560016 was found to be resistant to the local Turkish strain of ZYMV (*Table 1* and *Figure 4*). The current results are consistent with the results of previous studies conducted on ZYMV (Fidan et al., 2012; Helvaci et al., 2019; Guner et al., 2019; Ünlü et al., 2020). The researchers detected the presence of ZYMV in plant tissue by RT-PCR process using purified viral RNAs and ZYMV-F1 and ZYMV-R1 primer pairs. In this study, a 791 bp band marker for ZYMV infection was observed in all infected plant samples by agarose gel electrophoresis (*Figures 3* and *4*).

Figure 3 Samples showing a positive and negative reaction after RT-PCR analysis using ZYMV-specific (ZYMVF / ZYMVR) primers.

*(M: DNA ladder, 1: Watermelon 56, 2: Watermelon F2, 3: Watermelon 75, 4: Watermelon 171, 5: Watermelon 199, 6: Watermelon 333, 7: Watermelon 354, 8-9-10-11-12: Watermelon negative control, 13-14-15: Ornamental pumpkin negative control, 16: Ornamental pumpkin 24, 17: Ornamental pumpkin 30).

Figure 4. RT-PCR results of five Citrullus lanatus var citroides accessions found resistant to ZYMV-FL by Guner et al (2019) infected with the Turkish local strain of ZYMV.

Ling and Levi (2007) tested 190 bottle gourd genotypes for ZYMV-FL and they found that 36 of which were fully resistant, 64 genotypes were partially resistant, and 90 genotypes were susceptible. ZYMV-FL resistance was found mostly in *L. siceraria* genotypes collected in India. The 36 resistant genotypes, 33 were from India, one from Indonesia, one from South Africa and one from Zimbabwe, Guner et al. (2019) tested watermelons in the gene bank of USDA against ZYMV-FL strain and they discovered that PI 595203, PI 386015, PI 386016, PI386024, PI 386025, PI 386026, PI 244018, PI 244019, PI 485583, PI 494528, and PI 494529 watermelon genotypes were resistant to ZYMV. In addition, serological tests with Indirect-ELISA in a study conducted in Bali showed that 75% of zucchini plants were infected with ZYMV and up to 8.33% were positive for CMV (Pandawani and Wıdnyana, 2021). In another study, researchers reported that resistance to ZYMV in watermelon is controlled by a recessive gene (Guner et al., 2018). This study demonstrates that ZYMV resistant lines can be developed by producing lines with improved purity from the watermelon populations tested in our study. Many other studies have been carried out to develop new varieties resistant to ZYMV and to reveal the genetic structure of the disease. Many researchers have attempted to analyze the inheritance of ZYMV resistance and identify markers associated with resistance genes (Harris et al., 2009; Pachner et al., 2015; Capuozzo et al., 2017, Guner et al., 2018).

4. *Conclusions*

In this study, 92 local watermelons, 14 zucchinis (seed pumpkins) and 29 ornamental pumpkins genotypes, collected from different parts of Turkiye and 12 *C. lanatus* var. *citroides* genotypes provided form gene bank were tested against local Turkish ZYMV strain. PI560016 was found resistance to ZYMV. Although different susceptibility levels were detected among watermelon genotypes, all pumpkin genotypes were found to be susceptible to the tested Turkish local strain of ZYMV. ZYMV still continues to have a significant threat to the cultivation of cucurbits species both in greenhouses and open fields in Turkiye. Since there is no effective chemical control of viruses, measures to prevent virus infection and spread are still important approaches in crop production. In the control of virus vectors, the use of pesticides is not recommended since there is a very short time between the infection of the virus by the vector and its transmission. The development of varieties resistant to biotic stress factors, especially viruses, is the safest way in terms of sustainable environment and plant production. Breeding studies to develop resistant cultivars to ZYMV are of great importance in Cucurbit species cultivation. Breeding studies related to resistance to ZYMV have been carried out in recent times and some genes responsible for resistance have been identified and reported. The new strategies aimed at producing resistant plants (through conventional breeding programs, biotechnological and molecular breeding techniques or pathogen-derived strategies) should be improved. More tolerant genotypes can be developed by inbreeding and virus testing in each generation from the tolerant watermelon genotypes in the current study. In addition, Guner et al. (2019) reported PIs should be included in Turkish watermelon germplasm and ZYMV-resistant breeding lines should be developed by combination and backcrossing methods. Developing and disseminating virus-resistant cucurbit varieties, preferring ZYMV-free seedlings, regularly observation of the seedlings for ZYMV at regular intervals after planting in the field, eradication of diseased plants and applying control methods to reduce the possibility of ZYMV transmission with aphids are the most effective strategies to reduce yield losses caused by ZYMV. In addition, no resistant or tolerant genotypes were found in the seed pumpkin and ornamental pumpkin tested in this study. However, all seed pumpkin and ornamental pumpkin, except *C. pepo* var. *ovifera* genotypes 19 and 23, showed severe (4-5 scale value) symptoms in a short time. It is thought that some of these genotypes can be used as indicator plants (susceptible control) for ZYMV propagation in further studies.

Acknowledgment

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Akife Dalda-Sekerci Data curation, Formal analysis, Writing – review & editing. Cemile Temur Cinar: Data curation, Writing – review & editing. Emel Ünlü: Data curation, Formal analysis, Writing – review & editing. Hakan Fidan: Review & editing, Supervision. Halit Yetisir: Conceptualization, Methodology, Funding acquisition, Resources, Project administration, Writing – review & editing, Supervision.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

An Evaluation of the Tersilochinae (Hymenoptera: Ichneumonidae) Fauna of Türkiye and the New Record of *Aneuclis incidens* (Thomson, 1889) for the Province of Bingöl (Karlıova)*

Türkiye Tersilochinae (Hymenoptera: Ichneumonidae) Faunası Hakkında Bir Değerlendirme ve *Aneuclis incidens* (Thomson, 1889) Türünün Bingöl (Karlıova) İli İçin Yeni Kaydı

Saliha ÇORUH[1*], Medeni DALAN[2]

Abstract

The present study was undertaken to examine of the subfamily Tersilochinae (Hymenoptera: Ichneumonidae) in Türkiye. The Tersilochinae, one of the 47 subfamilies are a worldwide subfamily of the parasitoid wasp family Ichneumonidae. This subfamily, are koinobiont endoparasitoids of Coleoptera larvae, (although Symphyta larvae are recorded as hosts of one genus). Hosts include Curculionidae and Chrysomelidae so Tersilochinae are used for biological control. Tersilochinae species constituting the study were collected from parts of Türkiye between 1995-2022. At the same time, the study also added before 1995 literature information. 42 species from genera of *Aneuclis*, *Barycnemis*, *Diaparsis* (*Diaparsis*), *Diaparsis* (*Nanodiaparsis*), *Gelanes*, *Heterocola* (*Heterocola*), *Heterocola* (*Heterocoloides*), *Phradis*, *Probles* (*Euporizon*), *Probles* (*Microdiaparpis*), *Probles* (*Probles*), *Tersilochus* (*Gonolochus*) and *Tersilochus* (*Tersilochus*) have been identified from Türkiye so far. The study consist of 217 samples. Among these species, *Heterocola* (*Heterocola*) *longipalpis* Kolarov & Beyarslan is endemic for Türkiye at now. With this, *Heterocola* (*Heterocola*) *nigrotibialis* Horstmann and Kolarov, *Tersilochus* (*Gonolochus*) *rugulosus* Horstmann, *Tersilochus* (*Gonolochus*) *nitens* Horstmann & Kolarov and *Tersilochus* (*Tersilochus*) *cognatus* Holmgren are rare species for Türkiye. Again, *Probles* (*Microdiaparpis*) Horstmann is dense in terms of number samples, *Phradis* Förster and *Tersilochus* (*Tersilochus*) Holmgren are dense in terms of number species. Among the species determined, *Probles* (*Microdiaparpis*) *anatolicus* Horstmann, *Aneuclis incidens* (Thomson) and *Barycnemis harpura* (Schrank) were recorded the most abundant species in research areas. In addition to all this, species composition has created for each species and new location information of this subfamily has been added. Bingöl (Karlıova) provinces is the new locality for this subfamily.

Keywords: Hymenoptera, Ichneumonidae, Tersilochinae, Türkiye, Species composition

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Öz

Bu çalışma, Türkiye'de Tersilochinae (Hymenoptera: Ichneumonidae) altfamilyasının incelenmesi amacıyla yapılmıştır. Bu altfamilya, parazitoid bir arı grubu olan Ichneumonidae familyasının 47 altfamilyasından biridir. Coleoptera (Symphyta bir cinsin konukçusu olarak kaydedilmiştir) larvalarının koinobiont endoparazitoiti durumundadır. Çalışmayı oluşturan tersilochineler 1995-2022 yılları arasında toplanmış, 1995 yılından önceki literatür bilgileri de çalışmaya dahil edilmiştir. Toplamda tespit edilen 42 tür *Aneuclis*, *Barycnemis*, *Diaparsis* (*Diaparsis*), *Diaparsis* (*Nanodiaparsis*), *Gelanes*, *Heterocola* (*Heterocola*), *Heterocola* (*Heterocoloides*), *Phradis*, *Probles* (*Euporizon*), *Probles* (*Microdiaparpis*)*, Probles (Probles), Tersilochus* (*Gonolochus*) ve *Tersilochus* (*Tersilochus*) cinslerine aittir. Çalışma toplam 217 örnekten oluşmaktadır. Bunların içerisinden, *Heterocola* (*Heterocola*) *longipalpis* Kolarov & Beyarslan türü şimdilik Türkiye için endemiktir. Bununla beraber, *Heterocola* (*Heterocola*) *nigrotibialis* Horstmann and Kolarov*, Tersilochus* (*Gonolochus*) *rugulosus* Horstmann*, Tersilochus* (*Gonolochus*) *nitens* Horstmann & Kolarov ve *Tersilochus* (*Tersilochus*) *cognatus* Holmgren 1860 türleri nadir türler arasındadır. *Probles* (*Microdiaparpis*) Horstmann cinsi örnek sayısı bakımından, *Phradis* Förster ve *Tersilochus* (*Tersilochus*) Holmgren cinsleri de tür sayısı bakımından en çok rastlanılan gruplardır. Teşhis edilen türler arasında, *Probles* (*Microdiaparpis*) *anatolicus* Horstmann, *Aneuclis incidens* (Thomson) ve *Barycnemis harpura* (Schrank) en çok sayıya sahip türler olarak tespit edilmiştir. Bütün bunlara ek olarak çalışmada, her bir türe ait tür kompozisyonu oluşturulmuş ve yeni lokalite verileri eklenmiştir. Bingöl (Karlıova), bu altfamilya için yeni lokalite durumundadır.

Anahtarkelimeler: Hymenoptera, Ichneumonidae, Tersilochinae, Türkiye, Tür kompozisyonu.

1. Introduction

The order Hymenoptera includes insects commonly known as ants, bees and wasps. Most authors believe that the name has been derived from the Greek hymen, meaning 'membrane' and referring to the parchment-like transparent wings, without any scales or hair, and which often may be clouded (Gupta, 2005).

Hymenopterans include famous examples of social insects, like bees and ants, with regimented social systems in which members are divided into castes. They are also important pollinators of flowering plants, some species of which have flowers specially adapted to attract and receive the attention of specific hymenopteran (Palma, 2010).

Ichneumons (family Ichneumonidae), any of a large and widely distributed insect group (order Hymenoptera) of considerable economic importance. Ichneumons parasitize many insect groups, especially Lepidoptera (moths and butterflies), Coleoptera (beetles) and other Hymenoptera. Some utilize spiders as hosts. Thus, the group as a whole is benefical to humans because it parasitizes many insect pests. A relatively small number of species parasitize beneficial insects (Viet, 2017)

Tersilochinae, one of the 47 subfamilies are a worldwide subfamily of the parasitic wasp family Ichneumonidae (Townes, 1971). Clypeus wide, separated from face by a groove, the apical margin with a fringe of long parallel setae. Mesopleuron with foveate groove extending from about mid height to metacoxa. Fore wing with areolet minute and open, pterostigma large and triangular. Metasoma laterally compressed. Metasomal tergite I slender, spiracle near apex. Ovipositor upcurved, short to very long, with dorsal subapical notch in this subfamily.

There are currently 34 genera and 524 species (Yu et al., 2016) in the world. Up to 1995 (Kolarov, 1995) only six species belonging to four genera have been documented in Türkiye (Sedivy, 1959; Horstmann, 1971, 1981; Kasparyan, 1981; Kolarov, 1989; Öncüer, 1991; Kolarov and Beyarslan, 1994). After 1995, with the contributions (Özdan, 2014; İneciklioğlu, 2022; Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2002; Gürbüz et al., 2008; 2011; Eroğlu et al., 2011; Çoruh et al., 2014a, b) the numbers of Tersilochinae fauna of Türkiye reached to 42 species and 14 genera.

The aim of this study is to analyze studies related to the Tersilochinae (Ichneumonidae) species collected so far from Türkiye by taxonomical and biogeographical. It is also to update the location data with Bingöl (Karlıova) added as a new location. In this way, our goal is to make this subfamily available with all its data to researchers and science volunteers interested in this subject and to contribute to biological diversity.

2. Materials and Methods

2.1. Material

Icneumonid species detected in Türkiye are classified in Table 1 according to the research data of the years 1995-2014. As stated in these literatures, samples were generally collected using a standart sweeping net and were collected from various regions of Türkiye (*Figure 1*). In addition, *Aneuclis incidens* (Thomson, 1889) collected from Bingöl (Karlıova) in the summer of 2022. The species is newly registered for Bingöl and has been added to the existing species in a a new locality.

2.2. Method

The ichneumonid specimens have been obtained in different researches. The genera and species are listed in the alphabetic order according the recent Interactive Catalogue of World Ichneumonidae (Yu et al., 2016). The distributional records were also used from this catalogue. In table 1, are included valid taxa names, individual numbers, zoogeographical distribution and reference data.

3. Results

In this study, all Tersilochinae samples that were previously collected from different locations in Anatolia after 1995 have been evaluated from different perspectives.

Figure 1. Research area

3.1. Faunistic Data

Evaluation was made according to the number of species and individuals of the existing genera. Accordingly it was recorded as one species and one samples belong to the genus *Allophroides*, three species and forty-seven samples belong to genus *Aneuclis*, two species and twenty samples belong to *Barycnemis*, five species and seven samples belong to *Diaparsis* (*Diaparsis*), two species and six samples belong to *Diaparsis* (*Nanodiaparsis*), two species and five samples belong to *Gelanes*, two species and seven samples belong to *Heterocola* (*Heterocola*), one species and one sample belong to *Heterocola* (*Heterocoloides*), six species and nine samples belong to *Phradis*, two species four samples belong to *Probles* (*Euporizon*), five species seventy-eight samples belong to *Probles (Microdiaparpis),* two species and two samples belong to *Probles* (*Probles*)*,* three species and fourteen samples belong to *Tersilochus* (*Gonolochus*) and six species and fourteen samples belong to Tersilochus (*Tersilochus*).

It is understood that, while Probles (*Microdiaparpis*) is higher in number in terms of samples (*Figure 2a*), *Phradis* and *Tersilochus* (*Tersilochus*) are higher in number in terms of species (*Figure 2b*). Among the species detected, *Probles* (*Microdiaparpis*) *anatolicus* (with 70 samples), *Aneuclis incidens* (37), and *Barycnemis harpura* (18) were found to be the most ones in the research areas (*Table 1*). In contrast, the findings have shown that *Allophroides boops*, *Diaparsis* (*Diaparsis*) *carinifer*, *D.* (*D.) multiplicator*, *D.* (*D.) rara*, *D.* (*D.) temporalis*, *D.* (*Nanodiaparsis*) *frontella*, *Heterocola* (*Heterocola*) *longipalpis*, *H.* (*H.*) *inguaria*, *Phradis decrescens*, *P. minutus*, *P. morionellus*, *P. nigritulus*, *Probles* (*Euporizon*) *exilis*, *P.* (*Microdiaparpis*) *caudiculatus*, *P.* (*Microdiaparsis*) *microcephalus*, *Probles* (*Microdiaparpis*) *neoversutus*, *Tersilochus* (*Gonolochus*) *rugulosus*, *Tersilochus (Tersilochus) cognatus*, *Tersilochus jocator* and *Tersilochus (Tersilochus) heterocerus* (with 1 individual) were rarely found in Anatolia (*Table 1*). The rate of the species collected as a single individual is 52%.

Table 1. Data of collected species

Names of Taxa	IN	VD	SD	GR	ZR	FRT
SUBFAMILY TERSILOCHINAE Schmiedeknecht, 1910						
Genus *Allophroides* Förster, 1869						
Allophroides boops (Gravenhorst, 1829)	1	A	Ap	CAR	E, WP	Gürbüz et al., 2011
Genus *Aneuclis* Förster, 1869						
Aneuclis anterior Horstmann, 1971	3	A	J, Aug, S	AR, MtR	EP, E, WP	Kolarov and Beyarslan, 1994
Aneuclis incidens (Thomson, 1889)	37	B, D, E, G	M, J, Jl, Aug, S, O	AR, BSR, EAR, MtR, MR, SAR	EP, E, WP	Sedivy, 1959
Aneuclis melanaria (Holmgren, 1860)	7	A, B	M, J, Jl	CAR, MtR	EP, E, WP	Horstmann, 1971
Genus *Barycnemis* Förster, 1869						
Barycnemis alpina (Strobl, 1901)	2	H	Jl	EAR	EP, E, WP	Çoruh et al., 2002
Barycnemis harpura (Schrank, 1802)	18	C, G, H	Jl, Aug	BSR, EAR	EP, E, NEAR, WP	Kolarov, 1989
Genus *Diaparsis* (*Diaparsis*) Förster, 1869						
Diaparsis (*Diaparsis*) *carinifer* (Thomson, 1889)	1	A	M	BSR	EP, E, NEAR, WP	Khalaim and Yurtcan, 2011
Diaparsis (*Diaparsis*) *multiplicator* Aubert, 1869	1	D	M	EAR	E, WP	Gürbüz et al., 2011
Diaparsis (*Diaparsis*) *nitida* Horstmann, 1981	3	A	Ap, M	BSR, MtR	EP, E, WP	Gürbüz et al., 2011
Diaparsis (*Diaparsis*) *rara* (Horstmann, 1971)	1	E	M	BSR	EP, E, WP	Khalaim and Yurtcan, 2011
Diaparsis (*Diaparsis*) *temporalis* Horstmann, 1979	1	A	A	MtR	EP, E, NEAR, WP	Khalaim and Yurtcan, 2011
Genus *Diaparsis* (*Nanodiaparsis*) Horstmann, 1971						
Diaparsis (*Nanodiaparsis*) *aperta* (Thomson, 1889)	5	E, F	J, Jl	AR, BSR, MtR	EP, E, WP	Horstmann, 1971
Diaparsis (*Nanodiaparsis*) *frontella* (Holmgren, 1860)	1	D	S	BSR	EP, E, WP	Çoruh and Khalaim, 2012
Genus *Gelanes* Horstmann, 1981						
Gelanes fusculus Holmgren, 1860	3	A, E	Mrc, J	MtR	EP, E, WP	Gürbüz et al., 2008
Gelanes simillimus Horstmann, 1981	2	A	Mrc, Ap	MtR	EP, E, WP	Gürbüz et al., 2011
Genus *Heterocola* (*Heterocola*) Horstmann, 1971						
Heterocola (*Heterocola*) *longipalpis* Kolarov & Beyarslan, 1994	1	G	Jl	EAR	WP	Kolarov and Beyarslan, 1994
Heterocola (*Heterocola*) *nigrotibialis* Horstmann & Kolarov, 1988	6	E	J, Jl	CAR	WP	Khalaim and Yurtcan, 2011
Genus *Heterocola* (*Heterocoloides*) Horstmann, 1971						
Heterocola (*Heterocoloides*) *inguaria* (Haliday, 1838)	1	G	Jl	EAR	E, WP	Kolarov and Beyarslan, 1994
Genus *Phradis* Förster, 1868						
Phradis brevis (Brischke, 1880)	3	A, E	Mrc, J	CAR, MtR	EP, E, WP	Gürbüz et al., 2011
Phradis decrescens (Thomson, 1889)	1	H	J	EAR	EP, E, WP	Khalaim and Yurtcan, 2011
Phradis minutus (Bridgman, 1889)	1	E	Jl	MtR	E, WP	Kolarov and Beyarslan, 1994
Phradis morionellus (Holmgren, 1860)	1	?	?	Anatolia	EP, E, WP	Horstmann, 1981
Phradis nigritulus (Gravenhorst, 1829)	1	E	M	AR	EP, E, WP	Kolarov and Beyarslan, 1994
Phradis rufiventris Horstmann, 1981	2	H	J	EAR	EP, E, WP	Khalaim and Yurtcan, 2011

Table 1. Continued

	IN	VD	SD	GR	ZR	FRT
Genus *Probles* (*Euporizon*) Horstmann, 1971						
Probles (*Euporizon*) *exilis* (Holmgren, 1860)	1	A	Aug	MtR	EP, E, WP	Sedivy, 1959
Probles (*Euporizon*) *rufipes* (Holmgren, 1860)	3	H	Aug	BSR	E, WP	Horstmann, 1981
Genus *Probles* (*Microdiaparpis*) Horstmann, 1971						
Probles (*Microdiaparpis*) *anatolicus* Horstmann, 1981	70	A, D, E	M, J, Jl, S, O	BSR, MR, MtR	WP	Horsmann, 1981
Probles (*Microdiaparpis*) *caudiculatus* Khalaim, 2007	1	G	O	EAR	EP, E, WP	Çoruh and Khalaim, 2012
Probles (*Microdiaparsis*) *microcephalus* (Gravenhorst, 1829)	1	A	J	BSR	E, WP	Çoruh et al., 2014b
Probles (*Microdiaparpis*) *neoversutus* (Horstmann, 1967)	1	E	S	BSR	EP, E, WP	Khalaim and Yurtcan, 2011
Probles (*Microdiaparpis*) *versutus* (Holmgren, 1860)	5	E	A	MtR	EP, E, WP	Eroğlu et al., 2011
Genus *Probles* (*Probles*) Förster, 1868						
Probles (*Probles*) *erythrostomus* (Gravenhorst, 1829)	2	A	Ap, J	MtR	E, WP	Khalaim and Yurtcan, 2011
Probles (*Probles*) *flavipes* (Szépligeti, 1899	2	H	Jl	BSR	E, WP	Khalaim & Yurtcan, 2011
Genus *Tersilochus* (*Gonolochus*) Holmgren, 1859						
Tersilochus (*Gonolochus*) *caudatus* (Holmgren, 1860)	4	A, F, G	M	EAR, MR	EP, E, WP	Khalaim and Yurtcan, 2011
Tersilochus (*Gonolochus*) *rugulosus* Horstmann, 1981	1	D	Ap	AR	E, WP	Khalaim and Yurtcan, 2011
Tersilochus (*Gonolochus*) *nitens* Horstmann & Kolarov, 1988	9	G, E	Ap, M, J	BSR, EAR	E, WP	Gürbüz et al., 2011
Genus *Tersilochus* (*Tersilochus*) Holmgren, 1859						
Tersilochus (*Tersilochus*) *cognatus* Holmgren, 1860	1	A	Ap	MtR	E, WP	Khalaim and Yurtcan, 2011
Tersilochus (*Tersilochus*) *jocator* Holmgren, 1859	1	F	Ap	MtR	E, WP	Gürbüz et al., 2011
Tersilochus (*Tersilochus*) *heterocerus* (Thomson, 1889)	1	A	J	MtR	E, WP	Khalaim and Yurtcan, 2011
Tersilochus (*Tersilochus*) *obscurator* (Aubert, 1959)	4	A, E, G	A, M	CAR, EAR, MR	E, WP	Khalaim and Yurtcan, 2011
Tersilochus (*Tersilochus*) *triangularis* (Gravenhorst, 1807)	3	A, E	A, M	AR, MR, MtR	E, WP	Gürbüz et al., 2011
Tersilochus (*Tersilochus*) *tripartitus* (Brischke, 1880)	4	A, D, E	A, M	AR, EAR, MR	E, WP	Khalaim and Yurtcan, 2011

Individual numbers (IN), vertical distribution (VD), seasonal dynamics (SD), geographical regions (GR), zoogeographical regions (ZR), first record of Türkiye (FRT) of specimens. Vertical distribution (VD) (metre): A: 0-500 m, B: 501-750 m, C: 751-1000 m, D: 1001-1250 m, E: 1251- 1500 m, F: 1501-1750 m, G: 1751-2000 m, H: 2001-2700 m. Seasonal dynamics (SD): Mrc: March, A: April, M: May, J: June, Jl: July, A: August, S: September, O: October. Geographical regions (GR): AR: Aegean Region, BSR: Black Sea Region, CAR: Central Anatolia Region, EAR: Eastern Anatolia Region, MR: Marmara Region, MtR: Mediterranean Region, SAR: Southeastern Anatolia. Zoogeographical regions (ZR): E: Europe, EP: Eastern Palaearctic, NEAR: Nearctic Region, WP: Western Palaarctic.

Table 2. Provinces and references of collected species in Türkiye

Names of Taxa	Distributions in Türkiye	Reference (s)
SUBFAMILY TERSILOCHINAE Schmiedeknecht, 1910		
Genus *Allophroides* Förster, 1869		
Allophroides boops (Gravenhorst, 1829)	Karaman	Gürbüz et al., 2011
Genus *Aneuclis* Förster, 1869		
Aneuclis anterior Horstmann, 1971	Adana, Antalya, İzmir	Kolarov & Beyarslan, 1994; Khalaim and Yurtcan, 2011
Aneuclis incidens (Thomson, 1889)	Adana, Adıyaman, Afyon, Antalya, Burdur, Edirne, Gaziantep, İçel, Osmaniye, Kastamonu, Kütahya, Van	Sedivy, 1959; Horstmann, 1971; Kasparyan, 1981; Öncüer, 1991; Kolarov, 1989; Kolarov, 1995; Kolarov and Beyarslan, 1994; Khalaim and Yurtcan, 2011
Aneuclis melanaria (Holmgren,1860)	Ankara, Çanakkale, Çankırı	Horstmann, 1971; Kolarov, 1989; Kolarov, 1995
Genus *Barycnemis* Förster, 1869		
Barycnemis alpina (Strobl, 1901)	Bayburt	Çoruh et al., 2002; Khalaim and Yurtcan, 2011; Çoruh et al., 2014a
Barycnemis harpura (Schrank, 1802)	Ardahan, Artvin, Erzurum, Rize, Van	Kolarov, 1989; Kolarov and Beyarslan, 1994; Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Genus *Diaparsis* (*Diaparsis*) Förster, 1869		
Diaparsis (*Diaparsis*) *carinifer* (Thomson, 1889)	Samsun	Khalaim and Yurtcan, 2011
Diaparsis (*Diaparsis*) *multiplicator* Aubert, 1969	Erzincan	Gürbüz et al., 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Diaparsis (*Diaparsis*) *nitida* Horstmann, 1981	Hatay, Kars, Samsun	Gürbüz et al., 2011; Khalaim and Yurtcan, 2011; Çoruh et al., 2014a
Diaparsis (*Diaparsis*) *rara* (Horstmann, 1971)	Kastamonu	Khalaim and Yurtcan, 2011
Diaparsis (*Diaparsis*) *temporalis* Horstmann, 1979	Edirne	Khalaim and Yurtcan, 2011
Genus *Diaparsis* (*Nanodiaparsis*) Horstmann, 1971		
Diaparsis (*Nanodiaparsis*) *aperta* (Thomson, 1889)	West Türkiye, Artvin, Hatay, Osmaniye	Horstmann, 1971; Kasparyan, 1981; Kolarov, 1995; Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Gürbüz et al., 2011; Çoruh et al., 2014a
Diaparsis (*Nanodiaparsis*) *frontella* (Holmgren, 1860)	Kastamonu	Khalaim and Yurtcan, 2011
Genus *Gelanes* Horstmann, 1981		
Gelanes fusculus Holmgren, 1860	Hatay, Isparta	Özdan, 2014; Gürbüz et al., 2008; Gürbüz et al., 2011
Gelanes simillimus Horstmann, 1981	Hatay	Gürbüz et al., 2011
Genus *Heterocola* (*Heterocola*) Horstmann, 1971		
Heterocola (*Heterocola*) *longipalpis* Kolarov & Beyarslan, 1994	Erzurum	Kolarov and Beyarslan, 1994; Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Heterocola (*Heterocola*) *nigrotibialis* Horstmann and Kolarov, 1988	Sivas	Khalaim and Yurtcan, 2011
Genus *Heterocola* (*Heterocoloides*) Horstmann, 1971		
Heterocola (*Heterocoloides*) *inguaria* (Haliday, 1838)	Erzurum	Kolarov and Beyarslan, 1994
Genus *Phradis* Förster, 1868		
Phradis brevis (Brischke, 1880)	Hatay, Sivas	Khalaim and Yurtcan, 2011; Gürbüz et al., 2011
Phradis decrescens (Thomson, 1889	Van	Khalaim and Yurtcan, 2011
Phradis minutus (Bridgman, 1889)	Isparta	Kolarov and Beyarslan, 1994; Khalaim and Yurtcan, 2011
Phradis morionellus (Holmgren, 1860)	Anatolia	Horstmann, 1981; Kolarov, 1995; Khalaim and Yurtcan, 2011
Phradis nigritulus (Gravenhorst, 1829)	İçel	Kolarov and Beyarslan, 1994; Khalaim and Yurtcan, 2011
Phradis rufiventris Horstmann, 1981	Van	Khalaim and Yurtcan, 2011

Table 2. continued

Genus *Probles* (*Euporizon*) Horstmann, 1971		
Probles (*Euporizon*) *exilis* (Holmgren, 1860)	Adana	Sedivy, 1959; Öncüer, 1991; Kolarov, 1995; Khalaim and Yurtcan, 2011
Probles (*Euporizon*) *rufipes* (Holmgren, 1860)	Trabzon	Horstmann, 1981; Kolarov, 1995; Khalaim and Yurtcan, 2011
Genus *Probles* (*Microdiaparpis*) Horstmann, 1971		
Probles (*Microdiaparpis*) *anatolicus* Horstmann, 1981	Isparta, Rize, Tekirdağ, Trabzon	Özdan, 2014; Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Probles (*Microdiaparpis*) *caudiculatus* Khalaim, 2007	Erzurum	Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Probles (*Microdiaparpis*) *microcephalus* (Gravenhorst, 1829)	Rize	Çoruh et al., 2014b, Çoruh et al., 2014a
Probles (*Microdiaparpis*) *neoversutus* (Horstmann, 1967)	Kastamonu	Khalaim and Yurtcan, 2011
Probles versutus (Holmgren, 1860)	Isparta	Eroğlu et al., 2011
Genus *Probles* (*Probles*) Förster, 1868		
Probles (*Probles*) *erythrostomus* (Gravenhorst, 1829	Afyon, Antalya	Khalaim and Yurtcan, 2011
Probles (*Probles*) *flavipes* (Szépligeti, 1899)	Trabzon	Khalaim and Yurtcan, 2011
Genus *Tersilochus* (*Gonolochus*) Holmgren, 1859		
Tersilochus (*Gonolochus*) *caudatus* (Holmgren, 1860)	Edirne, Erzurum	Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Tersilochus (*Gonolochus*) *rugulosus* Horstmann, 1981	Afyon	Khalaim and Yurtcan, 2011
Tersilochus (*Gonolochus*) *nitens* Horstmann & Kolarov, 1988	Erzincan, Hatay, Osmaniye, Kastamonu	Gürbz et al., 2011; Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Genus *Tersilochus* (*Tersilochus*) Holmgren, 1859		
Tersilochus (*Tersilochus*) *cognatus* Holmgren, 1860	Edirne	Khalaim and Yurtcan, 2011
Tersilochus (*Tersilochus*) *jocator* Holmgren, 1859	Hatay	Gürbüz et al., 2011
Tersilochus (*Tersilochus*) *heterocerus* (Thomson, 1889)	Bursa	Khalaim and Yurtcan, 2011
Tersilochus (*Tersilochus*) *) obscurator* (Aubert, 1959)	Ankara, Edirne, Erzurum, Konya	Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a
Tersilochus (*Tersilochus*) *triangularis* (Gravenhorst, 1807)	Afyon, Edirne, Hatay	Gürbüz et al., 2011; Khalaim and Yurtcan, 2011
Tersilochus (*Tersilochus*) *tripartitus* (Brischke, 1880)	Afyon, Edirne, Erzurum	Khalaim and Yurtcan, 2011; Çoruh and Khalaim, 2012; Çoruh et al., 2014a

a

b

Figure 2. Distribution of species: a) according to number samples, b) according to number speceis

3.2. Ecological Data

Both a-biotic (temperature, humidity, light) and biotic (host, vegetative biodiversity, crowding and diets) stresses significantly influence the insects and their population dynamics (Khaliq et al., 2014). In this study, Tersilochine specimens were collected from different altitudes in collection areas.

The insects to be examined in this study were collected from an altitude range of 0-2700 m. As shown in Table 1, nineteen species have formed habitats in the range of 0-500 (A) m, two species in the range of 501-750 (B) m, only one species in the range of 751-1000 (C) m, six species in the range of 1001-1250 (D) m, 15 species in the range of 1251-1500 (E) m, three species in the range of 1501-1750 (F) m, eight species in the range of 1751-2000 (G) m and six species in the range of 2001-2700 (H) m (*Figure 3a*). Based on the findings, we can say that the range of 0-500 m is mainly preferred by tersilochines.

a   b

Figure 3. Distribution of species: a) according to altitude, b) according to months

Among them, twenty-nine species were collected from only one altitude. While *Aneuclis incidens* were collected from four different altitudes, *Barycnemis harpura, Probles* (*Microdiaparpis*) *anatolicus, Tersilochus* (*Gonolochus*) *caudatus, Tersilochus* (*Tersilochus*) *obscurator* and *Tersilochus* (*Tersilochus*) *tripartitus* were collected from three different altitudes.

An evaluation was also made about the months in which the tersiochines were collected. These species were generally collected from March to October (*Figure 3b*). However, in May and June, the population was denser. As seen in *Table 1*, twenty-five species were collected in a single month, *Aneuclis incidens* was collected in six different months in a year. Our findings have shown that *Aneuclis incidens* is highly adaptable to different altitudes and different climate conditions.

3.3. Zoogeographical Data

Türkiye has a rich biodiversity with over nine thousand natural and more than 30% endemic plant species in its geography (Teymuroğlu and Çoruh, 2022; Zeybek and Tozlu, 2022) and has seven geographical regions (*Figure 1*). In *Table 1*, the regions where the species are distributed are given in detail. As seen in *Table 1*, most of the samples were collected from the Mediterranean region (18), the Black Sea region, and the Eastern Anatolia region (13), and the least number of samples were collected from the Southeastern Anatolia region. The names of the provinces where the samples were collected are summarized in *Table 2* (*Figure 4a*).

a

b

Figure 4. Distribution of species: a) according to geographic regions, b) according to zoogeographical regions

When *Table 1* and *Table 2* are analyzed in terms of the regions where the samples were collected, it shows that; *Aneuclis incidens* was collected from six regions, *Diaparsis (Nanodiaparsis) aperta, Probles (Microdiaparpis) anatolicus*, *Tersilochus (Tersilochus) obscurator*, *Tersilochus (Tersilochus) triangularis* and *Tersilochus (Tersilochus) tripartitus* were collected from three regions. However, *Allophroides boops*, *Barycnemis alpina*, *Diaparsis (Diaparsis) carinifer*, *D. (D.) multiplicator*, *D. (D.) rara*, *D. (D.) temporalis*, *D. (Nanodiaparsis) frontella*, *Gelanes fusculus*, *G. simillimus*, *Heterocola (Heterocola) longipalpis*, *H. (H.) nigrotibialis*, *H. (Heterocoloides) inguaria*, *Phradis decrescens*, *P. minutus*, *P. morionellus*, *P. nigritulus*, *P. Rufiventris*, *Probles (Euporizon) exilis*, *P. (E.) rufipes*, *P. (Microdiaparpis) caudiculatus, P. (M.) microcephalus, P. (M.) neoversutus*, *P. (M.) versutus*, *P. (Probles) erythrostomus*, *P. (P.) flavipes, Tersilochus (Gonolochus) rugulosus, T. (T.) cognatus, T. (T.) jocator* and *T. (T.) heterocerus* were collected from one single region.

The distribution in the world regarding the areas of the insects was also analyzed and proportioned. According to this analysis, the West Palearctic has 95,2%, Europe has 88,0%, the East Palearctic has 46,20% and the Nearctic has 4,4%. The insects that were the subjects of our study were not found in the Afrotopical, Australian, Oceanic, and Neotropical regions (*Figure 4b*).

According to these results, Western Palaearctic and Europe have the highest numbers of species. Among these species, *D. (D.) carinifer* and *D. (D.) temporalis* were found to be distributed in four different regions. Also, the research has shown that only three of them were distributed in a single zoogeographical region. As a result, it became apparent that *H. (H.) longipalpis* is an endemic species for Türkiye at the moment. At the same time, *H. (H.) nigrotibialis* was found in Bulgaria and Türkiye, *T. (G.) rugulosus* was found in Italy and Türkiye, *T. (G.) nitens* was found in Bulgaria, and Türkiye, *T. (T.) cognatus* was found in Germany, Sweden, and Türkiye. These species are considered rare species for Türkiye.

3.4. Data of host by adult:

Barichneumon sp. Thomson, 1893 was obtained from *Sesamia nonagrioides* which feed that *Zea mays* in Adana (Bayram et al., 2007).

3.5. New data for East Anatolia:

Aneuclis incidens (Thomson, 1889)

Materal examined: Bingöl: Karlıova, 2013 m, 21.X.2022, Leg: M. Dalan; 1775 m, 11.XI.2022, Leg: M. Dalan.

Hosts: *Anobium fagi*, *Meligethes aeneus*, *Meligethes viridescens*.

This species is new for Bingöl (Karlıova) Province.

Figure 5. Distribution of species in Türkiye.

4. Conclusion

Terslochinae species were detected in 30 provinces in our country (*Figure 5*). Türkiye has 81 provinces. In other words, this subfamily was detected in 24.3% of our country. With this study, the number of provinces increased by one to 31. Bingöl was recorded as a new locality for this subfamily (*Figure 6*).

Figure 6. Distribution of species in new locality.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Çoruh, S., Dalan, M.; Data Collection or Processing: Dalan, M.; Literature Search: Dalan, M.; Writing, Review and Editing: Çoruh, S., Dalan, M.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

The Effect of Grass Juices on The Agricultural Properties of Legumes Grown in Different Root Media

Farklı Kök Ortamlarında Yetiştirilen Baklagillerin Tarımsal Özellikleri Üzerine Çimsularının Etkisi

Banu KADIOĞLU[1]*

Abstract

The consumption of grass juices obtained from cereals for health purposes is increasing day by day. For this purpose, research on different plant solutions has gained momentum. In our study, barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) grass juices grown in hydroponic conditions were used. Obtained grass juices were applied to pea and lupine seeds in 100% soil (control), 100% pumice, 100% perlite, 100% tea waste and 100% cocopeat substrate. Germination percentage, germination rate, average daily germination, peak value, germination value, root dry and fresh weight, stem dry and fresh weight, root and stem length, plant yield, grass yield, plant yield seed rate, macro and micro (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) mineral substance contents were investigated pea and lupine. Grass juices were obtained by mowing barley and oat seeds sown in $30 \times 50 \times 7$ cm plastic tubs in ten days and passing them through a juicer. Pea and lupine 25 seeds, which were placed in each pot, were grown in different root media (100% soil, 100% cocopeat, 100% pumice, 100% perlite, and 100% tea waste) under in vitro conditions. 150 ml of water (control), barley grass juice, oat grass juice and barley + oat grass juice were applied to the seeds according to their subjects. The experiment was carried out in 400 pots with 10 replications x 2 species x 4 treatments x 5 media, according to the factorial fully randomised design. As a result of the research, germination physiology (germination percentage, germination rate, average daily germination, peak value and germination value), mineral substance content (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn), growth and yield parameters at (root dry and wet weight, stem dry and wet weight, root and stem length, plant yield, grass yield, plant yield seed rate) barley grass juice application was found to be more effective than other applications after control. In all parameters examined in the research, it was determined that the five different medias used as growing media followed the order of soil>cocopeat>pumice>perlite>tea waste, and after the control and the best growing medium was cocopeat.

Keywords: Germination, *Hordeum vulgare* L. and *Avena sativa* L. juice, Hydroponic, Cocopeat, Perlite, Yield

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Öz

Sağlık amaçlı olarak tahıllardan elde edilen çim sularının tüketimi günümüzde giderek artmaktadır. Bu amaçla farklı bitki çözeltileri üzerine yapılan araştırmalar hız kazanmıştır. Yürüttüğümüz çalışmada hidroponik ortamda yetiştirilen arpa (*Hordeum vulgare* L.) ve yulaf (*Avena sativa* L.) çim suyunun, %100 toprak (kontrol), %100 pomza, %100 perlit, %100 çay atığı ve %100 hindistan cevizi torfu sustratlarında bezelye ve acıbakla'da çimlenme oranı, çimlenme hızı, ortalama günlük çimlenme, pik değeri, çimlenme değeri, kök kuru ve yaş ağırığı, gövde kuru ve yaş ağırlığı, kök ve gövde uzunluğu, bitki verimi, çim verimi, bitki verimi tohum oranı, makro ve mikro (N, P, K, Ca, Mg, Fe, Cu, Zn ve Mn) minerel madde içerikleri üzerine etkisi araştırılmıştır. Çim suları 30 x 50 x 7 cm boyutunda plastik küvetlere ekilen arpa ve yulaf tohumlarının on günde biçilerek katı meyve suyu sıkacağında geçirilmesi ile elde edilmiştir. Her saksıya 25 adet konan bezelye ve acıbakla tohumları invitro şartlarda farklı ortamlarda (%100 toprak, %100 cocopeat, %100 pomza, %100 perlit, ve %100 çay atığı) yetiştirilmiştir. Tohumlara konularına göre 150ml su (kontrol), arpa çim suyu, yulaf çim suyu ve arpa+yulaf çim suyu uygulanmıştır. Deneme 10 tekerrür x 2 tür x 4 uygulama x 5 ortam olacak şekilde 400 saksıda tesadüf parselleri faktöriyel deneme desenine göre yürütülmüştür. Araştırma sonucunda, çimlenme fizyolojisi (çimlenme oranı, çimlenme hızı, ortalama günlük çimlenme, pik değeri ve çimlenme değeri), mineral madde içerikleri (N, P, K, Ca, Mg, Fe, Cu, Zn ve Mn), büyüme ve verim parametrelerinde (kök kuru ve yaş ağırlığı, gövde kuru ve yaş ağırlığı, kök ve gövde uzunluğu, bitki verimi, çim verimi, bitki verimi tohum oranı) arpa çim suyu uygulaması kontrolden sonra diğer uygulamalara göre daha etkili bulunmuştur. Araştırmada incelenen tüm parametrelerde yetiştirme ortamı olarak kullanılan beş farklı ortamın toprak>cocopeat>pomza>perlit>çay atığı sıralamasını takip ettiği ve kontrolden sonra en iyi yetiştirme ortamının cocopeat ortamı olduğu belirlenmiştir.

Anahtar Kelimeler: Çimlenme, *Hordeum vulgare* L. ve *Avena sativa* L. suyu, Hidroponik, Cocopeat, Perlit, Verim

1. Introduction

Cereal grass, which has a positive effect on human health, has been used as food for years. Laboratory studies on cereal grasses that are beneficial to human health are also increasing (Yadav et al., 2013). Cereal grass is rich in antioxidants. Young leaves are also quite healthy. For this reason, it has recently received more attention as a natural medicine (Urbonaviciute et al., 2009). Barley contains β-glucon and high amount of protein. It can also be used as barley malt and grass juice (Altuner et al., 2022). Barley grass extracts, which are an important source of antioxidants, are useful in the treatment of many diseases such as obesity, diabetes, blood circulation system disorders, anemia, arthritis, high cholesterol levels, kidney diseases and cancer (Paulickova et al., 2007). At the same time, oat grass juice is very rich in amino acids, vitamins B1, B2, B6, and B12, minerals, vitamins, antioxidants (tricin), chlorophylls and enzymes (Rexhepi and Renata, 2015). In a study using oat plant extract doses, the effects of oil plant seeds on germination and seedling growth were investigated and it was stated that the germination time of oil plants seeds was prolonged with increasing oat plant extract doses, and increasing doses of oat plant extract had a negative effect on seedling growth (Ergin and Kaya, 2020).

Soilless culture is a form of cultivation with an average of 60 years of history. Experiments on soilless aquaculture started in the 1600s. Attempts were made to determine more plant growth substances and plant compositions. It is known that the Egyptians cultivated plants in water a few centuries before Christ. Soilless culture consists of water and substrate culture. The process of growing plants using mineral nutrient solutions in water is called hydroponic system. In the study, the germination process of barley and oat seeds was provided by hydroponic system. The green parts, which can reach 20-25 cm in length in approximately seven to eight days, intertwine and take on the appearance of a carpet (Karasahin, 2015a). In hydroponic environment, water requirement is low, disease and pest control is easy, efficiency is high and environmental pollution is less (Uyeda et al., 2011). Seed type, water quality, pH, irrigation time, plant nutrient, temperature, light intensity are the factors affecting yield in hydroponic system (Dung et al., 2010; Fazaeli et al., 2012). In substrate culture, plants are grown in organic, inorganic or synthetic media. Soil (control), pumice, perlite, tea waste and cocopeat substrates were used in the study. In the study, the effects of barley, oat and barley+oat grass water treatments on pea and lupine were investigated in five different media. Water was used as control application. In the study, it was aimed to determine effect the grass water application on the mineral content of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn, germination percentage, germination rate, average daily germination, peak value, germination value, root dry and wet weight, stem dry and fresh weight, root and stem length, plant yield, grass yield, plant yield seed rate.

2. Materials and Methods

The research was carried out in invitro conditions (25±1°C) in 2023. Grasses were obtained by sowing barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) seeds in 30 × 50 × 7 cm plastic tubs in a hydroponic system. Harvesting was done with scissors ten days later. Grass juices were obtained by passing the grass through a juice extractor (Akgun et al., 2018). The research, in which pea (*Pisum sativum* L.) and lupine (*Lupinus albus* L.) seeds were used, was carried out according to the factorial fully randomised experimental design, as 2 species × 4 applications × 5 media × 10 replications in a total of 400 pots. 5% NaClO solution was used to ensure the surface sterilization of the seeds. The seeds were sterilized for 10 minutes. Sterilized seeds; 25 seeds were planted in each pot (25 × 20) in pots containing soil (control), pumice, perlite, tea waste and cocopeat. 150 ml of water (control), barley grass juice (BGJ), oat grass juice (OGJ) and barley + oat grass juice (BGJ + OGJ) were applied to each pot by tidal method (Karasahin, 2015b). Germination times of different species were determined according to the principles stated in ISTA (ISTA, 2003). After the seeds were placed in the germination medium, they were checked every day and the number of germinated ones was determined. The plants harvested after 50 days were dried at room temperature and then dried in an oven at 70°C until they reached a constant weight. The dried samples were ground to an average thickness of 2 mm in a teflon blade grinder and made ready for analysis (Kacar and İnal, 2008). The total nitrogen content of the plant samples was determined by the "Mikrokjeldahl Method" after wet burning with a mixture of H_2SO_4 (AOAC, 1990). Macro and micro mineral substance contents (P, K, Ca, Mg, Fe, Cu, Zn, Mn) were determined with Perkin Elmer (Optima 2100) Model ICP – OES device after wet burning with a mixture of $HClO_4$ (AOAC, 1990). Plant yield (g/pot) obtain by weighing the harvested plant parts with precision scales, grass yield by weighing the grass harvested from 1 cm height from the root zone on precision scales (g/pot), and plant yield by proportioning the amount of seeds used in planting after weighing the harvested plant parts on precision scales, seed rate was determined (Karasahin, 2015b). At the end of the germination period of the seeds,

the roots and stems of the seedlings were cut with a razor blade from their junctions and their lengths were measured with the help of a millimetric ruler. Root and stem parts of plants harvested weighed on precision scales, root and stem fresh weights were determined. The root and stem dry weights of the dried plants were kept in the drying oven at 70°C until they reached a constant weight (Kadioglu 2020). In the study, germination percentage (%), germination rate (days), average daily germination (day), peak value (%), germination value (%), root dry and fresh weight (g/pot), stem dry and fresh weight (g/pot), root and stem length (cm), plant yield (g/pot) grass yield (g/pot) and plant yield seed rate (%), macro (%) and micro (ppm) mineral substance contents were investigated (Czabator, 1962; Ellis and Roberts, 1981; Matthews and Khajeh-Hosseini, 2007; Gairola et al., 2011; Akgun et al., 2018; Kadioglu, 2021). Differences between analysis of variance and mean LSD multiple comparison tests were performed in JMP 5.0.1 program.

Germination percentage (GP): n/Σn x 100   (Eq. 1)

n = Number of germinated seeds

Σn= Total number of seeds

Germination rate (GR): n1/t1 + n2/t2 + ⋯ …   (Eq. 2)

n1, n2, … number of germinated seeds t1, t2, …days

Mean daily germination (MDG): Total number of germinated seeds / total number of days   (Eq. 3)

Peak value (PV): Highest seed count/highest seeding day   (Eq. 4)

Germination value (GV): Average daily germination x peak value   (Eq. 5)

The average root/stem length was calculated as cm/plant by dividing the sum of root and stem lengths in a petri dish by the number of seeds.

3. Results and Discussion

The germination percentage, germination rate, average daily germination, peak value, germination value, root dry and wet weight, stem dry and wet weight, root and stem length, plant yield, grass yield, total yield, mineral content of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn of pea (*Pisum sativum* L.) and lupine (*Lupinus albus* L.) grown using soilless farming techniques in vitro were determined in the study. The parameters were analyzed according to the factorial fully randomised design. It was determined that the control took the highest values in all parameters examined in applications and substrates.

3.1. Germination parameters

Compared to the control, the treatments had a negative effect on the germination parameters. There are significant differences between species. Lupine is more sensitive to applications. Cocopeat medium has better values in germination parameters. Germination parameters (GP%, GR day, MDG day, PV% and GV%) in cocopeat media were 76.5%, 7.48 days, 3.93 days, 1.35% and 4.92%, respectively. Barley grass juice is more effective on GP%, GR day, MDG day, PV% and GV% parameters. Species × media, species × application, media × application and species × media × application interactions got the lowest values from lupine × tea waste, lupine × BGJ + OGJ, tea waste × BGJ + OGJ, and lupine × tea waste × BGJ + OGJ applications (*Figure 1*).

Column shows germination parameters (GP%, GR day, MDG day, PV%, GV%) and bar shows plants (%/day) and applications (%/day)

Figure 1. Germination parameters for both plants and applications

3.2. Growth parameters

Growth parameters including root and stem dry weight, root and stem fresh weight, root and stem length were investigated in our research in which we investigated the effect of grass juices on pea and lupine on different substrates. The examined parameters had the lowest values in lupine with 0.32 g plant, 2.73 g plant, 0.94 g plant, 6.90 g plant, 16.54 cm and 62.39 cm, respectively. The growth parameters are in the order soil (control) > cocopeat > pumice > perlite > tea waste. BGJ + OGJ had the lowest values in all the parameters examined in the applications. Control application and control environment gave the highest values in the growth parameters examined in the interactions. Pea were found to be more effective in interactions (*Table 1*).

3.3. Yield parameters

In the study, plant, grass and plant yield seed rate of pea and lupine were examined. Grass juice applications (GJA) have a negative effect on yield parameters. Grass juice applications were effective between the environment and the species. It was determined that plant, grass and plant yield seed rate of pea had the highest values with 102.13 g/pot, 61.20 g/pot and 40.93%, respectively. Plant, grass and plant yield seed rate in the environments were 99.92 g/pot, 59.83 g/pot, 39.5% in cocopeat substrate. It was determined that the control got the highest efficiency values (133 g/pot, 76.5 g/pot, 57%) in the applications. It was determined that the control application was followed by barley grass juice (100.7g/pot, 62.4 g/pot, 38.33%), oat grass juice (80 g/pot, 48.5 g/pot, 31.50%) and BGJ + OGJ (75.1 g/pot, 47.8 g/pot, 27.27%) applications, respectively. In the interactions, the lowest values were obtained by lupine × teawaste, lupine × BGJ + OGJ, lupine × BGJ + OGJ, lupine × tea waste × BGJ + OGJ (*Figure 2*).

Table 1. Effect of S ×M × A of interaction on the growth parameters

Species	Media	Application	Root dry weight (g plant)	Stem dry weight (g plant)	Root fresh weight (g plant)	Stem fresh weight (g plant)	Root length (cm)	Stem length (cm)
Pea	Soil	Control	1.36	3.47	2.04	13.0	28.42	84.44
		BGJ	0.41	3.38	1.06	9.47	23.60	74.33
		OGJ	0.36	3.04	1.51	8.07	20.41	70.89
		BGJ + OGJ	0.32	2.84	1.09	7.01	18.56	64.44
	Cocopeat	Control	1.17	3.13	1.38	13.0	26.59	84.44
		BGJ	0.23	3.00	1.04	9.46	22.66	74.11
		OGJ	0.22	2.92	0.91	7.83	20.33	69.67
		BGJ + OGJ	0.17	2.37	0.88	7.01	17.78	63.56
	Pumice	Control	0.81	3.04	1.31	11.4	25.20	80.67
		BGJ	0.21	2.96	0.91	9.46	22.31	73.56
		OGJ	0.16	2.68	0.81	7.61	20.24	65.33
		BGJ + OGJ	0.13	2.26	0.62	6.99	17.76	63.33
	Perlite	Control	0.68	3.02	1.05	11.3	25.20	80.67
		BGJ	0.15	2.69	0.72	9.09	20.83	73.11
		OGJ	0.13	2.26	0.55	7.48	20.24	65.22
		BGJ + OGJ	0.13	2.10	0.48	6.99	17.62	63.33
	Tea waste	Control	0.46	2.93	0.76	9.79	24.37	77.00
		BGJ	0.13	2.31	0.72	8.44	20.80	71.00
		OGJ	0.13	2.12	0.51	7.24	18.97	64.44
		BGJ + OGJ	0.12	2.10	0.47	6.97	17.59	61.89
Lupine	Soil	Control	1.28	3.38	1.86	6.94	17.19	61.11
		BGJ	0.38	3.37	1.67	6.64	16.48	55.00
		OGJ	0.36	3.00	1.15	5.42	15.81	52.22
		BGJ + OGJ	0.17	2.82	1.13	3.74	14.91	51.00
	Cocopeat	Control	0.81	3.01	1.46	6.94	16.93	59.44
		BGJ	0.36	3.00	1.02	6.61	16.32	55.00
		OGJ	0.20	2.96	1.00	4.83	15.70	52.00
		BGJ + OGJ	0.16	2.71	0.91	3.27	14.26	50.22
	Pumice	Control	0.81	2.96	1.31	6.87	16.93	59.22
		BGJ	0.22	2.96	1.00	6.45	16.18	53.22
		OGJ	0.16	2.71	1.00	4.52	15.64	51.56
		BGJ + OGJ	0.15	2.69	0.89	3.26	13.43	49.89
	Perlite	Control	0.56	2.71	1.02	6.84	16.81	58.78
		BGJ	0.21	2.71	0.81	6.31	16.03	52.78
		OGJ	0.16	2.49	0.72	4.40	15.52	51.33
		BGJ + OGJ	0.14	2.40	0.47	2.79	3.37	49.56
	Tea waste	Control	0.46	2.65	0.76	6.71	16.61	57.78
		BGJ	0.15	2.36	0.61	6.29	16.00	52.33
		OGJ	0.15	2.10	0.55	4.06	15.26	51.00
		BGJ + OGJ	0.13	2.07	0.32	2.00	2.88	49.44
		Species	***	ns	***	ns	***	ns
		Media	***	***	***	***	***	***
		Application	***	***	***	***	***	***
		S * M	ns	***	ns	***	***	***
		S * A	***	ns	***	ns	ns	***
		M * A	***	***	***	***	***	***
		S * M * A	***	***	***	***	ns	***

Statistically significant differences at ***p ≤ 0.001; ns: indicate the non-significant difference. GJA: Grass juice applications; Control: Water; BGJ: Barley grass juice; OGJ: oat grass juice; BGJ + OGJ: Barley + Oat grass juice

Column shows yield parameters (plant yield, grass yield g/pots and plant yield seed rate %) and bar shows plants (g/pots, %) and applications (g/pots, /%)

Figure 2. Yield parameters for both plants and applications

3.4. Mineral substance contents

The effects of grass juices on macro and micro mineral substances in pea and lupine seeds were investigated; Among the macro elements N (0.31%), P (0.43%), K (1.14%), Ca (0.60%) and Mg (0.49%), pea had the highest values. Substrates used in macro elements are listed as soil > cocopeat > pumice > perlite > tea waste. Barley grass juice application in N, P, K Ca and Mg elements took the values of 0.24%, 0.44%, 1.53%, 0.59% and 0.41%, respectively. In the macro elements species × media, species × application, media × application interactions, pea × cocopeat, pea × BGJ, cocopeat × BGJ applications gave the highest values (*Table 1*). Micro elements as well; Fe (559 ppm), Cu (7.39 ppm), Mn (105 ppm) and Zn (49.37 ppm) had the lowest values in lupine. Fe (521 ppm), Cu (7.05 ppm), Mn (129 ppm) and Zn (45 ppm) had the lowest values in the tea waste substrate. In applications, BGJ + OGJ had the lowest values as 255 ppm, 4.61 ppm, 64 ppm and 40 ppm, respectively. In the interactions of species × media, species × application, and media × application, pea × cocopeat, pea × BGJ, cocopeat × BGJ applications were more effective than other applications (*Figure 3*).

Column shows macro (a) and micro (b) element contents (%/ppm) and bar shows plants (%/ppm) and applications (%/ppm)

Figure 3. Macro (a) and micro (b) element contents for both plants and applications

Interest in grass waters prepared using different plant components; Grass juice variety is increasing day by day due to the fact that there is no side effects, it strengthens the immune system, and it can be used in diets. The consumption of plants for health purposes has increased in recent years. Cereals and cereal products are consumed for health purposes. For this purpose, more grass juices are consumed. There are studies on grass juices, which are very rich in plant nutrients and vitamins (Akgun et al., 2018). Water (control) application had the highest values in all parameters examined in our study, followed by BGJ, OGJ and BGJ + OGJ applications. At the same time, the order of soil (control) > cocopeat > pumice > perlite > tea waste was realized in terms of substrates. It has been determined that the GJAs differ in the substrates used. As a result of our study in which we applied BGJ, OGJ and BGJ+OGJ on pea (*Pisum sativum* L.) and lupine (*Lupinus albus* L.) on different substrates, the reason why the control application was more effective than the grass juice application may be the allelopathic effect. Allelopathy is defined as the inhibitory effect of one plant on another plant with its chemical secretions. Chemicals secreted by plants are also called allelochemicals (Rice, 1984; Khalid and Shad, 1991; Callaway, 2002). Allelochemicals that affect metabolic events can affect plant growth. This situation may vary depending on the type and density of the allelochemical substance (Jose and Gillespie, 1998). In allelopathy, one plant may have a direct or indirect detrimental effect on another plant (Zeng et al., 2008). It was stated that the extracts used in the study, which determined the allelopathic effects of different weed extracts, had a negative effect on seed germination in vegetables compared to the control (Kadioglu et al., 2005). In another study, it was reported that extracts obtained

from the leaves and roots of *Chromolaena odorata* (christmas bush) (0.1% and 10%) adversely affect the seed germination and seedling growth of some herbaceous plants (Hu and Zhang, 2013). In the study, different doses of oat grass juice and oat grass juice (25%, 50%, 75% and 100%) were applied to leguminous seeds such as oats, barley, beans, wheat, and lentils; germination rate, germination index, average germination time, shoot and root length were examined and it was stated that the effects of oat grass juice application and grass water doses on germination and seedling development were negative (Karaman et al., 2021). In another study, wheat grass juice applied barley (*Hordeum vulgare*), corn (*Zea mays*), wheat (*Triticum aestivum*), bean (*Phaseolus vulgaris*), clover (*Medicago sativa*), perennial grass (*Lolium perenne*), sheep's ball (*Festuca ovina*) and sugar beet (*Beta vulgaris*) seeds, germination index, germination rate and root and stem length were examined and it was stated that wheat grass juice reduced the investigated parameters (Akgun et al., 2018). In our study, average germination parameters in pea (*Pisum sativum* L.) were determined as GP: 63.26%, GR: 6.14 day, MDG: 3.26 day, PV: 1.33%, GV: 4.29%. Root dry, stem dry, root wet, stem wet, root length and stem length parameters were 0.36 g/plant, 2.83 g/plant, 0.90 g/plant, 7.68 g/plant, 18.09 cm and 65.61 cm in barley grass juice application, respectively. It has been determined that the results of our research are in parallel with the other studies, and that barley, oat and BGJ + OGJ applications have a negative effect on germination physiology and plant growth compared to the control. Grain juices contain substances such as alkaloids, saponins, and gums (Ashok, 2011). Since cereal grass juices contain these substances, it is thought to have an allelopathic effect on the germination and development of other plant species. The allelopathic effect can affect plant growth by affecting metabolic events such as photosynthesis, respiration and ion uptake mechanisms in plants (Jose and Gillespie, 1998; Terzi, 2007). In the study in which the effects of different doses of oat plant extract on the germination and seedling development of oil plants seeds were determined, it was determined that the germination time of oil plants seeds was prolonged with increasing oat plant extract doses, and increasing doses of oat plant extract had a negative effect on seedling growth (Ergin and Kaya, 2020). It has been stated by the researchers that increasing doses of oat plant extract inhibit the growth of wheat and oat seedlings. Hydroxamic acid, scopoletin, L-tryptophan substances cause allelopathic effects in oat plant (Gürsoy et al., 2013). In a different study, it was reported that the extract obtained from the leaves of salvia and celery plant had a negative effect on the germination and seedling growth of lentil seeds and inhibited root development (Stratu et al., 2012). Macro and micro plant nutrients affect plant growth by changing plant morphology, anatomy and chemical composition. At the same time, they affect the resistance/tolerance of plants against diseases and pests positively or negatively (Yıldız, 2012), making them more beneficial for human health. Plants have a limited ability to selectively take in essential plant nutrients for their growth and development. In various studies using plants or plant parts (herbs, roots, fruits, etc.), it has been reported that plant extracts have a large amount of antioxidant compounds and their use reduces or encourages plant growth (Lin, 2004; Joseph et al., 2007; Pan et al., 2009). In our study, it was determined that the GJA application for macro and micro plant nutrients got the high values after the control, while the control application gave the highest values for the plant, grass and total yield parameters, followed by the BGJ > OGJ > BGJ + OGJ interaction. The data obtained in our study show parallelism with the results of other studies. In a study examining the effect of wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.) grass juices on the yield and nutrient content of some medicinal and aromatic plants in soilless medium, it was determined that grass water applications had a negative effect on yield and plant nutrient content (Kadıoğlu, 2022). In another study conducted in hydroponic environment, the yield and nutritional values of barley, wheat and corn grass juice were examined, plant height and root length were examined and it was determined that the highest root length belonged to the corn plant (Karaşahin, 2015a). In another study, the effect of plant extracts on the germination and seedling growth of pepper was examined and it was stated that the inhibition effect and ratio of plant extracts changed depending on the species and doses (Özbay, 2018). The alleopathic effect varies from plant to plant. In our study, it was determined that the response of plant species to grass waters was different, and pea species were more resistant to grass water applications in all parameters examined.

4. Conclusions

As a result, seed germination physiology, plant growth parameters, mineral substance contents and yield of pea (*Pisum sativum* L.) and lupine (*Lupinus albus* L.) grown in soil, perlite, pumice, tea waste and cocopeat substrates of barley and oat grass juices obtained by hydroponic system. It was determined that except control medium, the other growing media had a negative effect on the parameters. Pea (*Pisum sativum* L.) germination percentage, germination rate, average daily germination, peak value, germination value, root dry and fresh weight, stem dry

and fresh weight, root and stem length, plant yield, grass yield, plant yield seed rate, N, P, K, Ca, Mg, Fe, Cu, Zn and Mn values were found to be better than lupine (*Lupinus albus* L.) values. It was determined that GJA application and cocopeat substrate gave better results than other applications and substrates in the examined parameters. In addition, it was determined that the interaction of barley grass juice × pea × cocopeat was determined to be more effective in general, and the allelopathic effect different between plant species. Based on the data obtained from the results of the conducted study; It is suggested that barley grass water application can be applied in cocopeat medium to improve the agricultural characteristics of pea.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

There is no conflict of interest.

Authorship Contribution Statement

Concept; Design; Data Collection or Processing; Statistical Analyses; Literature Search; Writing, Review and Editing: BK.

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ARAŞTIRMA MAKALESİ   RESEARCH ARTICLE

Contribution of Sweetpotato Production to Economic Empowerment of Farming Households in Niger-Delta Area of Nigeria

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Abstract

Many farming households in Nigeria are deprived, and poor, hence, the need for economic empowerment intervention. Sweetpotato production, due to its short cycle and low input requirement could be projected on a larger scale for farming households' economic empowerment. However, data regarding ability of sweetpotato farming for farming households' economic empowerment is scarce. Therefore, farming households' sweetpotato production and economic empowerment in Nigeria's Niger-Delta Area was investigated. 364 sweetpotato farming households were chosen through a five-stage sample process. Descriptive statistics, Pearson Product Moment Correlation, T-test, and multiple regression were used to analyze the data that interview schedule was used to collect. Most respondents were female and aged 42.7±11.9 years. Farm size; farming experience; and income from sweetpotato, other crops, and non-crop livelihood activities were 2.6±3.4 hectares; 17.2±12.4 years; and ₦1,327,723.0±1,639,414.0 yearly, ₦2,385,815.0±2,615,273.0 yearly, and ₦1,828,004.0±2,336,270.0 yearly, respectively. The sweetpotato production and economic empowerment were low and high amongst farming households, respectively. Age, farm size and income from sweet potato were significantly related to economic empowerment. The economic empowerment status of high and low sweetpotato producers differed significantly. Between farming households with low and high income from sweetpotato production, their economic empowerment status significantly differed. Farming households' economic empowerment was mostly predicted by farming experience; and income from sweetpotato, and non-crop livelihood activities. Sweetpotato production influenced farming households' ability to prosper economically. Agricultural policies oriented towards improving economic empowerment should be promoted to emphasize increased sweetpotato production.

Keywords: Empowerment status, Rural farming households, Sweetpotato farmers, Empowerment intervention, Potential of sweet potato farming

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1. Introduction

Sweetpotato (SP) is a dicotyledonous plant and a storage root crop with herbaceous tuber. It is among the most significant food crops globally, as evident in its rank seventh place in terms of importance globally, after rice, wheat, maize, potato, barley and cassava (Odebode et al., 2021). Nigeria is the second leading SP producer globally (Mwanja et al., 2017). The country accounts for 3.7% of world's sweetpotato production-SPP with an approximately annual production of 3.46 - 3.92 million metric tonnes (FAOSTAT, 2017). In Nigeria, SP tuberous roots, shoots, and leaves are incredibly beneficial for both livestock and people. Thus, producing it is a feasible economic activity for increase food security, the alleviation of poverty, and income generation in rural households.

Sweetpotato is a crop with a lot of nutritional and therapeutic benefits, making it comparable with an extensive range of vegetables and fruits. The crop is a highly nutritious food crop, due to its ability to produce six classes of foods such as vitamins, minerals, fibre, fats, protein and carbohydrates (Oyibo, 2021). This explains why its leaves, shoots and storage roots are valuable sources of oil, vitamins, proteins, low crude fibre, carotene, calcium, iron, sodium, ascorbic acid, dietary insoluble fibre which helps to prevent constipation, lysine and soluble fibre that lowers cholesterol (Abdulkarim and Yunana, 2015). Apart from nutritional value, SP has other economic benefit. The importance of SP is not only seen in human nutrition but also serves in income generation, thus, it has the potential to be a key crop for a living. According to Adeyonu et al. (2019), the crop is notably among the cash and food crops of the crop sub-sector's that contributed 20% to the Nigeria's GDP in 2014. The foregoing suggests the economic role of SP to farming households (FHs) and at national level in emerging and/or developing nations. Therefore, farmers can experience economic empowerment (EE) as a result of producing SP for income generating and nutritional benefits.

Banmeke (2003) viewed EE as the process of enabling a group of underprivileged or socially-marginalised individuals to improve their physical, economic and educational situations, which inevitably results in an improvement in their livelihoods. It entails expanding one's ability to make strategic life choices, especially in the contexts in which this ability had been denied to the individual (Sraboni et al., 2014).

In Nigeria, including Niger-Delta area, rural FHs are responsible for 80% of food production (Akaakohol and Aye, 2014). However, rural households are characterised by food insecurity, limited access to capital and poor income, among others (Adebosin et al., 2013). These deplorable situations of rural FHs call for EE measures in order to improve their social and economic conditions. Several interventions-projects, policies, and programmes-have been made at the national level to economically empower rural households so as to improve their economic and social conditions. The interventions, among others, include the Poverty Alleviation Programmes (PAP), Family Economic Advancement Programme (FEAP), and Nigeria EE and Development Strategy (NEEDS). Despite these interventions by the government, rural households still remain in poverty.

Sweetpotato production (SPP) has the potential of being widely utilised for the EE of Nigerian farmers. Ogundele et al. (2009) identified it as a viable alternative to ensuring empowerment of farmers. This, according to Olapade and Ogunade (2014), is because SP is a crop with low input requirement. Earlier study revealed that it shows no marked seasonality and has the ability to produce reasonable yields in agro-ecological zones where other crops cannot thrive and/or would fail (Olapade and Ogunade, 2014). In spite of these desirable attributes of SP for EE, the crop in Nigeria's Niger-Delta has not gained the desired attention among cultivators/growers. Different nations around the world have employed crops with comparable attributes as EE means. For instance, in The Philippines, Myanmar, Indonesia and Thailand, the use of the production of rice as an intervention has given rice growers empowerment. The intervention in these countries, according to Akter et al. (2017), resulted in better access to income, enhanced ownership of productive resources and increased access to such resources. This indicates that farmers in Nigeria's Niger-Delta can receive EE if SP is given the appropriate policy support. However, there is dearth of information establishing a link between SPP and FHs EE.

The study's objective was to assess the contribution of SPP to FHs' EE. The specific objectives were to: describe the SP FHs demographic traits; establish the SPP level; ascertain how much income earned from SPP, other crops production, and non-crop livelihood activities by SP farmers; and establish the EE level of farmers among SP FHs. The following hypotheses stated in the null forms were tested based on the study's objectives: few chosen demographic traits have no discernible correlation with the EE level of SP FHs; income (SP, other crops,

and non-crop livelihood activities income) has no significant relationship with level of EE of SP FHs; the FHs with high and low SPP do not substantially differ in their EE status; the EE status of FHs with low and high income from SPP is not significantly different; and few chosen independent variables have no discernible contribution on SP FHs' EE status.

2. Materials and Methods

The investigation was conducted in Nigeria's Niger-Delta. The area comprises nine coastal southern Nigerian states. These are Edo, Delta, Bayelsa, Rivers, Akwa Ibom, Cross Rivers, Ondo, Imo and Abia (United Nation Development Programme- UNDP, 2006). The research area is situated between longitudes 6° 00[1] and 6° 28[1] east and latitudes 4°50[1] and 5°19[1] north of the Greenwich Meridian and equator, respectively.

All farmers producing SP in the Nigerian states of the Niger-Delta area consisted of the study's population. The study's respondents were chosen through a sampling procedure done in a multi-phase. Three out of the nine states were selected at random using a simple random sample technique to represent 35% of the states in the Niger-Delta area. These were Bayelsa, Delta and Edo. There are three agricultural development programmes-ADPs zones in each State. From the nine ADP zones, altogether, five zones were purposefully sampled because of prevalence of SPP. These include Edo-North from Edo State, Yenagoa and Sagbama from Bayelsa State, and Delta-South and Delta-Central from Delta State. The blocks in each of the zone that were selected were divided into SP and non-SP producing blocks, using stratified sampling. The blocks producing SP in Delta-South and Delta-Central zones were four and five, respectively; seven and three in Yenagoa and Sagbama zones, respectively; and six in Edo-North zone. In each zone that was chosen, 40% of the blocks that produced SP were randomly sampled using the proportionate sampling technique. The cells that are known for SP production in each of the selected block were identified. Altogether, in the selected blocks, 52 cells were identified. Using proportionate technique of sampling, 25% cells producing SP in each selected block were randomly sampled, resulting in 11 cells producing SP. Using the proportionate technique of sampling, from the SP FHs list in the selected cells, 20% SP FHs on the list of each designated cells were picked randomly for study. The overall randomly picked SP FHs was three hundred and sixty-four (364). Thus, the study's sample size was three hundred and sixty-four (364) respondents. In each selected household, interview with farmers overseeing SPP were conducted.

Primary data through the use of interview schedule were obtained. Utilised interview schedule captured demographics, income from SP and other sources, EE level, and level of SPP information.

The EE of SP FHs is the study's dependent variable. The empowerment Condition Index of Rashid et al. (2016) and the EE scale of Banmeke (2003) were modified to measure EE. Respondents were asked to indicate the extent to which economic changes have occurred. This was assessed by presenting a 17 probable economic changes list to respondents, which included, among other things, increased income, increased food production and enhanced payment of school fees. The response options provided to respondents were None at All (NA), Low Level (LL), Average Level (AL), and High Level (HL), with corresponding scores of 0, 1, 2, and 3 given. Scores per each respondent were summed. The obtained minimum and maximum scores were 17.0 and 51.0, respectively. Index of EE was generated by adding all responses and the mean index was computed. Respondents were categorized into high or low EE using the mean index (37.54 ± 7.40) as benchmark, high EE (37.54-51.00) and low EE (17.00–37.53).

Additional crucial study variables included SPP and income earned from SPP.SPP was measured at ratio level by asking respondents to state the amount of SP produced for the farming years 2016/2017, 2017/2018 and 2018/2019, expressed in Kg. Respondents provided estimations for crop output. Odebode et al. (2021) used the estimated value from rural farmers' memory approach to measure crop output. Farmers' memories' estimated variables were measured and operationalized. The amount of SP produced was calculated and operationalized as follow: an estimated 40kg bag output per plot planted. The given estimated amount was translated to bags made from the entire farmland that was planted, and this was then translated to tonnes (using a 40kg bag of SP equals 0.04 tonnes of SP). The average SP output for the three farming years was computed. 2.53 tonnes was the obtained minimum score, and 872.95 tonnes was the maximum. An SPP index was computed using the sum of all the responses. Respondents were categorised using the mean (53.48 tonnes) into, low production (2.53 – 53.47 tonnes) and high production (53.48 – 872.95 tonnes). Interval level was utilised in obtaining/measuring income from SPP by the respondents being asked to indicate from options, the real amount in Naira value realised from SP per period;

daily, weekly, monthly or annually. Information on daily, weekly and monthly income were converted to annual income. 55.500 naira was the obtained minimum score, and 21.823.667 naira was the maximum. An index of income from SP was calculated by adding all the responses. Respondents were categorised using the mean (1.327.724 naira) into: low income from SP, between minimum score and score a little beneath the mean (55.500-1.327.723 naira) and high income from SP, between mean score and highest score (1.327.724-21.823.667 naira).

Standard deviation, mean, percentages, and frequency counts; and independent samples t-test, multiple linear regression, spearman rho, Pearson Product Moment Correlation-PPMC, and Chi-square were the descriptive and inferential statistics used to analysed the data, respectively. To ascertain EE significant causes, multiple linear regression method was utilised. The utilised model is stated as follow:

$$Y = a + b_1 X_1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots + b_n X_n + e \tag{Eq. 1}$$

Where: e = Error term; X_1, X_2……..X_n = regression parameters; b_1, b_2……b_n = regression coefficients; a = constant term or intercept; and Y = EE scores (dependent variable)

The model included the following regression parameters: X_1 = Size of farming household (exact number of people living in the home), X_2 = SP farm size, X_3 = SP farming experience, X_4 = SPP, X_5 = Income from SP, X_6 = Income from other crops, X_7 = Income from non-crop livelihood activities

3. Results and Discussion

3.1. Respondents demographic characteristics

The respondents' average age was 43 ± 12 years (*Table 1*). This suggests that most of the respondents were in their active ages and middle aged, hence, still possess stamina and/or strength to fulfill the labour demands of SPP. This result is consistent with the findings of Ahmed et al. (2014) that above 55.0% of Kano State SP farmers were in their active years and middle aged. The result aligns also with Oyibo (2020) findings, which indicated that more (44.2%) of Delta State rural farmers were middle-aged, with an average age of 46 ± 9 years old. Furthermore, the result supports Akouegnonhou and Demirbaş (2023) findings that most farmers in rural areas were between 41 – 60 years old. *Table 1* results show that female made up the majority of respondents (57.8%). This suggests that in the studied area, more female than male took part in SPP. The finding is consistent with Mmasa (2014) results that female were greater participants in SPP, compared to male in Mwanza, Coast and Shinyanga Regions of Tanzania.

9 ± 5 persons was obtained as the average size of household as depicts in *Table 1*. This suggests that the study area's SP farmers had large household. The finding agrees with big family size of SP FHs, as reported by Abdulkarim and Yunana (2015). Also, this is consistent with Falola et al. (2022) who found that amongst Nigeria's Ekiti State rural farmers producing pigeon pea, the mean size of family was nine persons. Furthermore, household size has implication for production of crop such as SPP and consequently for the FHs EE. Larger household could afford the FHs more SPP with regard to having more household members (as agriculture labour input) produce more output and earning more money for the household with the consequent favourable impact on the household EE. Earlier study by Ashimolowo (2005) found that household empowerment increased with household size.

Table 1 results show that the SPP average farm size was 2.6 ± 3.4 ha. It follows that the study area's SP farmers were majorly smallholder farmers. The finding corroborates Abdulkarim and Yunana's (2015) findings that farmers farm SP on a limited scale. Furthermore, the distribution of SP farm sizes among the respondents is probably going to impact EE, as small holders are likely to have low agricultural production status and/or output of cultivated SP, which reduce or decrease their SPP annual revenue, consequently, may result in less EE. Kurniati (2015) in a research carried out in Indonesia reported that the welfare/ empowerment (wealth) position of farmers increases with their land size. The average years of farm experience in SP cultivation was 17 ± 12 (*Table 1*). It can be inferred that respondents had relatively high number of years of experience farming SP. Hence, respondents were highly experienced in SP cultivation. The result corroborates Abdulkarim and Yunana's (2015) report that over half (60.0%) of farmers growing SP were involved in agricultural production for more than 10 years. The years of experience in farming SP might probably influence EE. Respondents with high number of years of SP farming experience are likely to have high EE, as they are more likely to be efficient in transforming their outputs into income, hence may have high EE. Carr et al. (2015) posits that years of agricultural involvement (years in farming) plays a role in both empowerment and disempowerment.

Table 1. Distribution of respondents' demographic characteristics

Variables	Frequency	%	Mean ± SD
Age (years)			
≤ 20	2	0.5	
21 – 30	61	16.8	
31 – 40	122	33.5	42.7 ± 11.9
41 – 50	95	26.1	
> 50	84	23.1	
Sex			
Male	154	42.3	
Female	210	57.7	
Size of household/family (persons)			
1 – 5	110	30.2	
6 – 10	184	50.5	9 ± 5
> 10	70	19.2	
Size of SP farm (ha)			
≤ 1	120	33.0	
1.01 – 2.00	113	31.0	2.6 ± 3.4
> 2	131	36.0	
SP farming experience (yrs.)			
1 – 10	155	42.6	
11 – 20	112	30.8	17.2 ± 12.4
> 20	97	26.7	

Note: yrs. = years, % = Percentage, ha = hectare, SD = Standard deviation

3.2. Respondents SPP income

The annual mean SP farming income was ₦1.327.723.0 ± 1.639.414.0, according to *Table 2*. The yearly mean income from SPPs is reasonably high and appreciable when compared with what is obtainable among other FHs within Nigeria. Idris-Adeniyi (2021) found the yearly average income from indigenous vegetables production of farm families in Nigeria to be ₦551,787.0. The implication of the fairly high SP income is that producing SP is a reliable and/or good way for rural households to source income. This result corroborates Oyibo and Odebode (2023) who observed that SP income was high with a yearly average income of ₦2.637.552.0 ± 3.362.512.0.

The respondents' highly realised SPP income suggests that they would likely have high EE as a result of producing SP, as high income is inclined to influence individuals' ability/capabilities to take control over various economic aspects of life situations. This agrees with Qurra-tul-ain et al. (2016) assertion that empowerment rises with income.

Table 2. Respondents' distribution according to SPP income

Variable	Frequency	%	Mean ± SD
Annum income from SP (₦)			
≤ 800,000.0	177	48.6	
800,000.1 – 1,600,000.0	88	24.2	1,327,724.0 ± 1,639,414.0
> 1,600,000.0	99	27.2	

Note: % = Percentage, ₦ = Naira, SD = Standard deviation,

3.3. Respondents' categorization based on their SP income

Majority of respondents (69.5%) had low SP income (*Table 3*). It can be inferred that in the studied area there was low level of SP income. Low SP income might negatively influence EE; low SP income will influence individuals' ability/capabilities to take control over various economic aspects of life situations and impede high EE.

Table 3. Categorization of respondents' according to their SP income

SP income	Freq	%	Minimum	Maximum	Mean	SD
Low	253	69.5	₦55,500.0	₦21,823,667.0	₦1,327,724.0	₦1,639,414.0
High	111	30.5				

Note:SP = Freq. = Frequency, % = Percentage, ₦ = Naira, SD = Standard deviation

3.4. Income/earnings from additional sources

Table 4 result shows that, out of the 97.0% of respondents that had other crops production income, the respondents realized an annual mean income of ₦2.385.815.0 ± 2.615.273.0. The annual mean income from producing other crops is notably high and appreciable when compared with what is obtainable among other FHs

within Nigeria. Idris-Adeniyi (2021) found the yearly mean crop income of farm families in Nigeria to be ₦1.199.394.0. The inference of the fairly high other cultivated crops income is that non-SP crop enterprises were reliable income sources for the rural households.

Table 4 also shows that the respondents' non-crop livelihood activities average annual income was ₦1.828.004.0 ± 2.336.270.0. The results suggest that respondents' non-crop livelihood activities earnings were lower than from the production of other crops. In addition, 61.5% respondents did not engage in any non-crop income pursuits. This suggests that crop enterprises were the only source of income for the majority of SP farmers. Also, this implies that most respondents may not have the capacity to supplement their households' income with respect to non-crop livelihood activities income.

Table 4. Respondents' Distribution according to other sources income

Variables	Frequency	%	Mean ± SD
Annum income from other sources (₦)			
Non-SP crop			
None	11	3.0	
≤ 800,000.0	80	22.0	2,385,815.0 ± 2,615,273.0
800,000.1 – 1,600,000.0	91	25.0	
> 1,600,000.0	182	50.0	
Non-crop livelihood activities			
None	224	61.5	
≤ 800,000.0	63	17.3	1,828,004.0 ± 2,336,270.0
800,000.1 – 1,600,000.0	29	8.0	
> 1,600,000.0	48	13.2	

Note:% = Percentage, SD = Standard deviation, ₦ = Naira

3.5. Respondents SPP

The data on farmed SP output of the respondents, as shown in *Table 5*, indicates that the average SP output in tonnes per year was 53.5 ± 78.1. The findings pointed to low output of SP; modest-scale SPP. The inference is that in the research area the producers of SP were generally small-holders. Furthermore, the SP output will probably influence EE status. Respondents with lower SP output are likely to be less economically empowered, as lower output often tends to result in lower income. This supports a study by Wouterse (2016), which discovered that lower quantity of agricultural output resulted in less empowerment status due to lower income.

Table 5. Respondents' distribution based on their SP output

SP output (tonnes annum^{-1})	Frequency	Percentage	Mean ± Standard deviation
1 – 10	27	7.4	
11 – 20	89	24.5	53.5 ± 78.1
> 20	248	68.2	

3.6. Respondents categorization according to SPP

The majority of respondents (69.5%) had low SPP, as seen in *Table 6*. It can be inferred that the studied area had low level of SP production. Low SP production might negatively influence EE; low SP production will influence SP income and impede high EE. In variance to this finding, Nwanebo (2012) found medium/moderate SP production.

Table 6. Categorization according to SPP of respondents

SPP (tonnes annum^{-1})	Frequency	Percentage	Minimum	Maximum	Mean	SD
Low (2.53 – 53.47)	253	69.5	2.53	872.95	53.48	78.070
High (53.48 – 872.95)	111	30.5				

Note: SD = Standard deviation

3.7. Economic Empowerment

The results of respondents' extent of economic change in relation to their various life situations are shown in *Table 7*.

3.7.1. Change in food production

Over half of the responders (58.2%) agreed they had high level of increase in food and livestock production, as revealed in *Table 7*. Increase in food and livestock production ranked sixth (2.42 ± 0.77) amongst all the

economic changes. Result for this variable suggests that FHs producing SP have a fair increase in food and livestock production. This could be because earnings from SPP allowed them to attend to their food and livestock production needs. The finding aligns with Banmeke (2003) research on EE of rural farmers, which found that 71.1% of the farmers have increased food and livestock production. Increase in food and livestock production is very important to the EE of FHs. SPP earnings can help FHs in dealing with agricultural production, particularly food and livestock production, related cost.

3.7.2. Change in farming and other forms of business

Result in *Table 7* shows that more than half of responders (57.4%) agreed they had high level of more farm-labour to work with. More farm-labour to work with ranked fifth on the economic changes experienced list with an average score of 2.45 ± 0.73. The finding is a good description of the EE condition of SP farming homes. The results for more farm-labour to work with suggest that the majority of SP FHs re-invested the income they got from SP sales in farm-labour.

3.7.3. Access to capital

Table 7 reveals that a bit below the average (48.1%) of respondents agreed they had high level increased profit, and 33.2% had average level increased profit. Increased profit ranked tenth on the seventeen economic changes list with mean of 2.12 ± 0.76. The result implies a fairly profit increase, which positively influence access to (availability of) capital for production function in agriculture.

3.7.4. Access of credit for households farming

Credit worthiness (2.35 ± 0.81) ranked eighth amongst listed items, according to *Table 7* results. Over half of responders (52.7%) agreed they had high level of credit worthiness. This finding is not unexpected given the trend shown in respondents' average annual SPP income (*Table 2*). It was revealed earlier that respondents' average earned yearly SPP income was ₦1.327.723.0 ± 1.639.414.0. This would lead to credit worthiness.

3.7.5. Access to education by members of FHs

Two key EE obligations usually serve as challenges to most FHs, they are; payment of school fees and prompt purchase of educational materials. In *Table 7*, results reveal that improved/prompt purchase of educational materials (2.52 ± 0.67) ranked second on the economic changes list, with the majority (61.3%) of respondents agreeing they had high improvement in purchase of educational materials. The results suggest that a greater proportion of respondents possessed the financial capabilities to meet up the purchase of educational material. The inference is that many of SP FHs had no issues with meeting their children's educational requirements. According to Ogunbameru and Idrisa (2013), farmers utilized the money they earned from crop production to sponsor their children education either at secondary school level or tertiary education.

3.7.6. Access to healthcare

This aspect of the FHs EE is very important as it affects their life and work. A farmer who is not mentally and physically fit and does not have money or finance to pay for prescribed drugs will not be productive on the farm and in life (Samuel, 2020). The results in *Table 7* reveal that 66.5% of the respondents agreed that they had high level of improved payment for prescribed drugs. Improved/prompt payment for prescribed drugs (2.56 ± 0.69) ranked highest amongst all the economic changes. These results agreed with Ogunbameru and Idrisa (2013), who, in a study on empowerment of rural farmers, found that 94.4% of farmers agreed to have used a portion of income they got from crop production to attend to their family needs; the routine family problem included paying hospital bills of household members. The inference is that SPP earning can help FHs in dealing with healthcare related costs.

Table7. Respondents EE distribution

Categories	High Level	Average Level	Low Level	Not at All	Mean	Rank
Change in food production					**4.5**	
Increase in agricultural production	150 (41.2)	129 (35.4)	39 (10.7)	46 (12.6)	2.1±1.0	11
Increased in food and livestock production	212 (58.2)	100 (27.5)	46 (12.6)	6 (1.6)	2.4±0.8	6
Change in farming and other forms of business					**11.0**	
More land to cultivate (expansion of available land)	207 (56.9)	113 (31.0)	33 (9.1)	11 (3.0)	2.4±0.8	7
More farm-labour to work with	209 (57.4)	112 (30.8)	39 (10.7)	4 (1.1)	2.5±0.7	5
Increase in farm implements	163 (44.8)	154 (42.3)	34 (9.3)	13 (3.6)	2.3±0.8	9
Engaged in more income generating activities	125 (34.3)	130 (35.7)	54 (14.8)	55 (15.1)	1.9±1.0	15
Increased income generating activities	115 (31.5)	156 (42.9)	51 (14.0)	42 (11.5)	2.0±1.0	14
Access to capital					**6.1**	
Increased savings	128 (35.2)	125 (34.3)	84 (23.1)	27 (7.4)	2.0±0.9	13
Increased profit	121 (33.2)	175 (48.1)	60 (16.5)	8 (2.2)	2.1±0.8	10
Increased income	100 (27.5)	174 (47.8)	80 (22.0)	10 (2.7)	2.0±0.8	12
Access to credit for households farming					**5.9**	
Obtain loans more easily	98 (26.9)	116 (31.9)	103 (28.3)	47 (12.9)	1.7±1.0	17
Increased access to fund/credit	104 (28.6)	132 (36.2)	99 (27.2)	29 (8.0)	1.9±0.9	16
More credit worthiness	192 (52.7)	121 (33.2)	37 (10.2)	14 (3.8)	2.4±0.8	8
Access to education by member of households					**5.0**	
Prompt/improved payment of school fees	215 (59.1)	116 (31.9)	31 (8.5)	2 (0.5)	2.5±0.7	3
Improved/prompt purchase of educational materials (exercise books, text books etc.)	223 (61.3)	107 (29.4)	33 (9.1)	1 (0.3)	2.5±0.7	2
Access to healthcare					**5.0**	
Improved/prompt payment for prescribe drugs	242 (66.5)	89 (24.5)	29 (8.0)	4 (1.1)	2.6±0.7	1
Improved/prompt payment of medical/treatment bills	220 (60.4)	103 (28.3)	35 (9.6)	6 (1.6)	2.5±0.7	4

Note: Values enclosed in parentheses represent percentage scores.

3.8. Respondents EE categorization

Table 8 reveals that high EE was present in 51.6% respondents. This suggests many or more SP FHs were relatively high in their EE status. It can be inferred that producers of SP possessed relatively highly economically empowered. This demonstrates that FHs in Nigeria's Niger-Delta have been substantially and appreciably economically empowered through producing SP. This suggests respondents' relatively high or adequate economic ability or capacity to take control of economic aspect of their life situations. The findings correlate with Wouterse (2016) who found a relatively high EE among Niger Republic rural households.

Table 8. Respondents' categorization according to EE

EE	Freq.	%	Minimum	Maximum	Mean	SD
Low (17.00 – 37.53)	176	48.4	17.00	51.00	37.54	7.40
High (37.54 – 51.00)	188	51.6				

Note:Freq. = Frequency, % = Percentage, SD = Standard deviation

3.9. Relationship between a few demographic traits and SP FHs' EE status

The results presented in *Table 9* show that respondents SP FS (r=0.20) was significantly (*p*<0.05) correlated with EE. This suggests SP producers SP FS and EE status had a significant correlation. Positive relationship of SP FS and EE status implies that SP FHs' EE status improves as SP FS increases. This corroborates the position put forth by Rashid et al. (2016) that the bigger the FS, the more empowered the farmers. Increased SP farm may could translate increased SP output and income and, consequently, high EE status. Increased land increases the quantity of agricultural output (Wouterse, 2016), and this can influence EE status.

Table 9. Chi-square and PPMC evaluations of a few chosen demographic traits and SP farmers' EE

Variables	Df	χ^2	r-value	*p*-value
Sex	1	0.27	-	0.60
Farm size for SP	-	-	0.20*	0.00
SP farming experience	-	-	-0.09	0.07

Note: *Significant at *p*≤0.05, df = Degree of Freedom, χ^2 =Chi-square Coefficient, r=Correlation coefficient.

3.10. Correlation between livelihood activities income and SP FHs' EE status

Table 10 Pearson Product Moment Correlation results show that SP income (r=0.24) and other produced crops income (r=0.10) were significantly (*p*<0.05) associated with SP farmers EE. This suggests that there was a strong relationship between EE and income of other produced crops and SP. Positive correlation of SP and other produced crops income with EE status implied that the SP producers' EE status increases as SP and other produced crops income increases. This implies that an increase in SP and other crops enterprise income would lead to EE status increase. Qurra-tul-ain et al. (2016) discovered a significant and positive contribution of income on empowerment status in Pakistan, which is consistent with this finding.

Table 10. Pearson Product Moment Correlation assessment of livelihood activities income and SP FHs' EE

Variables	r-value	*p*-value
SP income	0.239*	0.00
Other produced crops income	0.103*	0.05
Non-crop livelihood activities income	0.097	0.26

Note: *Significant at *p*≤0.05, r = Correlation coefficient

3.11. Difference between the EE status of FHs with low and high SPP

Table 11 reveals a significant difference between EE status of high (40.42 ± 6.15) and low (36.27 ± 7.55) SP farmers (t = -5.10, *p*< 0.05). This demonstrates that EE of SP FHs does differ significantly across low and high SP farmers. This suggests that higher-producing SP FHs were more economically empowered than lower-producing SP FHs. Therefore, it follows that SPP affects FHs EE. This conforms with the *apriori* expectation. The inference is that SPP have a favourably contribution on the EE of farmers. This supports Wouterse (2016) findings that agricultural production has significant effect on empowerment status.

Table 11. Difference in EE of SP FHs with low and high SPP

Variable	Production status	N	Mean	SD	Mean difference	t-value	Df	*p*-value
EE status	Low	253	36.27	7.55	-4.16	-5.10*	362	0.00
	High	111	40.42	6.15				

Note: df = Degree of Freedom, SD = Standard deviation, *Significant at *p*≤0.05.

3.12. Difference between the EE status of FHs with high and low SPP income

Table 12 shows that a significant difference between EE status of FHs with high (40.16 ± 5.99) and low (36.23 ± 7.69) SPP income (t = -4.92, *p*< 0.05). This displays that the EE of SP FHs does differ significantly across farmers with low and high SP farming income. This means that compared to SP FHs having low SPP income, those having high SPP income were more economically empowered. Hence, it follows that the respondents SPP income affects their EE status. The result conforms with the *apriori* expectation. The inference is that SP enterprise has a positive contribution to the EE of farmers.

Table 12. Difference in EE status of SP FHs with high and low SP income

Variable	SP income	N	Mean	SD	Mean difference	t-value	Df	*p*-value
EE status	Low	243	36.23	7.69	-3.93	-4.92*	362	0.00
	High	121	40.16	5.99				

Note: SD = Standard deviation, *Significant at *p*≤0.05, df = Degree of Freedom.

3.13. Contributors to SP FHs EE status

R^2 value was 0.101 (*Table 13*). This indicates that 10.1% of the EE status was explained by the chosen independent factors/variables. Furthermore, Table 13 showed household size (β = -0.209, *p*< 0.10), SP farming experience (β = 0.086, *p*< 0.10), SP income (β = 1.469, *p*< 0.10), and non-crop farm activities income (β = 4.785, *p*< 0.10) significantly contributed to EE status.

This entails that household size, SP farming experience, SP income and non-crop farm activities income were major contributors to respondents' EE status. The significant and negative contribution of household size to EE status establishes that with increase in the SP producers' family size or number, there is decrease in EE status. The result disagrees with the study of Ashimolowo (2005), which showed that household empowerment increased with

household size. The significance of SP production experience implies that with more production experience, EE status increases. According to Burgess (2014), involvement years in agriculture (years in farming) are a factor in empowerment. Increased SP farming experience implies increase in production and marketing experience, which boosts output and realized SPP income, giving farmers the ability to take control over his or her own economic life situations and EE. This finding disagreed with Carr et al. (2015), who found no significant association between South-West Uganda's smallholder farmers' farm experience and their empowerment. SP and non-crop livelihood activities income influences respondents EE and with high non-crop *vis a vis* SP livelihood activities income, there is significant increase in EE status. This is suggestive that improved non-crop as well as SP livelihood activities income will enhance high level of EE. This agrees with findings of Ballo et al. (2018), that crop production income has a significant and positive influence on FHs welfare status.

Table 13. Regression analysis on particular independent factors contribution to SP FHs EE status

Variables	β- value	t- value	*p*-value
Educational attainment	3.133	1.498	0.14
Household size	-0.209*	-1.848	0.07
Farm size for SP	-0.154	-0.361	0.72
SP farming experience	0.086*	1.865	0.06
Sweetpotato production-SPP	-0.008	-1.011	0.31
SP income	1.469*	1.784	0.08
Other crops enterprises income	-3.541	-0.184	0.85
Non-crop livelihood activities income	4.785*	1.816	0.07
Summary			
R-value	0.32		
R^2	0.10		
Adjusted R Square	0.04		
Standard Error of the estimated	6.22		
F-value	1.72		
P-value	0.09		

Note: *Significant at $p \leq 0.10$.

4. Conclusions

In Nigeria's Niger-Delta Area, SPP influenced FHs EE. Farm size for SP, farming experience, output and SP income enhanced SP FHs EE.

Agricultural programmes and policies aimed at improving EE and improving social and economic conditions should be promoted to emphasize increased SPP. Given the established nexus between SPP and EE, there should be a deliberate focus on rapid development of SP farm enterprise industry in the Niger-Delta area as a strategy to improve social and economic condition and stimulate rural development, particularly now that there is focus on diversification of the economy away from the crude oil-dependence. The study's result showed that FHs involved in SP in the studied area are mainly smallholders and that the EE of FHs increased with farm size and production experience. Therefore, it is recommended that during intervention programmes for SP FHs, SP production experience and farm size be strictly focused. It is suggested that effort be made by empowerment agencies to organize and/or increase campaign, sensitization, workshop and training on increasing/enhancing SPP income (maximization of producers returns on SP investment), as this have potential of contributing to EE.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Determination of Antioxidant and Antimicrobial Activities of Cell-Free Supernatant (CFSKC27L) and Exopolysaccharide (EPSKC27L) obtained from *Ligilactobacillus salivarius* KC27L

Ligilactobacillus salivarius KC27L'den Elde Edilen Hücresiz Süpernatant (CFS$_{KC27L}$) ve Ekzopolisakkaritin (EPS$_{KC27L}$) Antioksidan ve Antimikrobiyal Aktivitelerinin Belirlenmesi

Kubra CELIK[1], Zehranur YUKSEKDAG[2]*, Berat CINAR ACAR[3], Filiz KARA[4]

Abstract

Twenty-six lactic acid bacteria were obtained from poultry feces sampled located in the Ankara area (Türkiye) and belong to the *Lactobacillus* genus according to the results obtained by biochemical methods. This study screened these isolates for exopolysaccharides (EPS) production. EPS production was detected in these isolates, varying from 8 mg L^{-1} to 353 mg L^{-1}. The highest EPS-producing isolate (KC27L) was selected for further studies. The isolate was identified as *Ligilactobacillus salivarius* by 16S rRNA analysis. Furthermore, the anti-biofilm and antioxidant abilities of the cell-free supernatant (CFS$_{KC27L}$) and different concentrations (0.5 mg L^{-1} and 1 mg L^{-1}) of EPS belonging to the KC27L strain (EPS$_{KC27L}$) that exhibited high EPS production were determined. CFS$_{KC27}$ and different concentrations (0.5 mg L^{-1} and 1 mg mL^{-1}) of EPS$_{KC27L}$ determined the anti-biofilm impact on *Escherichia coli* ATCC 11229, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* EB-1. The highest anti-biofilm effect in 1 mg mL^{-1} EPS$_{KC27L}$ was detected at *E. coli* ATCC 11229 with 87 % inhibition. Three different methods (1.1-Diphenyl-2-picrylhydrazyl radical (DPPH) removal impact, Fe^{2+} ion chelating and superoxide anion radical scavenging activity) designated antioxidant activity. The highest 1.1-Diphenyl-2-picrylhydrazyl radical (DPPH) removal impact, Fe^{2+} ion chelating, and superoxide anion radical scavenging activity were found in 1 mg mL^{-1} EPS$_{KC27L}$ (79.6%, 24.9%, and 61.6%, respectively). Both anti-biofilm and antioxidant activities of 1 mg mL^{-1} EPS$_{KC27L}$ were higher than postbiotic. Finally, its molecular characterization was done following the partial purification of the EPS$_{KC27L}$. The EPS$_{KC27L}$ has two fractions with molecular weights of 1.6x10^3 and 6.4 x10^4 Da. Monosaccharide components of EPS$_{KC27L}$ were found to be glucose (53.1%), fructose (18.5%), arabinose (14.6%) and mannose (13.8%). CFS$_{KC27L}$ and EPS$_{KC27L}$ obtained from *L. salivarius* can be antioxidants and anti-biofilm agents.

Keywords: *Ligilactobacillus salivarius*, Cell-free supernatant, Exopolysaccharide, Anti-biofilm, Antioxidant

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Öz

Ankara bölgesinden (Türkiye) alınan kanatlı dışkılarından biyokimyasal yöntemlerle elde edilen sonuçlara göre *Lactobacillus* cinsine ait 26 laktik asit bakterisi izole edilmiştir. Bu çalışma, bu izolatları ekzopolisakkarit (EPS) üretimi açısından taramıştır. Bu izolatlarda 8 mg L^{-1} ila 353 mg L^{-1} arasında değişen EPS üretimi tespit edilmiştir. Bundan sonra yapılacak çalışmalar için en yüksek EPS üreten izolat (KC27L) seçilmiştir. İzolat 16S rRNA analizi ile *Ligilactobacillus salivarius* olarak tanımlanmıştır. Ayrıca, yüksek EPS üretimi sergileyen KC27L suşuna (EPS$_{KC27L}$) ait hücresiz süpernatantın (CFS$_{KC27L}$) ve farklı konsantrasyonlardaki (0.5 mg L^{-1} ve 1 mg L^{-1}) EPS'nin anti-biyofilm ve antioksidan yetenekleri belirlenmiştir. CFS$_{KC27}$ ve farklı konsantrasyonlarda (0.5 ve 1 mg mL^{-1}) EPS$_{KC27L}$, *Escherichia coli* ATCC 11229, *Enterococcus faecalis* ATCC 29212 ve *Staphylococcus aureus* EB-1 üzerinde anti-biyofilm etkisini belirlemiştir. 1 mg mL^{-1} EPS$_{KC27L}$'de en yüksek anti-biyofilm etkisi % 87 inhibisyon ile *E. coli* ATCC 11229'da tespit edilmiştir. Üç farklı yöntem ile (1.1-Difenil-2-pikrilhidrazil radikali (DPPH) giderim etkisi, Fe^{2+} iyon şelatlama aktivitesi ve süperoksit anyon radikal temizleme aktivitesi) antioksidan aktivite belirlenmiştir. En yüksek 1.1-Difenil-2-pikrilhidrazil radikali (DPPH) giderim etkisi, Fe^{2+} iyon şelatlama aktivitesi ve süperoksit anyon radikal temizleme aktivitesi 1 mg mL^{-1} EPS$_{KC27L}$'de (sırasıyla %79.6, %24.9 ve %61.6) bulunmuştur. 1 mg mL^{-1} EPS$_{KC27L}$'nin hem anti-biyofilm hem de antioksidan aktiviteleri postbiyotikten daha yüksek çıkmıştır. Son olarak EPS$_{KC27L}$'nin kısmi saflaştırılmasının ardından moleküler karakterizasyonu yapılmıştır. EPS$_{KC27L}$, molekür ağırlıkları $1.6x10^3$ ve $6.4x10^4$ Da olan iki fraksiyona sahiptir. EPS$_{KC27L}$'nin monosakarit bileşenleri glikoz (%53.1), fruktoz (%18.5), arabinoz (%14.6) ve mannoz (%13.8) olarak bulunmuştur. *L. salivarius*'tan elde edilen CFS$_{KC27L}$ ve EPS$_{KC27L}$ antioksidan ve anti-biyofilm ajanları olabilir.

Anahtar Kelimeler: *Ligilactobacillus salivarius*, Hücre içermeyen supernatant, Ekzopolisakkarit, Anti-biyofilm, Antioksidan

1. Introduction

Lactic acid bacteria (LAB), which commonly exist in nature, are probiotics, and it has been proven that LAB have also essential roles in poultry health (Piqué et al., 2019; Rajoka et al., 2019; Cano et al., 2021). The health benefits of probiotics come through direct or indirect interactions between cells or their released metabolites (Aguilar-Toalá et al., 2018; Ke et al., 2021). The soluble products or metabolites, comprised of the cell-free supernatant (CFS) composed of bacteriocins, organic acids, H$_2$O$_2$, short-chain fatty acids, carbohydrates, enzymes, vitamins, cofactors, and complex agents, excreted by probiotics that have health benefits for the host. These are postbiotics (Nataraj and Mallappa, 2020; Salminen et al., 2021). Since the precise compositions and mechanisms of postbiotics have yet to be fully understood, and there needs to be more information about their methods of preparation and analysis, there are fewer studies about postbiotics than probiotics (Cicenia et al., 2014; Hossain et al., 2021). Because of the food matrices' physicochemical properties and food processing, it isn't easy to directly use live starter/probiotic cultures on food. Hence, postbiotics could be used as an alternative to living microorganism to benefit from their wide-spectrum antimicrobial activity and avoid the negative interactions among primary and secondary starters and food (Arıcı, 2005; Pujato et al., 2014; Moradi et al., 2021). Currently, postbiotics have drawn interest because of their valuable impacts on the host, directly or indirectly (Żółkiewicz et al., 2020). Postbiotics, which are rich in high and low molecular weight biologically active metabolites, have drawn a great deal of attention from multiple researchers due to the convenience of use and hiding, persistence in a vast range of pH and temperature, and broad spectrum of antimicrobial activity (Campana et al., 2019; Jamwal et al., 2019). Also, the obtained goods of diverse signaling molecules of various regulative functions like anti-inflammatory, immunomodulatory, anti-proliferative, and antioxidant activities are remarkable for researchers (Barros et al., 2020; Moradi et al., 2021).

Polysaccharides classified into capsule polysaccharides, lipopolysaccharides, and exopolysaccharides (EPSs) concerning their composition are common in multiple organisms, including microorganisms, plants, and animals (Min et al., 2019). Generally, EPS can either be a homopolysaccharide in which one type of monosaccharide repeating units is included or a heteropolysaccharide in which different monosaccharide repeating units are included (Abarquero et al., 2021). Some lactic acid bacteria (LAB) strains produce EPS during their normal metabolic processes (Min et al., 2019), thus affecting foods' rheological, textural, functional, and sensory features. Also, they have protective functions in the natural environment against antibiotics or toxic compounds (Ayyash et al., 2018). The general properties of any EPS depend on its monosaccharide composition, molecular weight, connection type, and degrees of branching, and these contribute to determining its biological activity (Zhou et al., 2019).

Oxidative stress plays a role in many diseases, including heart disease, comprising atherosclerosis, diabetes, Parkinson's and Alzheimer's disease, inflammation, and cancer (Hajam et al., 2022). Numerous molecules, such as EPS and beta-glucan, are considered antioxidant agents (Sengül et al., 2011). In food products, due to the negative effects and toxicity of synthetic antioxidants on human health, harmless and nontoxic antioxidants obtained from natural sources for safety in humans are preferred (Choi et al., 2021; Albaş et al., 2022). EPSs are considered important candidates for functional foods as a natural source of antioxidants (Zhang et al., 2016).

In many industries, biofilms are one of the significant sources of contamination. The conventional cleaning and disinfection regimes cause inadequate control of biofilms and the spreading of resistance. Many methods have been developed to control biofilms and biofilm-associated infections; however, new, and effective strategies are still needed worldwide to prevent biofilms formed by pathogens, especially by multidrug-resistant bacterial strains (Raftis et al., 2011; Sarikaya et al., 2017). Since the ability to form biofilm increases the survival and permanence of pathogenic bacteria, chronic and repeated infections, and rising antibiotic resistance, it causes safety concerns in the Feedstuffs (Prete et al., 2021). LAB-derived EPSs are a promising alternative anti-biofilm agent to prevent biofilm formation (Abarquero et al., 2021; Prete et al., 2021). Although the biological properties of EPSs produced by LAB (e.g., antioxidant, anti-biofilm, antimicrobial, anticancer, immunomodulatory) have been investigated (Sharma et al., 2018; Min et al., 2019; Rajoka et al., 2019; Tukenmez et al., 2019; Bikric et al., 2022), non-lactic acid bacterial EPSs have been used commercially in poultry food additives. Therefore, there is a need to investigate LAB-derived EPSs that can be used as food additives for poultry. The purpose of this study is 1) to screen the EPS generation of lactobacilli isolated from chicken feces, 2) to investigate the anti-biofilm and antioxidant abilities of cell-free

supernatant (CFS$_{KC27L}$) and different concentrations (0.5 and 1 mg mL^{-1}) of exopolysaccharide (EPS$_{KC27L}$), for characterizing the lyophilized EPS of *L. salivarius* KC27L.

2. Materials and Methods

2.1. Isolation of Lactobacilli

The fecal samples of free-range chickens were collected from seven different districts of Ankara/Türkiye. Five g of fresh feces collected from chickens at different intervals was homogenized in 50 mL of phosphate-buffered saline (PBS, pH 7.2) with a mixer for 1 min to count and isolate the lactobacilli. After homogenization, 0.1 mL of proper dilutions of the homogenates spread in MRS agar (Man-Rogosa and Sharp, pH 6.2±0.2, Merck, Darmstadt, Germany) plates. All plates were incubated aerobically for 48–72 h at 37°C. Dominant slimy colonies were selected and purified by sequential streaking using the same medium. A total of 26 lactobacilli isolates were initially examined with Gram stain, and their cell morphology, motility, and catalase activity were determined (Li et al., 2021).

2.2. Screening of high EPS-producing lactobacilli, extraction, and partial purification of the EPS

The bacterial cultures were kept at 100°C for 15 min and were removed by centrifugation at 4000 g for 20 min. Trichloroacetic acid (TCA, Merck, Darmstadt, Germany) was added to 1.7 µL of the supernatant at a final concentration of 85% (w/v), stored at 4 °C for two h, and the precipitated proteins were removed by centrifugation at 12000 g for 30 min at 4 °C. The clear supernatant was gathered, and ethanol was put into the supernatant at a 3:1 (v/v) ratio and held overnight at 4 °C. The pellet, including EPS, was centrifuged at 12000 g for 30 min at 4 °C. The EPS was treated with cold pure ethanol, without vortexing, and alcohol was flushed at 40 °C (Li et al., 2016). The residue was dissolved in ultrapure water and stored at −40 °C for further analysis. Using glucose as a standard, the phenol-sulfuric acid method determined the total quantity carbohydrates in the EPS (Dubois et al., 1956). The protein ingredient of EPS was designated with the Bradford method (Takakuwa et al., 2023).

2.3. Molecular identification of the selected isolate

Lactobacillus sp. KC27L was selected among the 26 isolates because of the highest EPS production capacity. Molecular identification performed for *Lactobacillus* sp. KC27L. DNA was extracted using a Genomic DNA purification kit (Thermo Scientific, Waltham, Massachusetts, USA). The purity and amount of genomic DNA was specified in the ELISA (Epoch) (OD$_{260/280}$).

The amplification of 16S rDNA was carried out using universal primers (dos Santos et al., 2019). PCR analysis practised at RefGen Gene Research and Biotechnology Company. The sequence information obtained was analyzed with FinchTV (Version 1.5.0, United States) software, and the similarity with which microorganism sequence was determined by comparing it with other databases of known sequences in NCBI (National Center for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool) program.

2.4. Preparation of CFS$_{KC27L}$ and EPS$_{KC27L}$

For the cell-free supernatant (CFS$_{KC27L}$) preparation, *L. salivarius* KC27L density was arranged to be 10^9 CFU mL^{-1}. The culture was inoculated in MRS broth and incubated at 37 °C for 24 h. The homogeneous suspension was centrifuged at 10,000 × g, 4 °C for 15 min. The CFS$_{KC27L}$ was gathered by filtration owing to a cellulose acetate membrane according to the Loh et al. (2009) method and stored at -20 ºC for later use.

For the partial purification of EPS$_{KC27L}$, extracted EPS$_{KC27L}$ was dialyzed through a ten kDa membrane against distilled water at 4 °C for 72 h to eliminate low molecular weight impurities, and the remnant was lyophilized overnight. Lyophilized EPS (EPS$_{KC27}$) was prepared from the 10 mg/mL stock solution to a final volume of 0.5 or 1 mg mL^{-1}.

2.5 Anti-biofilm activity

Three strains (*Escherichia coli* ATCC 11229, *Enterococcus feacalis* 29212, and *Staphylococcus aureus* EB1) that previously exhibited the highest biofilm capacity (cut-off value: 1.052<2.073) in our laboratory (Bikric et al., 2022) selected for anti-biofilm experiments. The assessment of CFS$_{KC27L}$ and 0.5 and 1 mg mL^{-1} EPS$_{KC27L}$ showed their potential to prevent biofilm formation of three reference pathogen strains by adapting the microplate protocol described by Chaieb et al. (2011). Inulin (Sigma, St. Louis, Missouri, USA) (0.5 and 1.0 mg mL^{-1}), a plant-sourced prebiotic, was used as a control. Wells containing only medium were utilized as a negative control, and wells containing pathogen

bacteria were utilized as a positive control. The density of the bacteria was adjusted to 1.0×10^8 CFU mL^{-1}, and then pathogens were added (180 μL) to the plate well together with 20 μL CFS$_{KC27L}$ / EPS$_{KC27L}$. After incubating at 37 °C, 100 rpm for 24 h, the plate well was treated with distilled water. According to crystal violet method anti-biofilm activity was determined.

The anti-biofilm activity was determined using the below formula:

$$\text{Anti} - \text{biofilm} \% = [a/(a+b)] \times 100 \tag{Eq.1}$$

a: biofilm OD $_{570}$ nm, and

b: planktonic OD $_{570}$ nm.

2.6 Antioxidant activity

Three different methods designated to detect antioxidant activity. CFS$_{KC27L}$ and different concentrations (0.5 and 1.0 mg mL^{-1}) EPS$_{KC27L}$, inulin, and ascorbic acid (control) were used as samples.

DPPH scavenging activities of the samples designated utilizing the method declared by Li et al. (2014). Water is utilized as a blank, and DPPH in methanol is regarded as a control. The percentage of free radical scavenging activity detected from the following equation:

$$\% \text{ Scavenging } [A0 - (A1 - A2)]/A0 \times 100 \tag{Eq.2}$$

A_0: the absorbance of the control (water)

A_1: the absorbance of the sample

A_2: the absorbance of the sample under identical conditions as A_1 with water instead of DPPH solution.

The metal chelating ability of the samples was detected according to the method declared by Qiao et al. (2009). Sterile distilled water and EDTA-Na (Merck, Darmstadt, Germany) were used as the blank and positive control, respectively.

Results are given as the percentage chelating ability of ferrous ions by applying the following formula:

$$\text{The chelating ability on ferrous ion } (\%) = [(\text{Ablank} - (\text{A sample} - \text{A0})/\text{Ablank})] \times 100 \tag{Eq.3}$$

A_0: The absorbance of the sample under identical conditions

A_{sample}, with water instead of FeCl$_2$ solution.

In the superoxide radical scavenging activity, 50 mM phosphate buffer (pH 8.34) and samples were mixed and stored for 20 min at 25°C. 3 mM pyrogallol (Merck, Darmstadt, Germany) was incorporated. The absorbance value was measured at 325 nm (Wang et al., 2015). Results are given as the percentage of superoxide radical scavenging activity applying the formula below:

$$\text{Superoxide radical scavenging activity } (\%) = [(A0 - A1)/A0] \times 100 \tag{Eq.4}$$

A_1: The absorbance value of the samples

A_0: The absorbance values of the blank.

2.6 Characterization of EPS$_{KC27L}$

The molecular mass (Mw) of EPS$_{KC27L}$ designated by size-exclusion chromatography (SEC) with an Agilent 1200 series system fitted out with a PL aqua gel-OH MIXED-H column and a refractive index detector at a National Nanotechnology Research Center (UNAM) at Bilkent University according to the method of Boymirzaev et al. (2013). First, 1.0 mg mL^{-1} EPS$_{KC27L}$ was dissolved with ultra-pure water and filtered by a 0.45 μm filter (Millipore). Then, ten μL of EPS$_{KC27L}$ was injected and eluted with 0.2–0.8 M NaNO$_3$ (PubChem, Bethesda, USA) at a flow rate of 0.6 mL min. Pullulan (Merck, Darmstadt, Germany) is used as standard.

Analysis of monosaccharide composition, EPS$_{KC27L}$ was solved with ultra-pure water. Hydrolysis of EPS$_{KC27L}$ (10 mg mL^{-1}) was performed with 4 M HCl (Merck, Darmstadt, Germany) at 90 °C for three h and neutralized with 1 M

NaOH (pH 7.0) (Ledezma et al., 2016). After the hydrolysate was dried under vacuum, aliquots of 0.5 mL of methanol (Merck, Darmstadt, Germany) were put to remove the remnant. Monosaccharides determined by HPLC (AGILENT 1260 series) with Metacarp 67 °C (300 mm x 6.5 mm) column using water as mobile phase at a flow rate of 0.5 mL min^{-1}. The elution was performed with a detector (VARIAN 350 RI) at the Middle East Technical University Central Laboratory.

Nuclear Magnetic Resonance (NMR) spectroscopy was analyzed at Çankırı Karatekin University Research Center to determine the sugar residues. ^{1}H-NMR spectra of EPS$_{KC27L}$ recorded at 25 °C on an Agilent, 600 MHz, 14.1 Tesla Premium Compact NMR instrument with the chemical shifts (δ) informed in parts per million (ppm). Lastly, 30 mg of EPS$_{KC27L}$ sample dissolved in 500 μL deuterium oxide (D$_2$O).

2.7 Statistics

SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was utilized for the analyzing data. The statistical analysis was performed to calculate variance analysis (ANOVA). All tests are carried out in triplicate. In the anti-biofilm study, the Tukey test was used to designate the significant differences between pathogenic bacteria. The variances were equally distributed in the antioxidant activity study; the Tukey test was applied to determine significant differences between the methods.

3. Results

3.1. Screening of high EPS-producing lactobacilli and bacteria identification

In the current research, a total of 26 isolates from free-range chicken fecal samples were used. A total of 26 Gram-positive and catalase-negative isolates were isolated from MRS agar and considered lactobacilli. Following the primary characterization, the isolates were examined for their capacity to produce exopolysaccharides. Different amounts of exopolysaccharide production were determined for each isolate (*Table 1*). The maximum efficiency of EPS production was 353 mg L^{-1} for the KC27L isolate, while the minimum yield of EPS was found at 8 mg L^{-1} for the KC21L isolate. In further studies, the highest exopolysaccharides-producing isolate (KC27L) was used. BLAST screening was performed on both "forward" and "reverse" sequences in high-EPS production isolate. The "forward" sequences and the "reverse" sequence with the "reverse complement" were concatenated in Bioedit (Version 7.2, United States) software, and the consensus sequences obtained after this concatenation were compared with known sequences in the NCBI database using BLAST. The highest EPS-producing isolate (KC27L) is defined as *Ligilactobacillus salivarius* based on the 16S rRNA gene sequence (Accession No: SAMN24016084).

Table 1. Exopolysaccharides (EPS) production by isolates of lactobacilli

Isolate No	EPS (mg L^{-1})	Isolate No	EPS (mg L^{-1})
KC11L	78±7	KC46L	38±7
KC21L	8±0	KC56L	338±0
KC12L	19±0	KC66L	350±0
KC23L	32±0	KC76L	15±3
KC33L	58±0	KC86L	237±4
KC43L	117±0	KC96L	280±0
KC15L	213±0	KC116L	256±0
KC25L	189±0	KC126L	52±0
KC35L	119±0	KC136L	296±9
KC45L	226±0	KC17L	64±2
KC65L	277±6	KC27L	353±6
KC16L	45±1	KC37L	77±2
KC36L	161±0	KC47L	276±6

3.2. Anti-biofilm effect

Biofilm prevention capacity of the CFS$_{KC27L}$, EPS$_{KC27L}$, and inulin (control) tested on three pathogen test bacteria, which demonstrated a robust biofilm formation (cut-off value: 1.052<2.073). The results obtained were expressed as inhibition percentages of biofilm development and presented in *Table 2*. In cell-free supernatant, the highest antibiofilm effect was 47% at *E. coli* ATCC 11229. The EPS$_{KC27L}$ (1 mg mL^{-1}) reduced 87% biofilm on *E. coli* ATCC 11229. While inulin (1 mg mL^{-1}) inhibited 72% of biofilm formation by *E. coli* ATCC 11229.. The amount of biofilm inhibition enhanced as the exopolysaccharide concentration increased. According to the statistical analysis, a significant difference of 0.05 level was found between the biofilm inhibition of the pathogenic bacteria used. Also, a statistically significant difference of 0.05 level was found between CFS$_{KC27L}$, EPS$_{KC27L}$, and inulin (1 mg mL^{-1}) applications to prevent biofilm formation of pathogenic bacteria, depending on the type of microorganism used (*Table 2*). Also, biofilm removal percentages were lower at CFS compared to both concentrations of EPS.

Table 2. Antibiofilm activities of postbiotic, prebiotic (EPSKC27L), and inulin

Test Bacteria	Inhibition of biofilm (%)				
	Postbiotic [a,b,c]	Prebiotic (EPS$_{KC27L}$) [a,b,c]		Inulin [a,b,c]	
		0.5 mg mL^{-1}	1 mg mL^{-1}	0.5 mg mL^{-1}	1 mg mL^{-1}
S. aureus EB1 [a]	28±4	67±5	76±5	53±1	67±4
E. coli ATCC 11229 [b]	47±2	79±4	87±2	68±4	72±3
E. faecalis ATCC 29212 [c]	46±1	66±2	82±1	51±5	59±6

The mean difference in [a,b,c] is significant at the 0.05 level.

3.3. Antioxidant effect

In the current research, the antioxidant activity of CFS$_{KC27L}$, different concentrations (0.5-1 mg mL^{-1}) of EPS$_{KC27L}$, 0.5-1 mg mL^{-1} inulin (control), and 0.5-1.0 mg mL^{-1} ascorbic acid (control) evaluated by diverse methods: DPPH, superoxide anion scavenging, and metal ion chelating. Among the three methods, the highest activity was determined as 79.6% for 1 mg mL^{-1} EPS$_{KC27L}$ in DPPH radical scavenging activity, while the lowest activity was 18.6% for 0.5 mg mL^{-1} EPS$_{KC27L}$ in Fe^{+2} chelating activity (*Table 3*). Inulin, used as a control at a concentration of 0.5 mg mL^{-1}, exhibited no antioxidant activity. According to the statistical analysis, a significant difference of 0.05 level was detected between the methods used. Also, a statistically significant difference of 0.05 level was found between the applications used (CFS$_{KC27L}$, 1 mg mL^{-1} EPS$_{KC27L}$, 1 mg mL^{-1} inulin, and 1 mg mL^{-1} ascorbic acid).

Table 3. Antioxidant activities of postbiotic, prebiotic (EPSKC27L), and inulin

Methods	Antioxidant activity (%)						
	Postbiotic [a,b,c]	Prebiotic (EPS$_{KC27L}$) [a,b,c]		Inulin [a,b,c]		Ascorbic acid [a,b]	
		0.5 mg mL^{-1}	1 mg mL^{-1}	0.5 mg mL^{-1}	1 mg mL^{-1}	0.5 mg mL^{-1}	1 mg mL^{-1}
DPPH radical [a]	75.7±1.0	73.4±1.9	79.6±4.7	-	73.2±2.7	84.3±0.5	93.2±0.2
Fe^{2+} Chelating activity [b]	19.3±0.0	18.6±3.0	24.9±1.2	-	23.2±1.3	78.6±0.4	89.3±0.2
Superoxide anion radical [c]	61.3±1.9	44.8±2.5	61.6±3.3	-	32.3±1.1	82.4±0.4	91.4±0.1

-: No activity
The mean difference in [a,b,c] is significant at the 0.05 level.

3.4 Characterization of EPS$_{KC27L}$

In characterization studies, size-exclusion chromatography is used to forecast the Molecular weight (Mw) of EPS$_{KC27L}$. The Mw of EPS$_{KC27L}$ was determined as 1.6x10^3 and 6.4x10^4 Da, consisting of two fractions.

HPLC designates the monosaccharide composition of EPSKC27L. The monosaccharide composition of EPS$_{KC27L}$ indicated that the ratio of each sugar varied in percentages, such as galactose (0%), mannose (13.8%), and arabinose (14.6%), fructose (18.5%), and glucose (53.1%).

1H NMR was mainly used to designate polysaccharide molecules of glycosidic bond configuration. The 1H NMR spectrum of a polysaccharide could usually separate into three major regions: The anomeric region (5.5–4.5 ppm), the ring proton region (3.0–4.5 ppm), and the alkyl region (1.2–2.3 ppm).

4. Discussions

Microbial exopolysaccharides, a multifunctional compound with many applications in the pharmaceutical and food industries, are essential for human, animal, and poultry health (Rajoka et al., 2019). The exopolysaccharides generated by LAB have been declared to have several physiological functional properties, including antioxidant, antitumor, immunomodulatory, anti-inflammatory, anti-biofilm, and cholesterol-lowering potential (Wang et al., 2020). In the current study, the isolates showed EPS production capacity in the 8-353 mg L^{-1} (*Table 1*). Previous studies reported the quantity of EPS generation as 151 mg L^{-1} for *L. salivarius* ZNY9 (Yuksekdag et al., 2014), 24 mg L^{-1} for *L. paracasei* HCT (Xu et al., 2010), and 430 mg L^{-1} for *L. salivarius* E2 strain (Mercan et al., 2015). In this study, the amounts of EPS production by *Lactobacillus* isolates showed similar results to those reported in the literature. The EPS production amounts of the LAB vary and depend on the species, type of strain, isolation sources, and growth conditions (medium, temperature, pH, carbon source, concentration, incubation time, and inoculum size) (Ren et al., 2016).

It determined that the isolate with the highest EPS capacity (353 mg L^{-1}) selected for other studies was *Ligilactobacillus salivarius* because of molecular identification. There is minimal information regarding the topological differences between lactobacilli in the chicken gastrointestinal system and feces. *Lactobacilli* are the most widespread species of chicken, fattening pigs, beef cattle, and humans. *L. salivarius* strains are important members of the animal microbiota in the gastrointestinal tract (Raftis et al., 2011).

Three pathogenic bacteria that exhibited a robust biofilm formation (1.052<2.073) were selected to be used in anti-biofilm studies among 12 pathogenic bacteria whose ability for biofilm formation was previously determined by Bikric et al. (2022). In the current study, biofilm prevention capacity was detected by three pathogenic bacteria (*E. coli* ATCC 11229, *E. feacalis* 29212, and *S. aureus* EB1). Using biofilms produced by three pathogenic bacteria, the inhibition activity of CFS$_{KC27L}$ at two different concentrations (0.5 and 1 mg mL^{-1}) of EPS$_{KC27L}$ and inulin was determined (*Table 2*). In all conditions, the highest biofilm inhibition was realized by *E. coli* ATCC 11229. The highest biofilm inhibition at CFS$_{KC27L}$ was detected as 47% by *E. coli* ATCC 11229. Sevin et al. (2021) determined the anti-biofilm activity of postbiotics against ruminant mastitis-causing pathogens (*Staphylococcus aureus* (MRSA) ATCC 43300, *Streptococcus agalactiae* ATCC 27956, and *S. dysgalactiae* subsp. *dysgalactiae* ATCC 27957). The highest inhibition was determined at 25 mg mL^{-1} (96.2% by MRSA 43300, 95.10% by *S. agalactiae* ATCC 27956), and 17.5 mg mL^{-1} (92.65% *S. dysgalactiae* subsp. *dysgalactiae* ATCC 27957). On the other hand, in cell-free supernatants in this study, the biofilm inhibition value was lower compared to Sevin et al.'s (2021) results. Probiotics' primary mechanism of action on pathogens depends on the production and number of bioactive compounds produced during metabolism (Ohshima et al., 2019). This could be due to the different strain resources, metabolite differences, and their amount in cell-free supernatant. This study did not investigate the chemical composition of CFS$_{KC27L}$, limiting identification of the specific metabolite(s) responsible for the observed biofilm inhibition. The EPS$_{KC27L}$ has different rates of antibiofilm effect against the pathogen bacteria tested, and the highest inhibition was found in 1 mg mL^{-1} as 87% by *E. coli* ATCC 11229. Biofilm activity in three pathogen bacteria decreased by more than 0.5 mg mL^{-1} in 1 mg mL^{-1} in inulin and EPS$_{KC27L}$. Biofilm removal was higher in EPS$_{KC27L}$ than inulin (*Table 2*). The results showed that EPS$_{KC27L}$ has a broad anti-biofilm activity against biofilm-forming bacteria. Therefore, EPS$_{KC27L}$ could be used as an alternative to inulin to inhibit the growth of biofilm-forming bacteria. The anti-biofilm effect against biofilm-forming bacteria was higher in both EPS$_{KC27L}$ concentrations than in CFS$_{KC27L}$, whose metabolites and their concentrations are unknown. While EPS (a single metabolite) acts directly, all metabolites (CFS) may interact, and biofilm inhibition may be reduced. EPS may exert its anti-biofilm activity through several mechanisms. EPS could exert its anti-biofilm activity by altering bacterial cell surfaces (Rendueles et al., 2013), Preventing bacterial cells from adhering to surfaces (Valle et al., 2006), or also acting as signaling molecules to suppress gene expression involved in biofilm formation (Kim et al., 2009; Wang et al., 2015).

Because of their potential for scavenging free radicals and binding transition metal ion catalysts, besides their reducing activity and capability to prohibit chain initiation, natural antioxidants play an essential role against various diseases and aging processes (Wang et al., 2015). The DPPH free radical has an unpaired valence electron at one atom of its nitrogen bridge, and this causes a significant decrease in exposure to the proton radical scavenger. Also, it has a powerful, damaging effect on body organs (Li et al., 2014).

This research carried out antioxidant activities of CFS$_{KC27L}$, EPS$_{KC27L}$, inulin, and ascorbic acid (positive control). DPPH radical antioxidant activity of CFS$_{KC27L}$ was determined as 75.7%. Izuddin et al. (2020) reported cell-free

supernatant produced from *L. plantarum* RG14, RG11, and TL1 used for antioxidant activity of DPPH, and values obtained as TL1 67%, RG11 72%, and RG14 74%. Our study and other results (Izuddin et al., 2020) are close. The scavenging activities of different concentrations (0.5 and 1 mg mL^{-1}) of EPS$_{KC27L}$ on DPPH radicals changed depending on the concentration. Maximum scavenging activity was observed at 1 mg mL^{-1} at EPS$_{KC27L}$ (79.6%). Similarly, the DPPH radical scavenging activity of the commercial prebiotic inulin was found to be 73.2%. Our results demonstrated that all applications had a higher scavenging activity of DPPH and were much better than previously reported results for lactobacilli themselves at 1 mg mL^{-1}: 78.7% for 4 mg mL^{-1} EPS (Sevin et al., 2021), 75.98% for 4 mg mL^{-1} EPS (Rani et al., 2018), 53.63% for 5 mg mL^{-1} EPS (Trabelsi et al., 2017).

Metal chelating activity, one of the antioxidant mechanisms, decreases the concentration of the catalyzing transition metal in lipid peroxidation. Iron is an essential lipid oxidation pro-oxidant among transition metals because it shows high reactivity (Min et al., 2019; Rajoka et al., 2019). In the current research, Fe^{2+} chelating activity of CFS$_{KC27L}$ was 19.3%. The chelating activity was higher than in 1 mg mL^{-1} EPS$_{KC27L}$ (32.3%) than inulin (23.2%). Until now, ferrous ion chelating activity has rarely been utilized to evaluate cell-free supernatant's antioxidant capacity and LAB-derived EPS. Min et al. (2019) reported that the ferrous ion chelating activities of EPS103 rose to the maximum value of 69.7% when its concentrations arrived at 10 mg mL^{-1}. In another study, EPS111 exhibited a much higher ferrous ion chelating impact at 4 mg mL^{-1} concentration (72.8%) (Rajoka et al., 2019). The low activity in our study could be due to the low concentrations used.

The superoxide anion is a free radical and is evaluated as a reactive oxygen species (ROS) interfering with biological macromolecules, resulting in tissue damage. It supports the generation of other ROS, such as hydroxyl radical, hydrogen peroxide, and singlet oxygen (Rajoka et al., 2019). Therefore, in this study investigated the superoxide radicals scavenging capacity of CFS$_{KC27L}$ and different concentrations of EPS$_{KC27L}$. The superoxide anion scavenging activity observed in CFS$_{KC27L}$ was 61.3%. The superoxide anion scavenging activity was higher than in 1 mg mL^{-1} EPS$_{KC27L}$ (61.6%) compared to 1 mg mL^{-1} inulin (32.3%). Researchers reported the superoxide anion scavenging activity of some lactobacilli EPS; *L. plantarum* EPS (18%) (Zhang et al., 2016), *L. rhamnosus* EPS115 (5.9%), and *L. rhamnosus* EPS 111 (29.4%) (Rajoka et al., 2019). In our study, the superoxide anion scavenging activity was higher at both 0.5 and 1 mg mL^{-1} concentrations compared to these studies. The scavenging capacity is mainly attributed to the direct binding of the EPS molecules with superoxide radicals to form steady radicals, which could end the radical chain reaction (Rajoka et al., 2019). However, our results in three different antioxidant determination methods are higher and/or similar to other studies, with lower values observed than the ascorbic acid used as the positive control group. Since antioxidants harm the body (Wang et al., 2023), new safe and green antioxidants must be investigated. CFS$_{KC27L}$ and EPS$_{KC27L}$, whose antioxidant activities are examined in this study, may be natural antioxidant agents. As free radical scavenging activity is utilized for a preliminary evaluation, *in vitro* and *in vivo* experiments sometimes give different results. Therefore, a definitive judgment can be made after more comprehensive research on the *in vivo* antioxidant activity of CFS$_{KC27L}$ and EPS$_{KC27L}$.

The Mw of EPS$_{KC27L}$ produced by LAB is a crucial characteristic influencing its bioactivities. Since the low molecular weight of EPS could easily pass through multiple cell membrane barriers, it exhibits better bioactivity (Li et al., 2016). In the current study, the molecular weight of EPS$_{KC27L}$ was determined as 1.6×10^3 and 6.4×10^4 Da consisting of two fractions. Similarly, Tukenmez et al. (2019) detected the molecular weight of the EPSs, which was isolated from *L. plantarum* GD2, *L. rhamnosus* E9, and *L. delbrueckii* ssp. *bulgaricus* B3 consisting of two fractions ranging from 10^2 to 10^4 Da. However, some studies on the structure analysis of LAB-derived EPS reported that the EPS had a one fraction with a molecular weight of 1.86×10^5 for *L. gasseri* FR4 (Izuddin et al., 2020) and 3.32×10^5 Da for *Streptococcus thermophilus* EPS333 (Ren et al., 2016). In contrast, some researches declared that the EPS had three fractions (Li et al., 2014). Bikric et al. (2022) declared that the Mw EPS$_{BIS312}$ obtained from the *L. salivarius* BIS312 strain was determined as 9.0×10^3, 2.8×10^4, 7.2×10^5, and 6.8×10^6, while the Mw of the EPS$_{BIS722}$ obtained from *L. salivarius* BIS722 strain was designated to be 1.3×10^4, 5.4×10^4, 8.9×10^5, and 6.9×10^6 consisting of four fractions. Diverse bacterial resources, culture conditions, and heritable characters could cause differences in the molecular weight of EPS (Min et al., 2019).

LAB-derived EPS mainly comprises glucose, galactose, and mannose with different molar ratios (Wang et al., 2010; Li et al., 2014). In this research, EPS$_{KC27L}$ contained different monosaccharides (glucose, fructose, arabinose, and mannose) and showed varying percentages (53.1%, 18.5%, 14.6%, and 13.8%, respectively). Similarly, Rani et al.

(2018) noticed that the EPS generated by *L. gasseri* FR4 obtained from the native chicken was primarily composed of glucose (65.31%), mannose (16.51%), galactose (8.45%), rhamnose (6.55%), and a small fraction of fucose (3.18%). As announced by Bikric et al. (2022), the EPS_{BIS312} and EPS_{BIS722} involved glucose (34.53, 35.31%), mannose (26.41, 27.60%), rhamnose (13.58, 11.28%), and galactose (25.47, 25.82%), but the other monosaccharides (fructose and arabinose) were not determined. The difference in the monosaccharide composition of LAB-derived EPS could be related to different strain types, growth conditions, and carbon-resourced media content (Wang et al., 2010).

The anomeric region NMR signals, which can be divided into two categories, are frequently used to distinguish the anomeric protons of sugar residual in polysaccharides (5.5–4.9 ppm for α-anomers and 4.9–4.5 ppm for β-anomers) (Zhao et al., 2020). In the anomeric region of the 1H NMR spectrum of EPS_{KC27L} (Data not shown), three proton signals occurred at 5.2, 5.0, and 4.9 ppm, indicating that EPS_{KC27L} was mainly composed of three types of sugar residues. The peaks at 5.2 ppm correspond to the anomeric proton of α-D-mannose, 5.0 ppm to α-D-glucose and 4.9 ppm to β-D-glucose indicating that EPS_{KC27L} contained α and β configurations (Ismail et al., 2013; Ai et al., 2016). In general, α- and β-configurations are simultaneously present in hetero-polysaccharides from LAB (Ai et al., 2016). In addition, the strong signal at 4.7 ppm is presented in the spectrum attributed to the solvent hydrogen-deuterium oxide HOD (Shu et al., 2020). The signals procured in the spectrum between 4.4 and 3.2 ppm were owing to the ring protons bonded to C2–C6 and insufficiently eliminated because of the chemical shifts of the complex and heterogeneous nature of EPS_{KC27L} (Ayyash et al., 2018).

5. Conclusions

Although using different species of lactobacilli as probiotics in chickens has shown beneficial effects, there still needs to be more effective data to select promising lactobacilli and their exopolysaccharides and cell-free supernatant. This study determined that cell-free supernatant (CFS_{KC27L}), EPS obtained from *L. salivarius* KC27L (EPS_{KC27L}), had anti-biofilm and antioxidant activity. The study of Yildiz et al. (2023) determined that EPS_{KC27L} (12.50, 25.00, 50.00, and 100.00 μg mL^{-1}) did not cause a remarkable genotoxic impact in chromosome aberration (CA), sister chromatid exchange (SCE), micronucleus (MN), and comet assays. EPS_{KC27L} significantly reduced the CA, SCE, and MN frequency induced by mitomycin-C (0.20 μg mL^{-1}) methyl methanesulfonate (5.00 μg mL^{-1}). EPS_{KC27L} also significantly decreased DNA damage caused by hydrogen peroxide (100 μM). Utilizing naturally derived EPS as a poultry feed additive holds promise, potentially offering superior or comparable results to commercially available prebiotics like inulin. However, more studies should be conducted to recommend EPS obtained from strains as an additive in poultry feeding, and animal trials should also support research. With their antioxidant and anti-biofilm activities, the EPS_{KC27L}, regarded as a functional food ingredient candidate, could add value to the food and reduce the use of artificial additives.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Authors Contribution Statement

Concept: Yuksekdag, Z., Design: Celik, K., Data Collection or Processing: Celik, K., Yuksekdag, Z., Cinar Acar, B., Literature Search: Celik, K., Cinar Acar, B., Writing, Review and Editing: Celik, K., Yuksekdag, Z., Cinar Acar, B., Kara, F.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Effect of Flood on Poverty Status: Evidence from Sugar cane Farmers in Kwara State and Osun State of Nigeria

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Abstract

In Nigeria, sugarcane is a prominent crop and the primary crop used to produce sugar. However, floods have been a major tragedy that has troubled Nigeria's small-scale farmers. The bulk of rural poor people are these small-scale farmers, and as poverty rates have been rising over time, corrective action is required. This study was therefore carried out to assess the poverty status of sugar cane farming households (affected by flood, and unaffected by flood) in Kwara/Osun State of Nigeria. The study employed two-stage sampling technique to select 120 sugarcane farming households in the study area. Descriptive statistics, the Flood Loss Estimation, Logit Regression and Foster Greer Thorbecke (FGT) are the empirical analytical tools employed to analyze the data collected for the study. The results of the poverty index analysis revealed that 46 percent of the respondents are relatively poor, the poverty depth was 0.16 which implies that 16 percent were below the poverty line and the poverty severity was 0.07 which implies that 7 percent of the respondents were severely poor. Also, it revealed that flood shocks, gender, household size, household income, access to credit and membership to social organization were significant determinants of poverty. The study then came to the conclusion that many coping mechanisms people employ are corrosive because they have detrimental long-term implications on the sustainability of household subsistence. For future risk assessments and flood mitigation, the study advises using a holistic strategy and adept models. The remedies outlined in this paper would help the government, private sector, and sugar cane farmers economically.

Keywords: Coping strategy, Disaster, Environmental impact, Farming households, Mitigation

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1. Introduction

Africa's most populous country is Nigeria. The agricultural industry is comparable to a factory operating in atmospheric circumstances (Simsek and Cakmak, 2010). In Nigeria, the agricultural industry is crucial, and raising living standards is the main objective of the country's agricultural production plan. Small-scale farmers make up the majority of the rural poor, nonetheless, the rate of poverty has been increasing over time, necessitating intervention (Yaro, 2004; McCusker, 2006). Thus, targeting rural farmers could help reduce poverty in Nigeria. The nation is plagued by poverty, which has been worse since the late 1990s (Chimobi, 2010). According to estimates, 168 million Nigerians, or about 100 million people, live on less than $1 a day, and according to the United Nations Human Development Index, Nigeria was ranked 142 out of 175 countries in 2010 (World Bank Report, 2008). In 2010, 60.9% of Nigerians lived in absolute poverty, up from 54.7% in 2004 (World Bank Report, 2008). The situation is even worse in the North-Western and North-Eastern regions of the nation, where staggering poverty rates of 77.7% and 76.3 percent, respectively, are reported (World Bank Report, 2008).

Another important element is income inequality, which increased from 0.429% in 2004 to 0.447% in 2010 (Adenuga et al., 2014). In many low-income nations in Sub-Saharan Africa, including Nigeria, there is a high level of income disparity (World Bank Report, 2005). As a result, the atmosphere for economic growth and development has not been favorable.

Additionally, compared to urban poverty, rural poverty is more pervasive, severe, and deeper (Chaudhry et al., 2006). Due to a lack of commercial and industrial facilities or the resources required to build them, rural poverty is made worse by the lack of opportunities for employment and income generation (Adenuga et al., 2014). From a theoretical standpoint the rural parts of a region or country are located outside of the densely populated areas of towns, cities, and sub-urban villages, and their residents are largely involved in agriculture and the most fundamental components of secondary and tertiary activity (Adebayo, 1998).

Sugarcane is a significant crop grown in Nigeria (Wada et al., 2017). The main crop used to manufacture sugar is called sugar cane (*Sacchrum officinarium* L.), a perennial grass belonging to the Gramineae family. It is discovered that sugar cane is a necessary crop in sectors where the by-products are utilized for medications, pharmaceuticals, confectionary and beverages, electricity, and motor fuels. However, there are numerous issues with this crop's cultivation in Nigeria and other Northern African nations, including biotic and abiotic issues as well as social and environmental ones (including flooding) (Anonymous 2018; Anonymous, 2019). Lack of adequate funding, a lack of market investment, higher transportation and production costs for hauling harvested sugar cane to the mills, biotic and abiotic stresses, low building capacity, a lack of associations for sugar cane farmers and technologists, a shortage of regulatory structures, macro- and micro-environmental conditions, an absence of national and indigenous collaboration groups, and a lack of legislative capacity to enact the law are additional obstacles to the growth in sugar cane production in Nigeria. To overcome these issues and enhance sugar cane production, those involved in the sugar cane business in these nations are urged to start proactive efforts. The link between rural and urban is similar to development, (Gedik and Yilmaz, 2023).

Major and annoyance flooding episodes have increased across the country in recent years (Modupe et al., 2023). In terms of impact size and geographic scope, the flood of 2012 was regarded as the worst flood in modern times. It was proceeded by the historic-scale floods of 2017 and 2018. The federal, state, and local governments of Nigeria are compelled to look for solutions to enhance the holistic vulnerability of the communities as a result of the repeated destruction caused by flooding (Babatolu, 2014). Because of elements like climate change and some human-caused environmental impacts, there is no doubt that rainfall is extremely high (Solihu et al., 2022). According to a surveillance assessment conducted in several regions of Nigeria, heavy rains and the ensuing flooding also ruined agricultural products worth billions of naira. However, floods are not a new occurrence in the nation, and they may sometimes be incredibly destructive. In Nigeria's highly susceptible to flooding locations, floods significantly reduce a farmland's topsoil (Etuonovbe, 2011).

Kwara State and Osun State are two of Nigeria's most severely hit regions. The functioning of the Jebba and Kanji hydroelectric projects, as well as drainage from the reservoirs, control the River Niger's flow system beneath the Jebba dam in Kwara State (Adeniyi, 1973). The yearly "white floods" phenomenon, which typically start in July and climax in September, no longer follows the same pattern or regularity because it now happens approximately every four years, causing the dam to overrun its shoreline. The flooding events have a variety of

repercussions on rural residents since they not only lose money on their investments but also struggle to feed themselves, which has an impact on their way of life (Oyekale, 2008).

The presence of rivers ensured access to and from the coast by the sea, water supply, crop irrigation, and the production of electricity. Landscapes are harmed structurally and environmentally by flooding (Philips et al., 2004; Rehman et al., 2019).

Flooding could have both negative and positive effects. A number of farmers have suffered serious consequences as a result of the terrible incident, which has increased poverty levels in both rural and some urban areas. Nevertheless, this catastrophe causes more harm than good, leaving the majority of impacted households destitute and poor. Nigeria is still one of the world's poorest nations, therefore it's critical to think about effective coping mechanisms (Sanchez-Martinez, 2014). Therefore, the actual objectives of this study are to: examine the effect of flood on sugar cane farmers; assess the poverty status of the affected and unaffected sugar cane farmers; identify the factors determining their poverty status; and determine the suitable coping strategies.

2. Materials and Methods

2.1. Study area

The study was conducted in Kwara State and in Osun States of Nigeria. One of the two largest sugar cane hubs in Nigeria is Kwara State, which is located in an area with excellent soil for growing sugar cane. The river valley is advantageous for the production of sugar cane in the sugar cane hubs. Kwara State is located at 80,301°N and 50,001°E North central region of Nigeria. It has a total population of 2.37 million people (National Population Commission, 2006). It shares local boundary with Osun, Oyo, Ondo, Kogi, Ekiti, and Niger States and international boundary with Benin Republic. There are 16 known Local Government Areas in Kwara State among which Ilorin Local Government is one.

Osun State is located 7°30'N 4°30'E/7.500°N 4.500°E of South West part of the country. The Capital is Osogbo and has the total land mass of 9,251km^2 (3,572sq mi). It has a total population of 3,416,959 with 30 local government areas and head-quarters. Both states have rich and diversified agricultural products, including sugar cane, rice, yams, beans, cassava, potatoes, maize, Soy beans, sorghum, millet, and coco-yams, and they share a common boundary (Oyekale, 2012).

2.2. Sources of data

Through the use of structured surveys and interviews, the study used primary data from respondents. Primary information about the respondents' socioeconomic traits, farmer welfare, and farm characteristics was gathered and used for analysis.

2.3. Sampling technique

The data for this investigation were gathered using a two-stage sampling procedure. The households that grow sugar cane were the target ecology. The villages that does not produce sugarcane or where sugarcane is not grown in the target ecology were eliminated from a list of all the villages in the hubs, leaving just the villages where sugarcane is grown in the target ecology. The remaining communities were then divided into two classes (strata) based on criteria such as flood experience. Both agricultural households affected by flooding and farming families not affected by flooding are listed here. Villages with a very small number of sugarcane plantations were not taken into consideration due to the high intensity of the field operations. Two-stage sampling technique was used to select 120 sugarcane farming households in the study area. The first stage involved selection of six sugar cane producing villages in each state that is; three affected sugar cane producing villages and three unaffected sugar cane producing villages, in each state. The second stage involved random selection of ten farming households in each sampled village. Thus, a total of 120 sugarcane farming households were sampled and administered questionnaire for the study.

2.4. Method of data collection

Data was gathered utilizing questionnaire schedules regarding the experience of households that grow sugar cane during floods. A focus group discussion and a household survey questionnaire were used to deliver the questionnaire, which asked questions about the socioeconomic and demographic traits, as well as the level of poverty in each of the sugarcane farming homes.

2.5. Method of data analysis

For this study, a variety of empirical analytical methods and instruments were used. The demographic and socioeconomic features of the respondents were explained using descriptive statistics, such as percentages, frequencies, and means. Foster- Greer Thorbecke (FGT) was used for poverty analysis among the respondents and it is written as equation 1.

$$P_\alpha(y, z) = \frac{1}{n} \sum_{i=1}^{q} \left(\frac{z - y_i}{z} \right). \tag{Eq. 1}$$

Flood Loss Estimation Model was used to determine the effect of flood on the poverty status of the Sugarcane farmers. Logit Regression model was used to analyze the factors determining the poverty status and Descriptive Statistics was used to analyze the coping strategies employed after the flooding event. For estimating flood losses, a grid-based mathematical model was employed. It makes use of the identical uniform grid network as the hydraulic model. The flood loss estimates model calculates damages in both rural and urban areas using flood loss equations (Guetchine, 2013). Through stage-damage functions, the calculated maximum flood water depth is fed into the loss calculations. For specific water depth conditions, these functions are used to determine the unit damage percentage to any object (be it a building or a crop). In this work, the standard stage-damage functions developed by Dutta et al. (2003) for the calculation of rural and urban damages are applied.

In this study, the degree of flood damage is evaluated. The damage to the agricultural sector includes damage to crops and vegetables in any grid cell (I, j) in $\mathbb{N}$, $AD(I, j)$ is estimated based on Equations (1)-(2):

Damage to crops and vegetable- $AD(I, j) = D_m(I, j, k) \times CRP_a(i, j, k) \times L(k)$ \hfill (Eq. 2)

$D_m = CP_k \times Y_k \times DC_k(i, j)$ \hfill (Eq. 3)

Where; $AD(I, j)$ = Agricultural damage (₦)

n = Total number of crops

(I, j) = Grid cell

$D_m(I, j, k)$ = Damage to crop type k per unit area (₦ m^{-2})

$CRP_a(i, j, k)$ = Total area of cultivation of crop type k (m^2)

$L(k)$ = Loss factor for crop type k depending on the time period of the year (%)

CP_k = The estimated cost per unit weight of crop type k (₦ kg^{-1})

Y_k = The normal year yield of crop type k per unit area (kg m^{-3})

DC_k = The stage-damage function for crop type k (%)

The total damage to farmland infrastructure in any grid cell (I, j) in $\mathbb{N}$, $D_f t(i, j)$, is estimated as follows;

Damage to farm-land infrastructure - $D_f t(i, j) = TA(i, j) \times C_f t(i, j)$ \hfill (Eq. 4)

Where; $D_f t(i, j)$ = Damage to farmland infrastructure (₦)

$TA(i, j)$ = Total farm area occupy by the infrastructure (m^2)

$EC_f t$ = The estimated cost of complete replacement of farm infrastructure (₦ m^{-2})

$C_f t$ = The stage-damage function (%)

Logit Regression Model which is a binary regression model was used to determine the factors influencing the poverty status of the respondents. It was found that Logit regression was suitable for this study owing to its unique ability to account for both categorical and dichotomous dependent variables. According to Pampel, (2000), the model equation is given as:

$Logit(E[Y]) = Logit(P) = XT\beta$ \hfill (Eq. 5)

Where

$Logit(E[Y])$ = the binary response/dependent variable

$Logit(P)$ = the natural log of the odds of success

XT = the explanatory/independent variables

β = the regression co-efficient

Y = poverty status

The dependent variable was a dichotomous variable depicting the respondent' poverty status and takes the value of 1 if the respondent was poor and 0 if the respondent was not poor. The independent variables were the socio-economic factors. The hypothesized independent variables were:

X1 = Gender (Female/ Male)

X2 = Age (years)

X3 = Marital status (Married/ Single/ Widowed/Divorced)

X4 = Education level (Primary/ Secondary/ Tertiary/ No formal education)

X5 = Farming experience (years)

X6 = Household size (number)

X7 = Experience of flood shock (dummy; 1= affected, 0 = otherwise)

X8 = Farm income (₦)

X9 = Farm land ownership status (dummy; 1 = owned, 0 = otherwise)

X10 = Access to credit (dummy; 1 = yes, 0 = otherwise)

The Foster-Greer-Thorbecke (FGT) model was used to analyze the poverty status of the sugar cane farmers. FGT poverty index was used to measure the poverty status among the rural farming households and is given as:

$$P_\alpha (y, z) = \frac{1}{2} = \sum_{i=1}^{q} \left(\frac{z-yi}{z} \right) \qquad \text{(Eq. 6)}$$

Where n= total number of households in population

q= number of poor households

z= the poverty line for the household

yi= household income

α= poverty aversion parameter and it takes on value 0, 1, 2

$(\frac{z-yi}{z})$ = proportion shortfall in income below the poverty line

Determining the poverty index

When α= 0 in FGT, the expression become

$$P_\alpha (\frac{1}{n}) q = (\frac{1}{n}) \qquad \text{(Eq. 7)}$$

This is called incidence of poverty or headcount index, which measures the proportion of the population that is poor i.e falls below the poverty line (Aseel, 2017).

When α= 1 in FGT, the expression becomes:

$$P_1 = \frac{1}{2} = \sum_{i=1}^{q} \left(\frac{z-yi}{z} \right) \qquad \text{(Eq. 8)}$$

This is called poverty depth or poverty gap index, which measures the extent to which individuals fall below the poverty line as a proportion of the poverty line.

When α= 2 in FGT, the expression becomes:

$$P_2 = \frac{1}{2} = \sum_{i=1}^{q} \left(\frac{z-yi}{z} \right)^2 \qquad \text{(Eq. 9)}$$

This is called poverty severity index which measures the squares of the poverty gaps relative to the poverty line. To aid in cross-country comparisons, in 2008 the World Bank revised its international poverty line to $1.25/day at 2005-based purchasing-power parity and according to the report of the National Bureau of Statistics, this will help to design holistic policy in poverty alleviation, (Ravallion et al., 2009; NBS, 2013).

Construction of poverty line: Using the two-third mean per-capital income as the benchmark, which was used from the studies conducted by (Igbalajobi, et al., 2013), it was done to divide the respondents into poor and non-poor categories. Households are considered poor if their mean per-capital income is below the poverty line, whereas non-poor households are those whose mean per-capital income is above the benchmark.

Per-capital income (PCI) = Income/Household size

Total per-capital income (TPCI) = Summation of PCI

Mean TPCI = $\frac{TPCI}{Total\,number\,of\,Households}$ = MTPCI

Poverty Line (PL) = $^2/_3$ x MTPCI (Igbalajobi, et al., 2013).

3. Results and Discussion

3.1. Socioeconomic and Demographic Characteristics of the Sugarcane Farming Households

Table 1 gives a summary of the socioeconomic characteristics of the sugarcane farming households in the study area. The modal age is 36-40years. The result indicates that most of the respondents were still in their active age. This helps in increasing production through adoption of modern technologies and coping strategies for farming.

3.1.2 Distribution of respondents by gender

The study reveals that only 6% of the farmers are female while 94% farmers are male. This implies that the sugar cane farming in the study area is dominated by males. This result agreed with Olutunmise and Ajibefun, (2019) but in contrast to the study conducted by Ehinmowo et al. (2017). About 83% of the respondents are married. This was consistent with the research by Ehinmowo et al. (2017), which suggests that they are responsible enough to care for their families. This may also be the case given how strongly encouraged early marriage is in the research area. The largest number of people per household was 25, with an average household size of 8 people. Due to the vast family systems that are prevalent there and the demand for family work, the study area has a rather high family size. Only 48% of the respondents had tertiary education, while up to 27% had no formal education at all. About 63.33% of the farmers had farming experience between 1-5years, 28.33% had farming experience between 6-10years, and 4.17% had farming experience between 11-15years while 0.83% had more than 20years of farming experience. This implies that majority of the respondents were experienced farmers. Years of farming experience of a farmer contributes to his ability to manage his holding efficiency through trial and error. Thus, the higher the experience of a farmer, the higher the adoption rate of new technology will be.

Among the homes growing sugarcane, just 42% claimed membership in a farmers' group. The research area had a low average level of association membership among sugarcane farmers. The ineffectiveness of the associations, according to several farmers, has prevented them from joining any organization. The respondents, or about 48% of them, had access to non-agricultural income. Trading, motorcycle transportation, and other non-agricultural sources of income are available. Approximately 21% of those surveyed had access to credit. This suggests that the sugarcane growers in the study area have extremely limited access to formal loans. The survey also revealed that only a very small portion of loans sought were really used to finance farm operations, with some likely going toward home consumption.

Table 2 reveals the cost of damage by the flood, the income before the flood and after the flood. The income class was grouped based on their respective income. High income class represents those farmers with annual income of more than ₦500000, while the medium of the middle class are those with income between ₦100000 and ₦500000 and those with less than ₦100000 were classified as low income class (Igbalajobi et al., 2013). The result shows that the average cost of damage stood at ₦124893.35. The average income before damage was ₦1468741.36 and after flood was ₦1022847.51. The net loss as a result of flood is ₦445,893.85.

Table 1. Socioeconomic and Demographic Characteristics of the Respondents

Age (years)	Affected Frequency	Percentage (%)	Non-Affected Frequency	Percentage (%)	Pooled Frequency	Percentage (%)
<21	2	2.94	1	1.92	3	2.50
21-25	8	11.76	5	9.62	13	10.83
26-30	4	5.88	8	15.38	12	10
31-35	20	29.41	9	17.31	29	24.17
36-40	23	33.82	13	25	36	30
>40	11	16.18	16	30.77	27	22.50
Total	68	100	52	100	120	100
Gender						
Male	64	94.03	49	94.23	112	94.12
Female	4	5.97	3	5.77	7	5.88
Total	67	100	52	100	119	100
Marital status						
Single	8	15.38	7	10.29	15	12.50
Married	39	75	60	88.24	99	82.50
Divorced	5	9.62	1	1.47	6	5
Total	52	100	68	100	120	100
Household Size						
≤5	11	16.18	4	7.69	15	12.50
6-10	53	77.94	46	88.64	99	82.50
Above 10	4	5.88	2	3.85	6	5
Total	68	100	52	100	120	100
Education						
No Formal Education	13	19.12	19	36.54	32	26.67
Primary Education	1	1.47	3	5.77	4	3.33
Secondary Education	15	22.06	11	21.15	26	21.67
Tertiary Education	39	57.35	19	36.54	58	48.33
Total	68	100	52	100	120	100
Experience						
1-5	42	61.76	34	65.38	76	63.33
6-10	18	26.47	16	30.77	34	28.33
11-15	3	4.41	2	3.85	5	4.17
16-20	4	5.88	-	-	4	3.33
Above 20	1	1.47	-	-	1	0.83
Total	68	100	52	100	120	100

Source: Field Survey, (2019)

Table 2. Damage/Loss incurred by the respondents

Income class	Income before flood (₦)	Damage by flood (₦/ha)	Income after flood (₦/ha)
High	2125768.95	165280.55	1753320.69
Medium	906457.30	146234.20	623384.47
Low	150303.25	56720.22	32476.29
Average	1468741.36	124893.35	1022847.51

Source: Field Survey, (2019).

3.1.3 Analysis of poverty status of the respondents using FGT Poverty Index

Table 3. Summary of the Poverty Indices for the Respondents in the Study Area.

Poverty	Index
P_0	0.46
P_1	0.16
P_2	0.07

Source: Field Survey, 2019.

A proper assessment of poverty must include the measurement of the poverty line as well as three fundamental components, which are the head count ratio or poverty incidence (P_0), depth or gap of poverty (P_1), and severity or intensity (P_2) of poverty, as indicated in *Table 3 above*. The extent to which the household's or an individual's per-capita income is below the poverty line reflects this. The 120 respondents' combined per capita income was ₦9940909, and the mean per capita income was ₦6904.09. The median per capita income of the household, which was ₦46025.03, was used to calculate the poverty line. However, a household's income that is below the poverty line is considered to be poor, but a household's income that is exactly above the poverty line is considered to be non-poor.

As a result, the head count ratio or poverty incidence (P_0) was 0.46 with a poverty line of ₦46025.03. This suggests that 46% of the respondents in the research area were relatively poor and living below the poverty line. This was consistent with the findings of Ehinmowo et al. (2017). The poverty level or gap (P_1) was 0.16. This value shows that 16% of the respondents fell below the poverty line and needed to increase their income in order to reach the poverty line. In other words, a typical low-income sugarcane farmer would need to earn 16% of the poverty level in order to escape poverty. Severity or intensity of poverty (P_2) was 0.07. According to the data in the table above, this value indicates that 7% of the respondents in the study were extremely poor.

Table 4: Factors Affecting Poverty Status of Respondents using Probit Regression Model

Explanatory Variables	Coefficients	Marginal Effects	Standard Error	z	$P > |z|$
Experience of flood shock	0.107**	0.021	0.011	2.06	0.039
Age(years)	-0.036***	-0.007	0.003	-2.45	0.014
Gender	0.631	0.127	0.101	1.26	0.208
Years of schooling	-0.008	-0.002	0.007	-0.22	0.823
Household size	0.104**	1.423	0.699	2.04	0.057
Access to credit	0.212	0.039	0.071	0.56	0.576
Sugar cane farm income	-0.001**	-7.91^{-06}	0	-2.02	0.044
Organization membership	2.102	0.427	0.076	5.6	0
Constant	0.59	-	0.942	0.63	0.531

Note: ***' **' * indicates significance levels at 1% , 5%, 10% respectively; Number of observation= 120; Pseudo R^2=0.187, Log likelihood = -93.305, Prob> chi^2 = 0, Wald chi^2 (7) = 40.23; Dependent variable (Poor=0 and Non poor=1)
Source: Field Survey, 2019.

To determine the association between certain socioeconomic features of the respondents and their level of poverty, the Logit regression analysis was used. The coefficient and odd ratio value of the experience of flood shock are shown in *Table 4*, and they are positive and significant at the 5% level. The outcome suggests that as flood shock increases, there is a greater chance that study participants may become destitute. Additionally, at a 1% level of significance, the respondents' age coefficient is adversely significant. This suggests that the likelihood of becoming poor will decrease by 0.7% for every unit increase in respondents' age. The coefficient of agricultural income is shown in the table as negative and significant at the 5% level. It suggests that the likelihood of becoming will drop by 0.1% for every unit rise in farm income. This suggests that a rise in agricultural revenue will lessen the likelihood of poverty. The outcomes from Olutunmise and Ajibefun (2019) and this result concur. The likely

cause is that, as Shiferaw and Holden (1998) and Igbalajobi et al. (2013) also noted, farmers with greater money and assets are more likely to adopt innovative farming techniques.

A 5% level of significance was used to determine the significance of household size. The findings suggest that when household size increases, there is a greater chance that the respondents would become impoverished. This can be due to a rise in household members' needs and efforts to improve the family's standard of life.

At the 1% level of significance, the coefficient of social organization membership was similarly positive and significant. The findings suggested that a growth in social organization membership would increase the respondents' likelihood of being impoverished. In the study area, social capital is anticipated to have an impact on poverty. Other estimated variables were discovered not to have a substantial impact on respondents' participation choices.

Table 5: Factors that predispose the respondents to Flood

Predisposing Factors	Affected Frequency	Percent (%)	Pooled Frequency	Percent (%)
Time of planting	44	36.67	76	63.33
Inadequate government assistance	53	44.17	67	55.83
Inadequate finance authority assistance	55	45.83	65	54.17
Insufficient fund	52	43.33	68	56.67

Source: Field Survey, 2019.

From *Table 5*, result shows that 36.67% of the respondents claimed that time of planting predisposes them to flood, about 44.17% of the respondents claimed that inadequate government assistance predisposed them and about 45.83% claimed that inadequate financial authority support predisposed them to flood. The result indicates that most of the affected respondents were predisposed by a factor or two.

Table 6: Control measures adopted by the respondents

Control Measures	Affected Frequency	Percent (%)	Pooled Frequency	Percent (%)
Seeking help from friends and relatives	50	41.67	70	58.33
Involvement in non-farming activities	45	37.50	75	62.50
Purchasing food on credit	46	38.33	74	61.67
Others	4	3.33	116	96.67

Source: Field Survey, 2019.

Table 6 shows the control measures adopted by the respondents. About 42% of the affected respondents claimed that they seek help from family and friends. Also, 37.5% of the respondents claimed that they engage in one non-farming activity or the other in order to survive, 38.3% of the respondents purchased food on credit while 3.33% adopted other forms of coping strategies such as selling of farm asset, personal belongings among others. The findings, which concurred with that of (Opondo, 2013; Olutunmise and Ajibefun, 2019) on the use of corrosive coping techniques against flood, showed that the majority of the flood-affected respondents used one or more erosive control measures to mitigate the impact of the flood on their agricultural enterprise.

People's agency, resourcefulness, and capacity to occasionally provide a hand to one another both individually and collectively in order to achieve their fundamental demands are referred to as coping mechanisms (Wisner et al., 2003). They emerge from an understanding of the possibility of an event happening and pre-existing structures of reaction. They aim to maintain not only their own existence but also other human requirements like getting honor and respect as well as the coherence of the family, household, and society. It was confirmed in the area that some of the respondents purchased necessities on debt and paid it back when they sold their farm products at the end of the growing season.

Another frequent method by which agricultural households in the study area cope with the effects of poverty is borrowing money from cooperatives. According to the respondents, the majority of farmers got credit or loans from the cooperative society at the beginning of the season and paid them back at the end of the production season

in cash or kind. Because they are more susceptible to uncertainty and risk than other industries, such as producers of sugarcane, tree crops (particularly cocoa), arable crop farmers find it difficult to borrow money from commercial banks and occasionally from cooperative societies. Children were being transferred from private to public schools, and the local farms were being sold off. Even though there is poverty in the neighborhood, once people see the benefit of their chosen course of action, they employ a variety of techniques to cope with poverty syndrome.

Integrated disaster control measure.

• Capacity building including research and knowledge management.
• Preparedness to deal with any disaster.
• Prevention of danger or threat of any disaster.
• Prompt response to any threatening disaster situation or disaster.
• Assessing the severity or magnitude of effects of any disaster.
• Evacuation, rescue and relief.
• Rehabilitation and reconstruction *(National Policy on Disaster Management (NPDM))*

4. Conclusions

The study investigates the effect of flood on sugar cane farming households across Nigeria, using the lower river Niger basin area in Kwara State, and some sugar cane producing villages in Osun State as case of study. The outcome indicates that the poverty incidence (P_0) was 0.46, indicating that 46% of respondents in the research area were moderately poor and lived below the poverty line. The poverty depth (P_1) was 0.16, which also shows that 16% of the respondents were below the poverty line and will consequently need an increase in their income to do so. In other words, a typical low-income sugarcane farmer would need to earn 16% of the poverty level in order to escape poverty. The final measure of poverty (P_2) was 0.07. This number indicates that 7% of study respondents were extremely impoverished. Even though sugarcane is one of the crucial commodities that can help reduce poverty, the majority of farmers still struggle to make ends meet. The analytically most likely causes of poverty were emphasized as being flood shocks, gender, household size, not having access to finance, and membership in social organizations. These factors were also potential contributors to poverty. While the respondents' age, years of education, and farm income were statistically related to their lack of poverty. This means that women should be given the opportunity and the capacity to work in agriculture. It will be more facile to embrace new technology and coping mechanisms if more people have education, as 48% of the respondents claim to have. Increased loan availability or subsidies for agricultural supplies can help farmers earn more money relative to their outlays. For maximum efficacy, government initiatives to encourage the adoption of contemporary technologies should be combined with support services including education and training, extension services, credit, and market accessibility. The government should make news concerning climate change accessible so that farmers can plan their planting schedule and be protected from calamities brought on by climate change. The government ought to make available information on flood forecasting as well. In order to minimize human activities that exacerbate flood impacts in the River Niger basin, we advise an integrated watershed management policy for environmental preservation.

The policy should concentrate on increasing food production and environmental sustainability through land use afforestation, soil erosion control, and water management systems (which should include dams to control the flow of rivers and gather floodwater for agricultural, household, and industrial use). This will significantly reduce the number of fatalities caused by floods and other natural calamities (Opondo, 2013).

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Akinsola G.O., Ayinde O.E., Adewale E.T.; Design: Adewale E.T.; Data Collection or Processing: Adewale E.T.; Statistical Analyses: Adewale E.T.; Literature Search: Adewale E.T.; Writing, Review and Editing: Adewale E.T., Ayinde O.E., Akinsola G.O.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Seasonal Quality of Some Cool-Season Turfgrass Species in Cold Semi-Arid Climate

Bazı Serin İklim Çim Türlerinin Soğuk Yarı-Kurak İklimde Mevsimsel Kalitesi

Onur İLERİ[1*], Yasin ALTAY[2], Ali KOÇ[3]

Abstract

The study was carried out to determine winter hardiness, establishment speed, and seasonal variation in quality characteristics of some turf cultivars belonging to the common cool-season turf species in cold semi-arid climates. Nineteen different cultivars of perennial ryegrass, tall fescue, red fescue, Chewing's fescue, slender creeping red fescue, and Kentucky bluegrass were examined for six seasons in 2014-2016 years. The experiment was arranged in a randomized complete block design with three replications. The score-based measurements of turf texture, color, and general quality were taken in the middle of every season (2 summers, 2 springs, and 2 falls) for 2 years and the data were analyzed using non-parametric tests. Results showed that Stravinsky (perennial ryegrass) and Cardinal (red fescue) greatly lost their dark green color in autumn, while SR8600 (tall fescue) kept its satisfying green color. General quality decreased as the seasons proceeded, but Rosita (slender creeping red fescue) remained in high quality considering other red fescue species. Low adaptation ability was observed in Miracle and Evora cultivars of Kentucky bluegrass species due to their poor winter hardiness scores. These cultivars are also the slowest establishing among other cultivars. Results stated that Stravinsky, Esquire, Kokomo, Grandslam2 (perennial ryegrass), Maximal (red fescue), and Rosita (slender creeping red fescue) maintained high quality throughout summer and spring but decreased in autumn. However, Forte (tall fescue) and Evora (Kentucky bluegrass) presented better quality in autumn but the quality of Evora decreased in the next year. These cultivars should be used in a mixture to maintain high-quality turf through spring, summer, and autumn but Evora could require overseeding in some years. More and especially newly released cultivars should be tested to increase the turf quality of the mixtures.

Keywords: Cool-season, Cultivars, Seasonal variation, Turf quality, Turf species

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Öz

Bu çalışmada bazı yaygın serin iklim çim türlerine ait çeşitlerin yarı-kurak iklim koşulları altında kışa dayanıklılık tesis olma hızı ve çim kalitesi özelliklerinin mevsimsel değişimleri incelenmiştir. Çok yıllık çim, kamışsı yumak, kırmızı yumak, rizomlu kırmızı yumak, narin kırmızı yumak ve çayır salkımotu türlerine ait 19 farklı çeşit 2014-2016 yılları arasında incelenerek 6 mevsimde veri toplanmıştır. Deneme şansa bağlı tam bloklar deneme deseninde üç tekerrürlü olarak tasarlanmıştır. Çim tesisinde tekstür, renk ve genel kalite değerlerini belirlemek için skor temelli gözlemler her mevsimin (2 yaz, 2 güz, 2 bahar) ortasında yapılmıştır. Elde edilen skor verileri parametrik olmayan testler kullanılarak analiz edilmiştir. Bulgular tüm çeşitlere ait çim kalite özelliklerinin farklı mevsimsel değişim gösterdiğini ortaya koymuştur. Stravinsky (çok yıllık çim) ve Cardinal (kırmızı yumak) çeşitleri güz mevsiminde koyu yeşil renk özelliklerini büyük oranda kaybederlerken SR8600 (kamışsı yumak) çeşidi aynı mevsimde yeşil rengini korumuştur. Genel kalite yıl içerisinde bahardan güze doğru azalış eğilimde olurken Rosita çeşidi diğer kırmızı yumak çeşitlerine göre yüksek kaliteli tesis özelliğini korumuştur. Çayır salkımotu türüne ait Miracle ve Evora çeşitlerinin özellikle çok düşük kış dayanımı skorları nedeniyle yarı-kurak iklime adaptasyon yeteneklerinin çok düşük olduğu görülmüştür. Bu çeşitler aynı zamanda en yavaş tesis olmuşlardır. Stravinsky, Esquire, Kokomo, Grandslam2 (çok yıllık çim), Maximal (kırmızı yumak) ve Rosita (narin kırmızı yumak) çeşitleri bahar ve yaz mevsimi boyunca yüksek kaliteli tesis oluştururken güz ile birlikte görsel kalitede düşüşler tespit edilmiştir. Çayır salkım otu (*Poa pratensis*) türünün Forte ve Evora çeşitleri ise güz mevsiminde yüksek kalitede kalmışlardır ancak Evora çeşidinin kalitesi tesis sonrası 2. yılda düşüşe geçmiştir. Sonuçlar yıl boyu yüksek kalitede çim tesisi oluşturmak için bu çeşitlerin karışımlar halinde kullanılmasının daha uygun olduğunu göstermektedir. Evora çeşidi karışıma dahil edildiğinde 2. yıl üstten tohumlama yapılması gerekli görülmektedir. Karışımlarla tesis edilen çim alanların yarı-kurak iklimlerde yıl boyu yüksek kalitede tutulabilmesi için yeni tescil olan çeşitlerin de denenmesi faydalı olacaktır.

Anahtar Kelimeler: Çeşit, Çim kalitesi, Çim türleri, Mevsimsel değişim, Serin iklim

1. Introduction

The stress of daily life is challenging modern society in big cities and they are seeking greenness, especially turf areas, to blow off steam. These areas mostly consist of grasses and besides increasing the quality of human life (Russi et al., 2004; Ayanoglu and Orta, 2019), turf grasses are also fundamental for recreational areas, sports fields, landscapes, (Braun et al., 2021), and even for the conservation of soil from erosion (Ahn and Choi, 2013). Many other benefits were also stated by researchers as increasing the value of the property, providing aesthetics and cooling, mitigating runoff in urban, absorbing noise and pollutants, etc. (Stier et al., 2013; Monteiro, 2017; Balci and Orta, 2018). With the advancing civilization, turf areas are increasing in cities, and ensuring the high quality of these areas all year round is one of the most challenging issues for turf managers.

Maintaining high-quality turf is an issue where temperature significantly changes within a year like in temperate climates. In such areas, the seasons are generally distinct and the visual quality of turf grasses, especially color, may decline critically in response to a significant decrease or increase in temperature (Pinnix et al., 2018; Chen et al., 2021). Plant color is associated with chlorophyll content (Zhang et al., 2020) and high or low temperatures could cause dormancy in turf grasses, which leads to a decrement in leaf chlorophyll content (He and Huang, 2010). The turf quality of the species could seasonally change due to climatic variations, even among the cool-season species (Prokopiuk et al., 2019). Besides, Kir et al. (2010) indicated this seasonal variation in turf quality could differently occur among cultivars of the cool-season turf species. Therefore, turf performances of new cultivars could be investigated all year round.

Changing climate challenges all plants including turf species and thereby, the response of the species could change both spatially and temporally in the same ecology (Hatfield, 2017). Climatic variations triggered the release of new turf cultivars through increased breeding studies in the past decade, especially in countries like the USA, Canada, and the UK, where the turf industry advanced (Martiniello and D'Andrea, 2006; Meyer et al., 2017). There are plenty of turf species and cultivars in the market and the adaptation abilities of these species and cultivars should be tested under various environments because genotype × environment interaction significantly affects the turf quality (Russi et al., 2004; Gouveia et al., 2020). Turf managers prefer cultivars that are slowly growing, require low inputs, are tolerant of diseases, and are maintaining high quality (Hull et al., 1994; Christians et al., 2016). Cultivars belong to the cool-season species such as perennial ryegrass (PR), red fescue (RF), tall fescue (TF), and Kentucky bluegrass (KB) are highly preferred due to their high quality in Mediterranean countries (Martiniello and D'Andrea, 2006), North Europe (Sampoux et al., 2013), USA (Bonos and Huff, 2013), and in Turkey (Kir et al., 2010; Kara et al., 2020).

Turf quality is generally evaluated over visual parameters such as color and texture (Macolino et al., 2003; Bilgili and Acikgoz, 2011), but winter hardiness, density, and establishment speed are also important for the sustainability and functionality of these areas and they were included into turf measurements by researchers (Steinke and Stier, 2003; Hulke et al., 2007; Magni et al., 2014). Morris and Shearman (1998) revealed a rating-based method for the estimation of turf quality parameters and suggested the rating of color, density, texture, winter hardiness, etc., visually based on a 1-9 scale. This method is used by many researchers (Martiniello and D'Andrea, 2006; Sikorski et al., 2018; Prokopiuk et al., 2019; Ozkan and Kir, 2021) but analyzing these rating data is not proper by parametric tests because discrete data as in turf quality rates do not fulfill the assumptions of parametric tests as variance homogeneity and normal distribution of data. Therefore, it is suggested that nonparametric statistical tests can be useful to compare these data sets (Zar, 2013).

This study was conducted to determine the seasonal variation of turf quality in cold semi-arid climate conditions of Central Anatolia. Nineteen different cultivars belonging to the most common cool-season turf species were examined for two years and the data were analyzed using non-parametric tests.

2. Materials and Methods

2.1. Plant materials and study area

In the experiment, common cultivars of some cool-season turf grass species as perennial ryegrass (*Lolium perenne* L.) (PR), tall fescue (*Festuca arundinacea* Schreb.) (TF), red fescue (*Festuca rubra* ssp. *rubra*) (RF), slender creeping red fescue (*F. rubra* ssp. *trichophylla*) (SF), Chewing's fescue (*F. rubra* ssp. *commutata*) (CF), and Kentucky bluegrass (*Poa pratensis*) (KB) were used as plant turf materials. Cultivar names are given in *Table 1*.

Table 1. Cultivar names of the common turf grass species used in the experiment

Species	Cultivar	Species	Cultivar	Species	Cultivar
L. perenne	Stravinsky	*F. arundinacea*	SR8600	*F. rubra* ssp. *rubra*	Corail
	Esquire		Tomcat		Maximal
	Bizet-1		Avenger		Cardinal
	Kokomo		Forte	*F. rubra* ssp. *trichophylla*	Rosita
	Capri	*P. pratensis*	Miracle	*F. rubra* ssp. *commutata*	Maritza
	Grandslam2		Arrowhead		
	Monarch		Evora		

The study was carried out for two years from spring 2014 to spring 2016 in the experimental field of Eskisehir Osmangazi University, Faculty of Agriculture, which has an altitude of 795 m above sea level and was located within the borders of Eskisehir province, Türkiye. The long-term average (LTA) of precipitation is 352.4 mm for Eskisehir and it mostly falls as snow in winter due to temperature averages near or below zero (*Figure 1*). The cold semi-arid climate condition (BSk) prevails in the region according to the Koppen-Geiger classification method and the four seasons are distinct. Seasonal and long-term temperature averages are given in *Figure 1*. The soil of the experimental area has loamy characteristics with an 8.30 pH value, 0.49 dSm^{-1} EC, 3.02 % CaCO3, and 1.67% organic matter (Gozukara et al., 2022).

Figure 1. Seasonal variation of the temperature averages between the years 2014-2016 and LTA

2.2. Field studies and data collection

The seedbed was prepared by rotator and the soil was graded using the garden rake on 10 April 2014. Plots were prepared with the size of 2 x 2m ($4m^2$) and the experiment was arranged in a completely randomized block design with three replications. Cultivars of cool-season turf species were considered as treatment. Sowing was made into the plots by broadcast sowing on 11 April 2014 using the seeding rates of 25 g m^{-2} for KB and 40 g m^{-2} for the cultivars of the other species (Martiniello and D'Andrea, 2006). Nitrogen and P_2O_5 fertilizers were applied at the rates of 30 kg ha^{-1} and 72 kg ha^{-1} respectively while sowing (Martiniello and D'Andrea, 2006), and once turf was established, nitrogen fertilizer was applied in 6 rounds in a year (April, May, June, July, August, September) at the rate of 5 g m^{-2} (Bilgili and Acikgoz, 2007). After sowing, plots were covered at 1-2 cm thick of topsoil mixture containing 20% sieved-composted manure, 10% sand, and 70% silt loam soil and compressed using a lightweight roller to provide better establishment. Irrigation was applied by mini-sprinklers depending on the plant's requirements and with nearly 1-2 days intervals. Weeds were removed by hand. Plots were mowed from the height of 2-3 cm when they reached the height of 7-8 cm approximately (Demiroglu et al., 2010a).

Turf color, turf texture, general quality, and winter hardiness were assessed considering the 1-9 scale, and establishment speed was evaluated over coverage percent, which was suggested by the National Turf Evaluation

Program (NTEP) in the USA (Morris and Shearman, 1998). This program suggests the lowest rate (1) as very poor quality and the highest rate (9) as very good quality. Quality ratings were done visually on a seasonal basis (except winter) from the summer of 2014 to the spring of 2016 and every plot was rated in the middle of the seasons. Winter hardiness was rated in early spring by observing plant death and the cover rate was determined using a 1m transect, and a 75% cover rate was assumed as "established turf".

2.3. Statistical analyses

The study was designed in randomized plots design with three replications. The data were obtained using the visual rating method and they did not fulfill the assumptions of parametric tests as variance homogeneity and normal distribution (P<0.05). Therefore, data were analyzed using non-parametric tests in SPSS 18 software. Seasonal variation in turf quality parameters of the cool-season cultivars was analyzed by the Friedman test (P<0.05) because they are dependent, and the variations of winter hardiness and establishment speed among the cultivars were analyzed by Kruskal Wallis (P<0.05) test because the data are independent (Zar, 2013). Winter hardiness was analyzed considering two years and establishment speed was analyzed considering cultivars only. The means of the groups were compared using the Bonferroni-Dunn test (P<0.05).

3. Results and Discussion

3.1. Turf color

The turf color score of the cultivars varied between 4.65 – 5.67 and the seasonal variations were significant for Stravinsky and Esquire (PR), SR8600, Avenger, Forte (TF), Maximal, and Cardinal (RF), Rosita (SF), Miracle, Arrowhead, Evora (KB) (Table 2). Seasonal variation belonging to 8 out of 19 species was not statistically significant (P<0.05).

Turf color scores of PR and TF species were generally higher in spring 2015 and lower in summer seasons, but the fine fescue species (RF, SF, CF) presented the best turf color in summer 2015 (*Table 2*). KB species had the best results in autumn 2014 and spring 2015 but thereafter, significant decreases were observed (*Figure 2*). Turf color tended to decrease as the seasons advanced for all cultivars and the lowest color scores were recorded in the spring of 2016.

Turf grass species are well adapted to spring seasons in Mediterranean environments (Martiniello and D'Andrea, 2006). Well-adapted species present better color scores because turf color has a linear correlation with plant health and photosynthetic activity (Bell et al., 2002). Besides color is one of the most important visual parameters for both sports fields and recreational areas. In our study, PR and TF cultivars had a higher color score in spring, and Esquire and SR8600 cultivars showed good color performance (*Figure 2*). Bilgili and Acikgoz (2011) stated that cool-season turf mixtures had better color scores in spring, which was similar to our findings. However, the high color scores in spring greatly decreased in 2016, especially for Stravinsky and Esquire cultivars of perennial ryegrass (*Table 2*). This may be due to the stress caused by temperatures in 2016 (*Figure 1*) when the plants was growing rapidly. It is known that the response of the cultivars might be different even belong to the same species (Kir et al., 2010; Demiroglu et al., 2010b). There were different seasonal variations among the TF cultivars in terms of color but a slight decrement was observed for Avenger (*Figure 2*). Fine fescue species presented a higher seasonal variation in turf color and it was the lowest in autumn, especially in the first year (*Figure 2*). This might be due to the low adaptation of the species in the first year, but they presented better turf color in the autumn of 2015 (*Figure 2*). Wolski et al. (2021) stated fine fescue species; especially RF have better color scores in summer, which was similar to our findings (*Figure 2*). Arrowhead cultivar showed partly different seasonal variations in the second year considering other KB cultivars. Researchers stated different results for the seasonal variation of KB that indicate better color in autumn (Wolski et al., 2021), or in summer (Gul, 2015). Indeed this is due to ecological differences. In our study, cultivars of KB had the highest color score in the first year but in the second year, Miracle and Evora showed critical decreases (*Figure 2*). This might be due to their low adaptation abilities to cold semi-arid climate conditions.

Table 2. Turf color scores of the cultivars in different seasons

Cultivars	Summer14	Autumn14	Spring15	Summer15	Autumn15	Spring16	Mean	P
Stravinsky	8.33a	2.33c	8.33a	5.00bc	3.00c	3.67c	5.11	**0.041**
Esquire	7.00ab	5.67c	8.33a	6.33bc	2.33d	3.67d	5.56	**0.021**
Bizet-1	5.67	6.67	5.67	5.67	3.67	4.33	5.28	0.205
Kokomo	7.00	5.00	7.00	5.00	3.00	6.33	5.56	0.216
Capri	4.33	6.33	5.00	5.00	3.67	4.33	4.78	0.294
Grandslam2	7.00	4.33	8.33	5.67	3.67	5.00	5.67	0.221
Monarch	4.33	7.33	7.00	5.67	2.33	4.33	5.17	0.061
SR8600	4.33b	6.33a	7.00a	1.67b	7.00a	2.33b	4.78	**0.019**
Tomcat	4.33	5.33	7.00	1.00	5.00	5.00	4.61	0.092
Avenger	5.33b	4.00bc	7.67a	1.00d	5.67bc	3.67c	4.56	**0.041**
Forte	5.67bc	4.33c	7.00ab	1.00d	7.67a	3.67c	4.89	**0.021**
Corail	3.67	3.33	6.33	7.67	5.00	6.33	5.39	0.154
Maximal	6.00b	1.67c	5.67b	9.00a	5.00b	5.67b	5.50	**0.035**
Cardinal	6.33b	1.00d	5.67bc	9.00a	6.33b	5.00bc	5.56	**0.024**
Rosita	5.33b	2.00d	5.00b	9.00a	4.33bc	5.00b	5.11	**0.028**
Maritza	6.00	3.33	5.67	7.00	5.67	5.00	5.45	0.259
Miracle	5.67bc	8.67a	7.67ab	4.33c	1.67d	1.00d	4.84	**0.010**
Arrowhead	6.67a	7.67a	7.67a	1.67c	3.00bc	5.00b	5.28	**0.029**
Evora	6.67bc	9.00a	7.67ab	4.33cd	1.67de	1.00e	5.06	**0.007**
Mean	5.77	4.96	6.83	5.00	4.19	4.23	5.16	

Figure 2. Seasonal variation in turf color scores belongs to the cultivars of a) PR b) TF c) RF, SF, CF d) KB

3.2. Turf texture

The mean texture score of the cultivars varied between 3.17 - 7.61 (*Table 3*). Only Stravinsky (PR), SR8600, and Forte (TF) had significant seasonal variation among them. The texture score of these cultivars was similarly higher in summer and lower in autumn but Stravinsky showed lesser variation than SR8600 and Forte (*Figure 3*).

Turf texture defines the overall width of the leaves and it is preferred to be between 1.5 – 3.00 mm for better visual quality (Beard, 1973; Salman et al., 2019). None of the cultivars used in this study had very coarse or very fine texture

scores, considering the suggestions of NTEP for turf texture, where 1=very coarse and 9=very fine (*Table 3*). Texture differences are not only observed on a cultivar basis but a seasonal basis (Salman et al., 2011; Gul, 2015). In our study, texture scores of Stravinsky, SR8600, and Forte showed a significant seasonal variation. Turf grasses produce new leaf tissues after every mowing as long as the environmental conditions are favorable and the significant seasonal variation in texture score might be related to their rapid adaptation abilities to season-based changes in environmental conditions. However, lesser variation is preferred in green areas as observed in Stravinsky (*Figure 3*).

Table 3. Texture scores of the cultivars in different seasons

	Seasons							
Cultivars	**Summer14**	**Autumn14**	**Spring15**	**Summer15**	**Autumn15**	**Spring16**	**Mean**	**P**
Stravinsky	5.67b	3.00d	5.00bc	7.67a	4.33c	4.33c	5.00	**0.023**
Esquire	5.67	3.67	5.67	6.67	4.33	5.00	5.17	0.064
Bizet-1	3.67	3.67	7.00	3.67	5.00	5.00	4.67	0.075
Kokomo	5.00	3.67	5.67	6.33	5.00	4.33	5.00	0.288
Capri	3.00	3.67	5.67	6.33	5.67	4.33	4.78	0.203
Grandslam2	5.00	4.33	6.33	6.33	5.00	4.33	5.22	0.132
Monarch	3.67	5.00	7.00	6.33	7.00	6.33	5.89	0.352
SR8600	5.00b	1.00c	3.00bc	8.33a	1.00c	1.00c	3.22	**0.010**
Tomcat	3.00	1.00	4.33	7.67	2.33	2.33	3.44	0.087
Avenger	2.33	1.00	3.67	8.00	1.67	2.33	3.17	0.077
Forte	1.00d	1.00d	4.33ab	8.67a	1.00d	3.00bc	3.17	**0.017**
Corail	4.33	5.00	7.00	5.67	7.00	7.00	6.00	0.094
Maximal	5.67	6.33	7.67	4.67	7.00	8.33	6.61	0.065
Cardinal	7.00	6.33	7.67	6.00	7.00	7.00	6.83	0.887
Rosita	7.00	7.00	8.33	7.33	8.33	7.67	7.61	0.174
Maritza	6.33	7.00	8.33	7.00	7.00	7.67	7.22	0.459
Miracle	3.67	3.00	7.67	4.67	5.00	3.67	4.61	0.095
Arrowhead	2.33	4.33	4.33	6.00	3.00	1.00	3.50	0.061
Evora	3.67	3.67	7.00	5.00	5.00	4.33	4.78	0.230
Mean	4.37	3.88	6.09	6.44	4.82	4.68	5.04	

Figure 3. Seasonal variation in texture scores of Stravinsky, SR8600, and Forte

3.3. General turf quality

The general quality of the cultivars was determined as 4.98 in Eskisehir ecological conditions. Seasonal variation was significant (P≤0.05) for Stravinsky, Esquire, Kokomo, Grandslam2, Forte, Maximal, Rosita, Miracle, and Evora (*Table 4*). Cultivars of PR and KB showed a decreasing trend in general quality as the season advanced but the variation was irregular for fine fescue cultivars (*Figure 4*). The seasonal variation of the other ten cultivars was not statistically

significant.

The general quality is information about the visual quality of turf grasses. It was defined as the composite of all visual quality parameters such as color uniformity, texture, etc. (Pooya et al., 2013; Salman et al., 2019). NTEP suggested a score-based evaluation using a 1-9 scale and the lowest score indicates very poor, while the highest score indicates the ideal quality (Beard, 1973; Pooya et al., 2013). The general quality of nine cultivars belonging to PR, TF, RF, SF, and KB species showed significant seasonal variation (*Table 4*). Cultivars of PR (Stravinsky, Esquire, Kokomo, Grandslam2) and fine fescue (Forte, Maxima, Rosita) had similar seasonal variation but the quality of Forte, Maxima, and Rosita significantly increased in autumn 2015 unlike the cultivars of PR and Rosita was the only cultivar, which reached to maximum score among the others (*Figure 4*). Researchers stated that cultivars have different quality scores in different seasons because of different adaptation abilities under changing climatic conditions (Kir et al., 2010; Salman et al., 2011; Wolski et al., 2021). Turf quality decreases down to the lowest score if turf species are not adapted to the environment (Demiroglu et al., 2010a). This might be the reason for the continuously decreasing turf quality of Miracle and Evora cultivars in our study. Therefore, these cultivars might be assumed as not adapted to cold semi-arid climates.

Table 4. General quality scores of the cultivars in different seasons

Cultivars	Summer14	Autumn14	Spring15	Summer15	Autumn15	Spring16	Mean	P
			Seasons					
Stravinsky	7.67a	3.33cd	5.00ab	4.33abc	5.00ab	1.67e	4.50	**0.047**
Esquire	8.67a	4.00b	7.67a	4.00b	3.67b	1.00c	4.84	**0.018**
Bizet-1	6.67	5.67	4.00	4.00	4.33	3.00	4.61	0.294
Kokomo	8.00a	5.00bc	7.00ab	3.33c	2.33cd	1.00d	4.44	**0.028**
Capri	7.00	6.33	6.33	5.33	3.00	2.33	5.05	0.078
Grandslam2	8.33a	4.00bc	7.67a	5.00b	5.67b	1.67c	5.39	**0.013**
Monarch	7.33	5.67	8.33	4.00	4.33	1.67	5.22	0.112
SR8600	6.33	4.67	4.33	5.67	6.33	2.33	4.94	0.195
Tomcat	6.67	6.00	5.00	5.67	5.00	3.67	5.34	0.116
Avenger	6.67	5.67	6.33	6.33	5.67	2.33	5.50	0.250
Forte	7.67a	3.33d	5.00c	6.00bc	6.33ab	3.00d	5.22	**0.016**
Corail	5.00	5.00	6.33	3.67	5.67	4.33	5.00	0.191
Maximal	5.67ab	2.33c	6.33ab	3.67c	7.00a	5.00b	5.00	**0.035**
Cardinal	7.33	2.67	7.00	3.67	5.67	5.00	5.22	0.253
Rosita	7.00b	2.00d	7.00b	6.00bc	9.00a	5.67c	6.11	**0.035**
Maritza	7.33	3.33	6.33	4.67	5.67	4.33	5.28	0.087
Miracle	5.67a	6.33a	5.00ab	4.33ab	2.33b	1.00b	4.11	**0.025**
Arrowhead	6.33	7.00	3.67	5.00	3.00	1.67	4.45	0.062
Evora	5.67b	7.33a	7.00a	3.00c	3.00c	1.00d	4.50	**0.016**
Mean	6.90	4.72	6.07	4.61	4.89	2.72	4.98	

3.4. Winter hardiness

Winter hardiness scores of the cultivars showed a significant variation (P≤0.01) and changed between 1.0 and 6.3. Corail, Maximal, and Cardinal had the highest scores but they were statistically in the same group with all cultivars except the cultivars that belong to KB species (*Table 5*). Miracle and Evora had the lowest winter hardiness scores.

Expression of winter hardiness is suggested by NTEP on a 1-9 scale, where 1 indicates 100% winter damage and 9 indicates 0% winter damage on the leaves. In our study, none of the cultivars had the highest winter hardiness score because a 0% winter injury of the leaves is almost impossible for turf grasses in environments, where winter seasons are cold and harsh (Hulke et al., 2007), as in Central Anatolia. However, the winter hardiness of some species, even cultivars, could show significant variations in different environments (Martiniello and D'Andrea, 2006; Varoglu et al., 2015; Alagoz and Turk, 2017). For example, Rimi and Macolino (2014) stated that overseeding an established turf using RF (cv. Corail) increased the winter hardiness of the turf. Corail is one of the cultivars, which had the best winter hardiness score in our study. Researchers generally stated high winter

hardiness of KB (Aamlid et al., 2012; Zuk et al., 2012) but cultivars of KB, especially Miracle and Evora, had the lowest scores in our study (*Table 5*). This might indicate low adaptation of these cultivars to environments, where the winter is cold and harsh.

Figure 4. Seasonal variation in general quality scores belongs to the cultivars of a) PR b) TF, RF, SF c) KB

Table 5. Winter hardiness scores and establishment speed of the cultivars

Cultivars	Winter hardiness score	Establishment speed (days)
Stravinsky	4.7abc	32.0f
Esquire	3.7abc	24.7hi
Bizet-1	4.0abc	23.0ij
Kokomo	4.0abc	26.0h
Capri	3.0abc	20.3j
Grandslam2	4.3abc	26.3gh
Monarch	4.3abc	22.3ij
SR8600	5.0abc	45.3cd
Tomcat	6.0ab	40.3de
Avenger	5.3ab	31.7fg
Forte	5.3ab	34.0f
Corail	6.3a	39.7d
Maximal	6.3a	44.7d
Cardinal	6.3a	60.0a
Rosita	6.0ab	35.0ef
Maritza	5.3ab	41.0d
Miracle	2.0bc	54.7ab
Arrowhead	4.0b	53.0bc
Evora	1.0c	61.7a
Mean	4.6	37.6
P	**0.001**	**0.001**

3.5. Turf establishment speed

The mean establishment speed was 37.6 days and it significantly varied among the cultivars (P≤0.01) between 20.3 and 61.7 days (*Table 5*). The establishment speed of the cultivars belonging to PR species was higher, while it was the lowest for KB cultivars. Capri was the fastest established cultivar, while Evora was the slowest. However, Cardinal (RF) was also one of the slowest established cultivars (*Table 5*).

Results showed that species aligned as PR < TF < RF, SF, CF < KB in terms of the establishment speed. Other researchers also determined a similar variation of establishment speed among PR, TF, and KB (Valverde and Minner, 2007; Charif et al., 2021). However, significant cultivar-based variations were observed in our results. For example, Avenger and Forte cultivars of TF had similar establishment speeds to the cultivars of PR, but SR8600 and Tomcat were similar to the cultivars of fine fescue (*Table 5*). In addition, Cardinal significantly varied from other fine fescue cultivars in terms of establishment speed. This indicated that establishment speed could change significantly among the cultivars of the same turf species. Roche and Loch (2005) determined a significant variation among the cultivars of *Cynodon dactylon* in terms of establishment speed.

4. Conclusions

In conclusion, turf color and general quality of the cultivars did not vary significantly, but there were significant variations in texture. Cultivars of TF had lower texture scores than expected due to their rough structure. The results showed that seasonal variation of turf quality characteristics was significantly different among the most common cool-season turf species. PR and fine fescue species lost their visual quality, especially color in autumn just after the warm summer while KB remained high quality. Therefore, these species should be mixed to establish better-quality turf. However, it was observed that Miracle and Evora are not well adapted to cold semi-arid climates, due to their low winter hardiness. Therefore, the other cultivars of KB should be used in turf mixtures for these climatic conditions. Besides, KB was the slowest established species in our study, thus sowing date should be arranged considering the slow establishment speed of KB. Our results showed the seasonal variation in turf quality was not only varied species-based but also cultivar-based for the most common cool-season turf species. Therefore, other and newly released turf cultivars should be tested to ensure sustainable and high-quality turf year-round.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Ali Koc; Design: Ali Koc, Onur İleri; Data Collection or Processing: Onur İleri; Statistical Analyses: Yasin Altay; Literature Search: Onur İleri; Writing, Review and Editing: Ali Koc, Onur İleri.

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ARAŞTIRMA MAKALESİ   RESEARCH ARTICLE

A New Perspective on Türkiye's Sheep Population: Classification with Decision Trees

Türkiye Koyun Varlığına Yeni Bir Bakış: Karar Ağacı ile Sınıflama

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Abstract

Decision trees are data mining algorithms that make interpreting the cause-effect relationship or classification between features with visual diagrams easy and do not require parametric assumptions. The aim of this study is to classify different sheep breeds raised in Turkey according to their origin and tail structure characteristics using a decision tree algorithm. It has been seen that the CART (Classification and Regression Trees) algorithm is sufficient for the classification of sheep breeds by obtaining low-risk values. According to the results of the study, it is seen that domestic sheep breeds are distributed to Eastern Anatolia, Mediterranean, Black Sea, and Central Anatolia Regions and imported breeds are distributed to all regions of Türkiye. The total population of these breeds was determined as 43.889.918 heads. As it can be understood, the Akkaraman breed ranks first with a rate of 40.88% of the total sheep stock. Next comes the Morkaraman breed with a rate of 11.68%. Thirdly, the most cultivated breed is Kıvırcık, whose rate is 8.82%. Merino sheep comes in fourth place with 8.43%. Awassi sheep are among the most common breeds in the fifth place. Hereby the distribution of sheep breeds in Türkiye has also been revealed and the distribution of breeds according to environmental conditions has been determined. The decision tree model generated using the CART algorithm for the distribution of sheep breeds in Turkey based on tail structures has been found to have a value (0.47). The proportion of sheep with a thin tail structure is 52.2%, while those with a semi-fatty tail structure are 17.5%, and those with a fatty tail structure are 30.2%. It has been determined that sheep breeds with thin tails are more commonly raised in Turkey. In the study, a decision tree model was also created using the CART algorithm to analyze the distribution of sheep populations in regions based on their origin, and it was found to have a value (0.26) with an accuracy rate of 74%. According to the research, 73.9% of sheep are domestic, while 26.1% are imported. When examining the distribution of origin by region, the region with the least number of imported sheep is Eastern Anatolia, while the highest proportions are found in the Mediterranean and Black Sea regions. The region with the lowest proportion of domestic sheep is the Mediterranean and Black Sea (70.4%), whereas the highest proportion is in the Eastern Anatolia region (82.6%).

Keywords: Sheep number, Classification, Decision tree, Sheep breeds

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Öz

Karar ağaçları özellikler arasındaki neden-sonuç ilişkisini ya da sınıflandırmayı görsel diyagramlarla kolay yorumlanmasını sağlayan, parametrik varsayımlara gerek duymayan veri madenciliği algoritmalarıdır. Bu çalışmanın amacı, Türkiye'de yetiştirilen farklı koyun ırklarının orijin ve kuyruk yapısı özelliklerine göre karar ağacı algoritması kullanarak sınıflandırmaktır. Düşük risk değerleri elde edilerek, koyun ırklarının sınıflandırılmasında CART algoritmasının yeterli olduğu görülmüştür. Çalışma sonuçlarına göre, yerli koyun ırklarının Doğu Anadolu, Akdeniz, Karadeniz ve İç Anadolu Bölgelerine ve ithal ırkların ise Türkiye'nin tüm bölgelerine dağıldığı görülmektedir. Bu koyun ırkların toplam popülasyonu 43.889.918 baş olarak belirlenmiştir. Toplam koyun varlığı içerisinde Akkaraman ırkı %40,88 oranı ile ilk sırada yer almaktadır. Bunu %11.68 oranla Morkaraman ırkı takip etmektedir. Üçüncü sırada ise en çok yetiştirilen tür %8.82 ile Kıvırcık koyunudur. Merinos koyunu ise %8.43 ile dördüncü sırada yer almaktadır. İvesi koyunları ise en yaygın ırklar arasında beşinci sırada yer almaktadır. Böylelikle koyun ırklarının Türkiye'deki dağılımı da ortaya konmuştur ve çevresel şartlara göre ırkların dağılımı belirlenmiştir. Türkiye'deki koyun ırklarının kuyruk yapılarına göre dağılımına ilişkin CART algoritması ile oluşturulan karar ağacı modelinin risk değeri düşük (0.47) bulunmuştur. İnce kuyruk yapısına sahip koyunların oranı %52.2 iken, yarı yağlı kuyruk yapısına sahip koyunların oranı %17,5, yağlı kuyruk yapısına sahip koyunların oranı ise %30.2'dir. Türkiye' de ince kuyruklu koyun ırkları daha yaygın olarak yetiştirildiği belirlenmiştir. Çalışmada koyun varlığının kökenine göre göre bölgelere dağılımına yönelik CART algoritması ile oluşturulan karar ağacı modelinin risk değeri düşük bulunmuştur (0.26) ve doğru sınıflandırma oranı %74' dir. Araştırmada koyunların yüzde 73.9'u yerli, yüzde 26.1'i ise ithal koyunlardan olduğu tespit edilmiştir. Bölgelere göre köken dağılımı incelendiğinde ithal koyun sayısının en az olduğu bölge Doğu Anadolu'da, en yüksek oranda ise Akdeniz ve Karadeniz bölgeleridir. Yerli koyun oranının en düşük olduğu bölge Akdeniz ve Karadeniz (%70.4) olurken, en yüksek oranın ise %82.6 ile Doğu Anadolu bölgesi olduğu belirlenmiştir.

Anahtar Kelimeler: Koyun sayısı, Sınıflandırma, Karar ağacı, Koyun ırkları

1. Introduction

While the number of sheep and goats in Türkiye for the first period of 2022 is approximately 58.4 million, this number is 57.5 million in 2021, the number of sheep increased by 1.02% and the number of goats decreased by 0.99% (MFAL, 2023). The number of sheep milked is approximately 32 million heads, of which 19.8 million are sheep and 5.4 million are goats. According to the 2021 milk production data of the Turkish Statistical Institute, the total milk production is approximately 23.2 million tons, of which 2.1 million tons are obtained from sheep and goats. Of the total small ruminant milk obtained, 622.7 tons are sheep milk, 63.6 thousand tons are goat milk, 385.933 tons (19.77%) of the total red meat production (1.952.030 tons) are sheep meat, and 10,831 tons (5.54%) are goat meat (Sevinç et al., 2022; TÜİK, 2023).

The number of sheep and goats in Türkiye for the first period of 2022 was approximately 58.4 million, while the total number of sheep and goats in 2021 was 57.5 million heads, with a 1.02% increase in sheep and a 0.99% decrease in goats (MFAL, 2023). The number of sheep and goats milked is approximately 32 million heads, of which approximately 19.8 million are sheep and 5.4 million are goats. According to the milk production data of the Turkish Statistical Institute for 2021, total milk production was approximately 23.2 million tons, of which 2.1 million tons was obtained from sheep and goats. Of the total sheep and goats milk obtained, 622.7 tons of sheep milk and 63.6 thousand tons of goat milk, 385,933 tons (19.77%) of sheep meat and 10,831 tons (5.54%) of goat meat constitute the total red meat production (1.952.030 tons) (Sevinç et al., 2022; TÜİK, 2023).

Increases in ovine meat and milk production depend on the continuation of research on many issues such as animal breeding, management of the environment and natural resources, animal welfare, and social and cultural structure (Keskinkılıç, 2019; İzmir Commodity Excahnage, 2019; Yılmaz, 2019; Çiçek et al., 2022). It is important to have sufficient and accurate information about sheep breeding to meet the increasing need for meat and milk in the world in recent years and the sustainability of sheep breeding activities. Knowing how the number of ovine animals is dispersed across the geography of the country in terms of meat and milk production values affects the positive development of the factors affecting productivity (Cengiz et al., 2015; Özsayin and Everest, 2019; Kandemir et al., 2019; Cedden et al., 2020).

There are studies examining the effects of many factors on meat and milk production in sheep breeding. In the studies carried out, some yield characteristics and some structural characteristics were tried to be determined in sheep based on genotype or province (Özsayın and Everest, 2019; Kaymakçı and Taşkın, 2008; Sönmez et al., 2009; Kandemir et al., 2015; Semerci and Çelik, 2016; Tamer and Sarıözkan, 2017; Karakoç and Aygün, 2019; Kahraman et al., 2020; Demir and Aygün, 2021; Kırbaş et al., 2022; Hanoğlu et al., 2021). However, although there are studies that consider meat and milk production based on region and province, there are no studies that examine the genotype characteristics that directly affect these productions and the tail shape and distribution according to origins, which indirectly affect these productions, at the same time.

On the other hand, it is important to examine the effects of these factors with models using advanced software instead of classical approaches, in terms of making the results more reliable. Today, there are many new algorithms developed for processing data obtained from large information stacks. In this system known as data mining, artificial intelligence, and machine learning are especially valuable in the analysis of non-parametric data (Kononenko and Kukar, 2007). The decision tree method is a data mining algorithm that has been widely applied in various branches of science as a modern analytical technique (Abu-Hanna and De Keizer, 2003). This method has CHAID (Chi-square Automatic Interaction Detector), CART (Classification and Regression tree), QUEST (Fast Neutral Efficient Trees) and Exhaustive CHAID (Comprehensive Chi-square Automatic Interaction Detector) sub-algorithms for model analysis according to the research subject data structure. In the working principle of decision tree models, tree branches are formed like a tree structure with the answers taken from the basic simple questions of the independent variable. Tree branches formed in this way show which independent variable or variables affect the dependent variable (Temel, 2004; Sevgenler, 2019).

The classification and Regression tree method is a nonparametric analysis method that divides the data set into homogeneous subclasses and presents the relationship between dependent and independent variables visually (Kayri and Boysan, 2008; Akşahan and Keskin, 2015; Alev Çetin and Mikail, 2016). This method has some advantages such as not requiring any assumptions about the distribution of independent variables and not being negatively affected by multicollinearity, outliers, and missing observations (Mendeş and Akkartal, 2009).

This study aimed to classify the distribution of different sheep breeds in Türkiye in terms of origins and tail structures by considering the similarities and differences of the breeding areas, as well as to examine the current situation with the regression tree algorithm. It is thought that the findings obtained in the study will contribute to the creation of sheep breeding programs and sheep breeding maps to be made on a regional and provincial basis in Türkiye.

2. Material and Method

The material of the research consists of the data for the year 2021 obtained from the Livestock Information System (HAYBIS) belonging to the Republic of Turkish Ministry of Agriculture and Forestry (Anonymous, 2021a).

2.1. Study area

Seven geographical regions of Türkiye constitute the study area of the research. Four of these seven geographical regions are the name of the sea to which they are adjacent (Mediterranean Region, Black Sea Region, Aegean Region, Marmara Region), and the other three regions are named according to their positions in the whole of Anatolia (Central Anatolia Region, Eastern Anatolia Region, Southeastern Anatolia Region) (*Figure 1*). There are 81 provinces, 922 districts, 32175 neighborhoods, and 18292 villages in Türkiye. In this context, its population in 2022 is 84 million, and its surface area is 783,562 square kilometers (Yakar and Özgür, 2022).
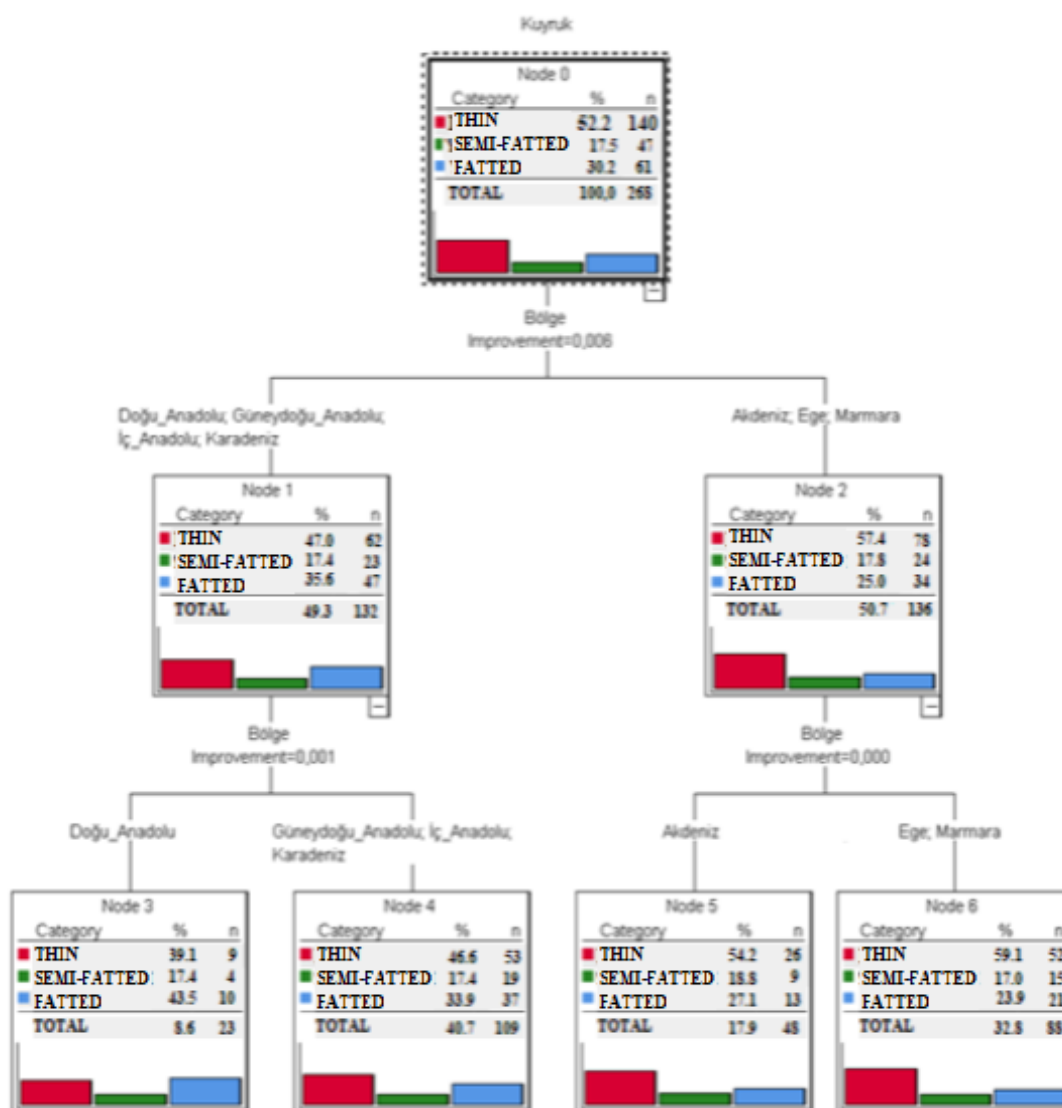

Figure 1. Map of Türkiye by geographical regions

Sheep breeds, tail shapes (thin, fatted, semi-fatted), and their origins (imported or domestic) bred according to regions in Türkiye. The number of sheep in the provinces in each region determined in the Türkiye Domestic Genetic Resources Promotion Catalogue published by the Ministry of Agriculture and Forestry (Anonymous, 2021b) and the tail structure of these breeds (thin, semi-fatted and fatted) and origin (imported and domestic) classified according to their characteristics and descriptive statistics were given (Kalaycı, 2006; Alpar, 2013).

2.2. Classification and regression tree

In this study, classification and regression tree algorithms were applied according to the tail structure and origin characteristics of sheep breeds. A classification and regression tree is an iterative partitioning tree procedure that is constructed by dividing data into binary subgroups using continuous or discrete arguments. When the dependent variable is categorical, it is called classification tree (CT), and when it is continuous, it is called regression tree (RT). The first node of the classification and regression trees is the root node where the dependent variable is located and has not yet been split. First, this root node of the regression tree splits into two parent branches and these two parts are called parent branches. Nodes formed because of separation and division are also called subsets (Kalaycı, 2006).

The basic principle in the construction of the regression tree is to iteratively split the response variable into two child nodes to ensure maximum homogeneity in the response variable. During the construction of the tree, the program tests all explanatory variables included in the model and determines the cut-off value (category if the explanatory variable is categorical) of the explanatory variable to ensure the highest homogeneity in the resulting node (Akşahan and Keskin, 2015). In this process, if homogeneity is ensured as much as possible in any progeny node that is recursively created in the response variables, the fragmentation process ends in these nodes and these nodes are called terminal nodes (Oruçoğlu, 2011).

Splitting points of nodes are usually determined based on splitting criteria such as Gini or Twoing. Gini is a classification method based on binary divisions; after each division, the smallest Gini value is selected. The tree is continuously divided using the Gini index and eventually the optimum tree is determined by evaluating it with independent test data. The minimum number of observations is important when determining tree depth, and 10% of the data set is generally preferred. Gini index is calculated with the following formula, C represents the number of classes and pi represents the proportion of data points belonging to class i:

$$Gini\ İndex = 1 - \sum_{i=1}^{C} P_i^2 \qquad \text{(Eq. 1)}$$

A splitting criterion called twoing is another splitting criterion used in the CART algorithm. Twoing takes into account the distribution of variable values when dividing the data set between two subgroups and tries to minimize this distribution. When splitting a node into two child nodes, Twoing calculates the probabilities for each of these child nodes separately. It then creates branches based on these possibilities. The Twoing method is especially preferred when there is imbalance in the data set and other criteria such as the Gini index are insufficient. The Twoing formula is as follows:

$$\text{Twoing} = \sum_{j=1}^{J} \frac{n_j}{N} * \frac{1}{4} \left(1 - \frac{n_j}{N}\right) \left(1 - \frac{\sum_{k \neq j}^{J} n_k}{N}\right) \qquad \text{(Eq. 2)}$$

N represents the total number of samples, and nj and nk represent the number of samples in j and k subgroups, respectively. J refers to the total number of subgroups (Breiman, 1984). In this study, the IBM SPSS v25 program was used to classify the tail structure and origin characteristics of sheep breeds with a regression tree.

3. Results

While the region with the highest number of sheep breeds in Türkiye was the Mediterranean with 48, the region with the least sheep breed was the Eastern Anatolia Region with 23. However, considering the number of sheep in Türkiye (43.889.918), the Eastern Anatolia Region ranks first among the regions with 11.803.377 heads (26.89%). This in order; It is followed by Central Anatolia with 23.07% and Southeast Anatolia with 14.74%. When the average number of sheep according to the regions is examined, the lowest 68,536 head belong to the Black Sea Region. The region with the highest average sheep presence is Eastern Anatolia with 513.190 heads. In the study, the presence of sheep according to the regions is given in *Table 1*.

Table 1. Some descriptive statistics of sheep presence by regions

Region	Number of races	Total number of sheep	Share in Türkiye (%)	Number of sheep in the provinces in the region	
				The province with the least sheep	The province with the most sheep
Aegean	43	5.298.59	12.07	1.933.18	5.298.59
Mediterrenian	48	3.599.19	8.20	1.267.39	3.599.19
Marmara	45	4.332.82	9.87	2.160.63	4.332.82
Black Sea	33	2.261.71	5.15	1.071.76	2.261.71
Southeastern Anatolia	30	6.468.77	14.74	3.118.12	6.468.77
Eastern Anatolia	23	11.803.38	26.89	6.382.28	11.803.38
Central Anatolia	46	10.125.45	23.07	6.499.14	10.125.45
Türkiye	**53***	**43.889.92**	100.00		

* It is the total number of races existing in Turkey, the races in each region vary.

When the sheep breeds bred in Türkiye are examined, it is determined that there are 53 different sheep breeds in total. Having so much diversity in the same species is a great wealth for Türkiye. Such a change in environmental conditions with global warming makes the future unknown. Being able to benefit from the different characteristics of each sheep breed will create an advantage for people living in future Türkiye. The number of sheep breeds reared in Türkiye and their proportions are given in *Table 2*. The total population of these breeds was determined as 43.889.918 heads. As it can be understood, the Akkaraman breed ranks first with a rate of 40.88% of the total sheep stock. Next comes the Morkaraman breed with a rate of 11.68%. Thirdly, the most cultivated breed is Kıvırcık, whose rate is 8.82%. Merino sheep comes in fourth place with 8.43%. Awassi sheep are among the most common breeds in the fifth place. In this way, other sheep breeds are bred in different regions of Türkiye, respectively.

The distribution of sheep breeds according to tail structures in Türkiye is given in *Figure 2*. Accordingly, the risk value of the decision tree model created with the CART algorithm for the distribution of sheep according to their tail structures was found to be low (0.47) and the correct classification rate was 53%. As seen in *Figure 1*, while the rate of sheep with thin tail structures is 52.2%, the rate of sheep with semi-fat tail structures is 17.5% and the rate of sheep with fatted tail structures is 30.2%. Thin-tailed sheep breeds are more commonly bred in a significant part or region of our country.

In the distribution of the tail structure according to the regions, a similar structure is observed in the Eastern, South-eastern, and Central Anatolian regions and the Black Sea. When the tail structures are examined, the total ratio of the East, Southeast, Central Anatolia, and Black Sea regions is 49.3%, while the total ratio of the sheep in the Mediterranean, Aegean, and Marmara regions is 50.7% (*Figure 2*). As shown in the section denoted by the 1st Node (Node 1), the ratio of thin, semi-fatted, and fat-tailed sheep in the East, Southeast, Central Anatolia, and Black Sea Regions, respectively; 47.0%, 17.4%, and 35.6%. In the 2nd Node, where the Mediterranean, Aegean, and Marmara Regions are located, when the distribution of sheep presence according to tail structures is examined; thin-tailed sheep have a rate of 57.4%, semi-fat 17.6%, and fat-tailed sheep have a rate of 25.0%. This situation is as expected. In other words, while thin and semi-fat-tailed sheep are dominant or more common in the Western Anatolia Region, the proportion of fat and semi-fat-tailed sheep is relatively high in the Eastern, Southeastern, and Central Anatolian Regions.

Eastern Anatolia region differs from Southeast and Central Anatolia Region and Black Sea Region (*Figure 1*). While the rate of fat tail sheep in the Eastern Anatolia region is 43.5% (3rd Node), this rate is 33.9% in the aforementioned regions (4th Node). The rates are close to each other in the Mediterranean (5th Node) and Aegean and Marmara regions (6th Node) where thin-tailed sheep are concentrated. While the rate of thin-tailed sheep is 54.2% in the Mediterranean Region, this rate is 59.1% in the classification that includes the Aegean and Marmara regions (6th Node). However, it is noteworthy that the ratio of fat-tailed sheep (27.1% and 23.9%, respectively) is higher than that of semi-fat-tailed sheep in both the Mediterranean, Aegean, and Marmara regions.

Table 2. Sheep breeds raised in Türkiye.

Breed	Presence	Rate	Breed	Presence	Rate
Acıpayam	9.579	0.02	Kıvırcık (3)	3.870.648	8.82
Akkaraman (1)	17.940.185	40.88	Koçeri	54.608	0.12
Alman Siyah Bas	8.184	0.02	Lacaune	8.405	0.02
Anadolu Merinosu	331.402	0.76	Langhe	279	0.0005
Assaf	868	0.00	Malya	18.623	0.04
Bafra	28.931	0.07	Menemen	23.622	0.05
Bandırma	732	0.00	Merinos (4)	3.698.157	8.43
Bergamasca	19	0.00	Mor Karaman (2)	5.124.472	11.68
Çine Çapari	73.427	0.17	Norduz Koyunu	33.451	0.08
Dağlıç	128.831	0.29	Orta Ana Merinosu	120.394	0.27
Dogu Friz (Mars)	3.967	0.01	Ödemiş	1.250	0.003
Dorper	7.226	0.02	Pırlak	2.722.164	6.20
Esme	137.395	0.31	Plevne	2.393	0.01
Gökçeada	112.666	0.26	Polatlı	7.133	0.02
Hamdani	1.677.950	3.82	Ramlıç	53.241	0.12
Hasak	1.023	0.00	Romanov	98.890	.0.23
Hasmer	1.199	0.00	Sakız	1.506.754	3.43
Hemsin	237.350	0.54	Sarole	6.045	0.01
Herik	72.082	0.16	Sönmez	10.292	0.02
Ile de Frans	26.578	0.06	Suffolk	18.550	0.04
İvesi (5)	3.002.326	6.84	Tahirova	155.309	0.35
Kangal Akkaraman	568.288	1.29	Teksel	2.253	0.01
Karacabey Merinosu	368.275	0.84	Tuj	32.156	0.07
Karagül	8.771	0.02	Turcana	4.288	0.01
Karakas	7.076	0.02	Türkgeldi	281	0.0005
Karayaka	1.153.923	2.63	Zom	308.768	0.70
Karya	99.239	0.23	**TÜRKİYE**	**43.889.918**	**100.00**

In the study, the distribution of sheep existence by origin is given in *Figure 2*. Accordingly, the risk value of the decision tree model created with the CART algorithm for the distribution of sheep to regions according to their structures of origin was found to be low (0.26) and the correct classification rate was 74%. In the study, 73.9% of the sheep are domestic, while 26.1% are imported. When the distribution of origin by region is examined, Eastern Anatolia differs from other regions. The ratios of domestic and imported sheep in this region (Eastern Anatolia, 1st Node) are respectively; 82.6% and 17.4%. Whereas, in the group that includes the Mediterranean, Aegean, Southeast and Central Anatolia, and the Black Sea and Marmara Regions (2nd Node), respectively; 73.1% versus 26.9%. When the Mediterranean, Aegean, Southeast, and Central Anatolia and the Black Sea and Marmara regions (2nd Node) are examined within themselves, the ratio of domestic and imported sheep in the 3rd Node, where the Mediterranean and Black Sea is a group, is respectively; 70.4% and 29.6%. In the 4th Node group, which includes Aegean, Southeast, Central Anatolia and Marmara regions, the rates are respectively; 74.4% domestic and 25.6% imported. To summarize, the region with the lowest number of imported sheep in Eastern Anatolia, while the highest rate is in the Mediterranean and Black Sea regions. The region with the lowest rate of domestic sheep was the Mediterranean and Black Sea regions (70.4%), while the highest rate was the Eastern Anatolia region with 82.6%.

Figure 2. Distribution of sheep according to origine according to the decision tree method

4. Discussion

Regression trees are used to pre-select the features that affect the continuous dependent variable. Regression trees are algorithms that easily interpret the cause-effect relationship or classification between measurements in different structures of research with visual diagrams and do not require any parametric assumptions. On the other hand, although there are few studies on the use of decision trees in small cattle raised in Türkiye, different decision tree algorithms have been used in studies examining the effects of various factors on weaning weight, fleece weight, and birth weight. Olfaz et al. (2019) investigated the effects of gender, birth type, holding type, birth weight, and weight measurement time on weaning weight in Karayaka sheep with CART and CHAID decision trees.

In conclusion, according to the CHAID algorithm, the effect of measurement time, gender, and farm type on weaning weight was found to be significant. The results of the CART algorithm, on the other hand, determined

that the type of birth was effective on the weaning weight. (Eyduran et al., 2017). In addition, the CART method was applied to evaluate the effects of genotype, sex, birth type, year, and maternal age on birth weight in Eyduran et al. (2008), Karakaş, and Norduz lambs. While the effect of gender was insignificant in single-born lambs, the effect of genotype was significant in twin lambs. Balta and Topal (2018) examined the effects of year, flock type, maternal age, lamb gender, birth type, and lamb color on birth weight in Hemşin lambs by the CART method. The most important variables affecting birth weight are respectively, year, birth type, sex, herd type, and maternal age. In this study, the distribution and classification of different sheep breeds bred in Türkiye according to regions were examined according to the regression tree algorithm. Origin and tail structures were taken into consideration as classification criteria in sheep breeds. The low-risk values obtained according to the results of the study showed that the CART algorithm was sufficient in explaining the distribution of sheep breeds according to their tail and origin characteristics. This result was reported by Olfaz et al. (2019), Eyduran et al. (2008), and Balta and Topal (2018).

As a result, according to the CHAID algorithm, the effect of measurement time, sex, and holding type on weaning weight was found to be significant. The results of the CART algorithm showed that the type of birth was effective on weaning weight. Eyduran et al. (2017) estimated the fleece weight of Akkaraman and İvesi ewes from some physical properties of fleece with the help of CHAID analysis, which can be a useful method for determining high-yielding ewes. In addition, Eyduran et al. (2008) applied the CART method to evaluate the effects of genotype, sex, type of birth, year, and age of dam on birth weight in Karakaş and Norduz lambs. While the effect of sex was insignificant in single-born lambs, the effect of genotype was found to be significant in twin-born lambs. Balta and Topal (2018) examined the effects of year, flock type, dam age, lamb sex, birth type, and lamb color on the birth weight of Hemşin lambs using the CART method, and the most important variables affecting birth weight were found to be the year, birth type, sex, flock type, and dam age, respectively. In this study, the distribution and classification of different sheep breeds bred in Türkiye according to regions were analyzed by regression tree algorithm. Origin and tail structures were taken into consideration as classification criteria for sheep breeds. The low-risk values obtained according to the results of the study showed that the CART algorithm is sufficient to explain the distribution of sheep breeds according to tail and origin characteristics. This result is consistent with Olfaz et al. (2019), Eyduran et al. (2008), and Balta and Topal (2018) who applied the CART decision tree algorithm in sheep and goat breeds.

Feeding in sheep breeding in Türkiye is largely based on village common pastures. There are a total of 14.6 million meadow pasture areas in Türkiye, of which 1.45 million are meadows and 13.1 million are pastures. The Eastern Anatolia Region has the largest meadow and pasture area, with a rate of 37.54%. The Central Anatolia Region (29.68%) takes second place. Other regions, respectively; Black Sea Region (11.97%), Southeastern Anatolia Region (6.90%), Aegean Region (5.49%), Mediterranean Region (4.51%), and Marmara Region (3.90%) come from (Kuşvuran et al. 2011; Çaçan and Yüksel, 2016). In the research, the surface measurements of the meadow and pasture areas owned by the regions in Türkiye and the presence of sheep belonging to the regions are directly proportional and in harmony. The difference between the animal existence in Eastern Anatolia and Central Anatolia Regions and other regions is statistically significant. It is understood that these two regions are the locomotive of sheep breeding in Türkiye.

Although the decrease in agricultural production values in recent years has shown that the sector has contracted (Sezenler, 2013; Karaman, 2018), the high input costs that have occurred have had a significant impact on the sustainability of animal husbandry (Güler and Saner, 2021). Accordingly, Türkiye's imports of live animals and carcasses have also increased. In Türkiye, after 2010, especially to reduce meat prices, the import of livestock, mostly for fattening and slaughtering, has accelerated. Between 2010 and 2019, approximately 8 million heads of live sheep/cattle animals with a total value of 7.08 billion dollars were imported. Between 2010 and 2019, 296.487 tons of boned/boneless/carcass meat with a total value of 1.406 billion dollars were imported (Anonymous, 2020). Grants, loans, and support given to the livestock sector in the last fifteen years in Türkiye; it is seen that some provinces and most large-scale enterprises in these provinces benefit. It is aimed to increase the meat and milk productivity of large enterprises and to supply the animal products that Türkiye needs domestically thus preventing the increase in product prices (Oğul, 2022).

The research obtained it by crossing 24 domestic breeds, 16 imported breeds from 53 sheep breeds in Türkiye, and domestic breeds with imported breeds from the past to the present. It was determined that 13 native breeds

registered by the Ministry of Agriculture and Forestry were created (Anonymous, 2021a). Sheep breeding is a production branch that can be done in almost all regions of Türkiye. This branch of production is mostly made with local breeds and the feeding of these breeds is done by adhering to pasture (Semerci and Çelik, 2016; Acıbuca and Bostan Budak, 2018). In other words, the production system of sheep is predominantly based on the extensive production model. In Türkiye, state-supported small cattle enterprises have been established in many provinces and these enterprises continue to produce. Since these enterprises think that sheep breeding with domestic breeds is not very profitable, they approach the issue with the idea that it would be more appropriate to start with cultural breeds or crosses. As the breeding was done, there were animal sales from newly established enterprises from imported breeds to surrounding enterprises, and in this way, the dispersal of imported breeds to that region was realized. However, due to different climatic difficulties, it is analyzed that imported breeds could not become widespread in some regions due to climatic difficulties and remained limited to locally imported farms.

In the research, the Eastern Anatolia Region is the first to differentiate due to the high rate of domestic sheep breeds. The proportion of imported breeds in this region is quite low. The result of the study was determined by the regional study Kandemir and Taşkın (2022a). It has been determined that there are 23 breeds in total in the Eastern Anatolia Region, of which 19 breeds are domestic and 4 breeds are imported. This is because; It is thought that because of the very cold and long winters in the Eastern Anatolia Region and the very limited vegetation, it is thought that the races coming from outside cannot adapt to the difficult conditions. Imported breeds grown; It is thought that sheep breeds such as Charolais, Ile de France, and Romanov can be raised by intensive feeding in cold winter periods. The Black Sea Region is the second region to differentiate. Since the livestock enterprises in the region know that sheep breeding with local breeds is not very profitable, they act with the thought that it would be more appropriate to start with culture breeds or crosses. Karayaka sheep constitute half of the sheep presence in the region due to their adaptation to high rainfall and humidity (Kandemir and Taşkın, 2022b). The fact that imported breeds are not preferred over domestic breeds in intensive production in the Black Sea Region, which receives high rainfall, causes a differentiation. About 5 per thousand of the 10 million sheep assets in the Central Anatolia Region consist of imported breeds (Taşkın and Kandemir, 2022a). This is thought to be one of the reasons for the variation in sheep breeds bred in the region.

In sheep, the tail structure is a morphological feature and is a transmitted breed character that is transferred from generation to generation. It is accepted as a constant measure in the classification of sheep breeds (Taşkın et al., 2015). Tail structures in Türkiye are registered as fat tail, the half fat tail, and thin tail, and the distinctions are made accordingly (Anonymous, 2021b). In the research, the fat-tailed sheep breeds, which started from the Eastern Anatolia Region, which had the harshest winter, left their place to the thin-tailed sheep breeds in the milder regions. Eastern, South-eastern, Central Anatolian, and Black Sea Regions are in harmony with the regional studies that are predominantly oil-tailed (Kandemir and Taşkın, 2022a, Kandemir and Taşkın, 2022c, Kandemir and Taşkın, 2022d). Thin-tailed sheep breeds are mainly grown in the Marmara, Mediterranean, and Aegean regions. Similarly, this finding is in harmony with the regional sheep breeding studies (Kandemir and Taşkın, 2022c; Kandemir and Taşkın, 2022e, Taşkın and Kandemir, 2022a; Taşkın and Kandemir, 2022b).

On the other hand, decision trees are considered a powerful tool for data analysis and creating predictive models in terms of their advantages. As a matter of fact, since decision trees have a simple structure, it is quite easy to interpret and explain the results. The tree structure increases understandability as it helps visualize the decision process (Quinlan, 1985). Since decision trees can work with categorical and numerical data, they are used effectively in data sets where different types of data coexist (Breiman et al., 1984). Decision trees have fast training and prediction times even on large data sets. This feature provides a significant advantage in real-time applications and big data analysis (Genuer, 2010). In addition, decision trees can be used directly in data sets, which reduces data preprocessing steps (Hastie et al., 2009).

Besides the mentioned advantages, decision trees also have disadvantages. For this reason, some issues should be carefully evaluated when using it in small cattle breeding. Because decision trees tend to overfit the training data set, this leads to the drawback of reducing generalization ability (Domingos, 1997). Additionally, decision trees can be quite sensitive to small changes in the input data (Hastie, 2009). In unbalanced data, decision trees can focus on these classes and ignore others, considering that larger classes have more samples. Thus, while a high accuracy is achieved in the majority class, the small class may misclassify. Due to these drawbacks, when using decision trees, data quality should be increased with data pre-processing steps, tree depth should be limited and care should be taken to select the correct node division criteria for the nodes in the trees.

5. Conclusion

In this study, which shows the distribution of sheep breeds according to geographical regions in Türkiye, the distribution of sheep according to their origin and tail structure is shown with a statistical method, a decision tree. It has been determined that there is a wide diversity in the geographical regions of Türkiye depending on different environmental conditions. While the presence of fat-tailed breeds is high in regions with cold climatic environments, it is seen that tail structures become thinner in milder temperate regions. Sheep breeds that are not in different continents and Türkiye have been brought to Türkiye due to the importation that has been going on for many years. Although the Eastern Anatolia, Mediterranean, Black Sea, and Central Anatolia Regions differ by evaluating the sheep according to their origins in geographical regions, imported sheep breeds are bred in all regions of Türkiye, although predominantly local breeds.

In the accompaniment of this study, some suggestions that we think may be useful for the future of sheep breeding are given. These recommendations are as follows; In Türkiye, there are studies carried out to protect the diversity of local gene resources and to ensure their sustainability. However, further studies at the molecular level will contribute to the determination of genetic diversity as well as the level of inbreeding, the creation of gene resource protection programs and the development of breeding strategies, especially for the development of sheep breeding. To produce high-quality roughage, which is also an important problem in Türkiye's sheep breeding, it should be ensured that the forage crops culture in field agriculture is developed as well as the existing pasture areas. Finally, it should be ensured that shepherd or animal care should change the perspective of young people, especially in rural areas, towards sheep breeders, who do not have social security or a profession, to be respected in society. Instead of temporary solutions, necessary legal arrangements including the technical and economic dimension of the problem should be made as soon as possible. Otherwise, the number of people who leave or leave this production branch in many regions of our country where sheep breeding is carried out will increase gradually.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: ÇK, ÇT, TT; Design: ÇK; Data Collection or Processing: ÇK, TT; Statistical Analyses: ÇT; Literature Search: TT; Writing, Review and Editing: ÇK, ÇT, TT.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

The Effect of Heavy Metals in Potato (*Solanum tuberosum* L.) Genotypes for Some Physiological Parameters

Patates (*Solanumtuberosum* L.) Genotiplerinde Ağır Metallerin Bazı Fizyolojik Parametrelere Etkisi

Imer RUSINOVCI[1], Sali ALIU[2*], Sefer DEMIRBAS[3], Dukagjin ZEKA[4], Mimoza JAKUPI[5]

Abstract

The main aim of this study is to identify physiological characteristics, including Chl a, b, total Chl "a+b" and carotenoids, in potato genotypes under the influence of lead (Pb+2) and cadmium (Cd+2) heavy metals. Two potato genotypes from the Netherlands, Riviera and Agria, were used in the study. Potato tubers were transferred to 2 kg compost pots and placed in a controlled environment with a 12 photoperiod, a day/night temperature of 25/19°C and a relative humidity of 75%. Since Pb+2 and Cd+2 poisoning was greater than in the control and different fractions of heavy metal residues in the substrate were transferred to plant organs, the amounts of these two metals in each treatment (outside the control) were measured. Plant pigments were extracted from fresh leaves in amounts ranging from 60 to 100 mg and these were then extracted into samples containing 80/20% (v/v) acetone/water with 0.5% w/v $MgCO_3$ at room temperature for a full day. Photosynthetic pigments of each sample were extracted three times. The absorbances obtained at 663 nm, 644 nm and 452.5 nm for the maximum absorption of Chl a, Chl b and carotenoids, respectively, were used to measure the amount of chlorophyll and carotenoids. Differences between Pb+2 and Cd+2 and physiological markers were examined with the Duncan Multiple Range test. Information on Pb+2 and Cd+2 content in applications revealed wide variability. When potato genotype seedlings were exposed to varying levels of Pb+2 and Cd+2, the amount of chlorophyll and carotenoids in their leaves was lower than the control group. The results showed that there were significant and statistically significant changes in carotenoid and chlorophyll concentration at the LSD p = 0.01 level. Similar to Pb+2 and Cd+2 inhibiting plant growth, it had a negative effect on photosynthesis as well as chlorophyll and carotenoid contents. Moreover, these effects became more pronounced when the concentrations of two stress factors (Pb+2 and Cd+2) increased.

Keywords: Potato, Cd^{+2}, Pb^{+2}, Chlorophyll, Carotenoids, Heavy metals

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Öz

Bu çalışmanın temel amacı, kurşun (Pb+2) ve kadmiyum (Cd+2) ağır metallerinin etkisi altındaki patates genotiplerindeki Chl a, b, toplam Chl "a+b" ve karotenoidler dahil olmak üzere fizyolojik özelliklerin tanımlanmasıdır. Hollanda'dan iki patates genotipi, Riviera ve Agria, çalışmada kullanılmıştır. Patates yumruları 2 kg'lık kompost saksılarına aktarılarak 12 fotoperiyodu, 25/19°C gündüz/gece sıcaklığı ve %75 bağıl nemi olan kontrollü bir ortama yerleştirildi. Pb+2 ve Cd+2 zehirlenmesi kontrolden daha fazla olduğundan ve substrattaki ağır metal kalıntılarının farklı kısımları bitki organlarına aktarıldığından, bu iki metalin her bir uygulamadaki (kontrol dışında) miktarları ölçülmüştür.Bitki pigmentleri, 60 ila 100 mg arasında değişen miktarlarda taze yapraklardan ekstrakte edildi ve bunlar daha sonra, tam bir gün boyunca oda sıcaklığında %0,5 w/v MgCO3 ile %80/20 (h/h) aseton/su içeren örneklere ekstrakte edildi. Her örneğin fotosentetik pigmentleri üç kez ekstrakte edildi. Chl a, Chl b ve karotenoidlerin maksimum emilimi için sırasıyla 663 nm, 644 nm ve 452,5 nm'de elde edilen absorbanslar, klorofil ve karotenoid miktarını ölçmek için kullanılmıştır. Pb+2 ve Cd+2 ile fizyolojik belirteçler arasındaki farklar Duncan Çoklu Aralık testi ile incelenmiştir. Uygulamalardaki Pb+2 ve Cd+2 içeriğine ilişkin bilgiler geniş bir değişkenlik ortaya koymuştur. Patates genotipi fideleri değişen seviyelerde Pb+2 ve Cd+2 'ye maruz bırakıldığında, yapraklarındaki klorofil ve karotenoid miktarı kontrol grubuna göre daha düşük olmuştur. Elde edilen sonuçlar, karotenoid ve klorofil konsantrasyonunda LSD p = 0.01 düzeyinde belirgin ve istatistiksel olarak önemli düzeyde değişiklikler olduğunu göstermiştir. Pb+2 ve Cd+2'nin bitki büyümesini engellemesine benzer şekilde, klorofil ve karotenoid içeriklerinin yanı sıra fotosentez üzerinde de olumsuz bir etkisi olmuştur. Ayrıca, bu etkiler iki stres faktörünün (Pb+2 ve Cd+2) konsantrasyonları arttığında daha belirgin hale gelmiştir.

Anahtar Kelimeler: Patates, Cd^{+2}, Pb^{+2}, Klorofil, Karotenoid, Ağır metaller

1. Introduction

Potato (*Solanum tuberosum* L.), a member of the *Solanaceae* family, is one of the most widespread and used agricultural crops in the whole world and which has a great importance in reducing the growing hunger (Özdemir, 2023). The potato as an agricultural crop was introduced to Europe during the year 1570, and during the 17th century it spread to other countries of the world (Bradshaw and Ramsay, 2006). Pointed out that there was a need to improve the properties of the potato to fight hunger and various diseases at that time, so a greater interest in breeding wild potatoes began over 150 years ago. Nowadays, the potato plant is cultivated for human consumption and is the fourth most important food crop in the world, after rice, wheat, and corn (Farooq et al., 2008). Global potato production has increased significantly in recent years, particularly due to increased production in developing countries. Genetic variability offers plant breeders greater opportunities to discover new traits, through selection and breeding, as well as to improve various traits such as increased resistance to biotic and abiotic stresses (Shilpa et al., 2021). Kosovo has a land area of 10.887 km^2 or 1.1 million ha of this surface, about 430.000 ha are forested or 39% and 577.000 ha are agricultural land (52%), which is 31% is pasture and about 69% arable land. Potato is an important food source of Kosovo (Rusinovci et al., 2010; 2012 and 2015). However, the amount of heavy metals (HM's) affects environmental pollution due to various human activities such as: intensive agriculture, mining, energy transmission, etc. (Nedelkoska and Doran.,2000). Also, heavy metal pollution affects the biosphere in many countries around the world (Meagher, 2000). This type of heavy metal pollution is one of the most important environmental problems. This comes as a result of various agricultural and industrial activities including industrial fertilizers and pesticides, organic manure, emissions from smelters and incinerators, traffic, the distribution of mining waste, the use of contaminated sewage sludge, etc. (Aliu et al., 2013). Lead (Pb^{+2}) and cadmium (Cd^{+2}) can be considered to be the most dangerous in environmental pollution (Malkowski et al., 2005). In addition to having an adverse effect on plant growth and development, excessive plant absorption of Cd^{+2} can build up in various plant sections and subsequently enter human bodies through the food chain. Some results indicate that among the most dangerous heavy metals in the environment is cadmium (Jaishankar et al., 2014). Growing amounts of soil pollution from mining, sewage irrigation, air pollution, and fertilizer application harm crops and the health of people and animals. According to Cd^{+2} inhibits the process of photosynthesis and has an impact on the activity of the organism's protection enzymes or antioxidants in plants. Because agricultural soil surfaces contain amounts of heavy metals, they tend to bio-accumulate in the food chain (Quartacci et al., 2006). The main sources of agricultural land pollution are agronomic practices, the application of sludge as organic fertilizer, as well as the application of industrial effluents as a source of water for irrigation of agricultural plants (Singh and Sinha., 2005). Mining also plays an important role in contamination (Nedelkoska and Doran., 2000). Pollution with heavy metals affects the biosphere in different countries of the world (Meagher, 2000). The effect of Cd^{+2} and Pb^{+2} has also been observed in inhibiting the activity of photosynthesis (Baszyński et al., 1980), transpiration and carbohydrate metabolism (Moya et al., 1993; Pal et al., 2006; Paunov et al., 2018).

Plants show different patterns of heavy metal accumulation which would affect the biosynthesis of photosynthetic pigments (reduction of photosynthetic pigments such as chlorophylls and carotenoid content) (Mobin and Khan., 2007). However, there are also plants with heavy metal tolerance mechanisms, which accumulate and bind them to amino acids, peptides, and proteins (Horváth et al., 2007). Therefore, Pb^{+2} and Cd^{+2} cause inhibition in plant growth and development, ion absorption and transport disorders, inhibition of the photosynthesis process (Fargašová, 2001). The Chlorophyll a and b pigments usually use photosystem II to absorb light, which is followed by electron transport (Taiz and Zeiger., 2002). However, the mechanisms of heavy metal toxicity on photosynthetic activity cannot yet be considered a settled issue, due to variations in experimental design (Giardi et al., 1997). High concentrations of heavy metals (Cd^{+2}, Pb^{+2}) in agricultural areas have been identified as toxic and the situation is defined as heavy metal stress (Yıldırım et al., 2019). Similarly, heavy metal pollution is present in Kosovo, which is caused by various sources, including the Obiliq power plant, the Zveçan smelter and the ferronickel factory. In addition to soil surface pollution, they also affect the growth and development of plants during their vegetation (Aliu et al., 2013). In this experiment, we evaluated the effect of some heavy metals (Pb^{+2} and Cd^{+2}) in potato genotypes on different physiological traits.

2.Materials and Methods

2.1 Plant material and growth conditions

The plant materials included in our research were 2 different genotypes of potato (*Solanum tuberosum* L.); Potato genotypes (PG's) Riviera and Agria of Dutch origin. During this period, potting compost (minimum 1 kg/replications) was prepared for PG's cultivars and each treatment. In our research there were a total of 8 pots for heavy metals which were $PbCl_2$, $CdCl_2$ and control. Potato tubers were transferred to compost in pots weighing 2 kg in a controlled environment or controlled rooms with a photoperiod of 12 and a temperature of $25/19^{\circ}C$ day/night and a relative humidity of 75%. The compost consisted of pH $(CaCl_2)$ = 5.8; moisture= 65.11%, Humus= 30.9%, Organic Matter= 62.14%, Nitrogen(N)= 2.64mg100^{-1}, Phosphorus (P_2O_5)= 116 mg100^{-1}, Calcium (Ca)= 36.40 mg100^{-1}, Magnesium (Mg)= 15.58 mg100^{-1}, Zinc (Zn)= 0.12 mg100^{-1}, Copper (Cu)= 0.17 mg100^{-1}, Iron (Fe)= 2.73 mg100^{-1}, Molybdenum (Mb)= 0.34 mg100^{-1}.

In this experiment, during the growth period, were prepared different concentrations of heavy metals: ControlT0-dH$_2$O, T1- 55.62 mg kg^{-1} $PbCl_2$, T2-111.24 mg kg^{-1} $PbCl_2$, T3-166.86 mg kg^{-1} $PbCl_2$, T4-11 mg kg^{-1} $CdCl_2$, T5-22 mg kg^{-1} $CdCl_2$, T6-23 mg kg^{-1} $CdCl_2$.

2.2 Chl (a, b, and total) and carotenoid analysis

Plant pigments were extracted in amounts ranging from 60 to 100 mg of fresh leaves, which were then extracted into samples containing 80/20% (v/v) acetone/water with 0.5% w/v $MgCO_3$ at room temperature for a full day without knowledge. Each sample's photosynthetic pigments were extracted three times. Using the absorbance measured at 663 nm, 644 nm, and 452.5 nm for the maximum absorption of Chl a, Chl b, and carotenoids, respectively, in the UV-Vis spectrophotometer, BK-UV-10, the concentration of chlorophyll and carotenoid content was computed (Turfan, 2022). By using the absorption coefficient calculations outlined by Lichtenthaler(1986), Aliu et al. (2013) and Aliu et al. (2014) pigment content was determined in mg g^{-1} leaf dry weight.After 20 days of exposure, the following parameters were determined in different part of plants include heavy metal contents (HMC), Chl a, b, and carotenoid concentrations. All analyzes for the determination of HMs, including Pb^{2+} and Cd^{2+}, were achieved using a Spectrophotometer of atomic absorber (SAA) Thermo Elemental M, by U.S. EPA Method 245.5 Cold Vapor Atomic Absorption Spectroscopy.

2.3 Statistical analyses

The experimental design was a completely randomized design with three (3) replications. All changes for the investigated physiological parameters for Pb^{2+} and Cd^{2+} were tested through the statistical package MINITAB-14. Statistical differences were performed through Duncan's multiple tests.

3. Results and Discussion

Data regarding content of heavy metals (Pb^{+2} and Cd^{+2}) showed a large range of variability among the treatments. Results are given in *Table 1*. Based on the results of ANOVA (*Table 1*), the highest value for lead content for both Riviera and Agria potato genotypes was reached in the third treatment (T3-166.86 mg kg^{-1}PbCl$_2$). Values for the Riviera potato genotype at content of Leaf/Stem (L/S) were 0.091 mg kg^{-1}PbCl$_2$, while Root Lead Content (RLC) was 0.112mgkg^{-1}PbCl$_2$. While in the Agria potato genotype, the L/S values were 0.157 mgkg^{-1}PbCl$_2$ and RLC = 0.099mgkg^{-1}PbCl$_2$. Differences between genotypes for L/S content were D= +0.066mgkg^{-1} PbCl$_2$, and RLC= +0.022 mgkg^{-1} PbCl$_2$, highly significant differences at the 0.05 and 0.01 probability level. Also, the cadmium content for different treatments was different for the two potato genotypes that were in our research. The highest cadmium content for both genotypes Riviera and Agria was found in the sixth treatment (T6-23 mg kg^{-1}CdCl$_2$). The content of cadmium in the Riviera potato genotype for L/S was 0.007mg kg^{-1}CdCl$_2$, while in Agria 0.0079mg kg^{-1}CdCl$_2$. The differences between them were D= -0.0009mg kg^{-1}CdCl$_2$. While the root cadmium content (RCC) in the Riviera potato genotype was 0.0079mg kg^{-1}CdCl$_2$ and Agria 0.0089mg kg^{-1}CdCl$_2$. The differences between them were D= -0.001mg kg^{-1}CdCl$_2$. Results are given in *Table 1*. Heavy metals (HM's) were mostly observed accumulate in the roots. The content of Cd^{+2} and Pb^{+2} increased with increasing doses of these elements in the root (Kabata-Pendias, 2000; Cannata et al., 2013).

Table 1. The content of heavy metals in potato genotypes

Genotype	Treatments	Leaf/Stem	Roots
Riviera	Control	0.025[cd]	0.071[b]
	T1	0.040[c]	0.078[b]
	T2	0.083[ab]	0.057[c]
	T3	0.091[a]	0.112[a]
	T4	0.002[f]	0.009[d]
	T5	0.003[e]	0.004[f]
	T6	0.007[d]	0.007[e]
Agria	T1	0.032[c]	0.087[b]
	T2	0.080[b]	0.014[c]
	T3	0.157[a]	0.099[a]
	T4	0.001[f]	0.0036[f]
	T5	0.0029[e]	0.0038[e]
	T6	0.0079[d]	0.0089[d]

* – values within individual marked by at least one same letter are not significantly different at 0.05probability level

The reaction of the potato genotypes to Pb^{+2} and Cd^{+2} resulted in a difference (reduction) in the content of the physiological parameters of chlorophyll and carotene in the leaves, which were significantly different compared to the control. The levels with different concentrations of heavy metals consisted in their differences in the content of chlorophyll and carotenoids, which were different and significantly higher at the probability level of LSDp = 0.0.For both potato genotypes Riviera and Agria the effect with a high reduction in the content of Chla was found to treatment of third content (T3-166.86 mg kg^{-1} $PbCl_2$) on values 0.04mgg^{-1} FW(Riviera) and 0.26 mgg^{-1} FW(Agria). The differences between potato genotypes were D=-0.22 mgg^{-1} FW. The average value for control was 0.86 mgg^{-1} FW, compared to values (0.04 and 0.26) for content of Chla. The differences were 0.82 mgg^{-1} FW and 0.60 mgg^{-1} FW. Also, content for Chla affected by Cd^{+2} with a higher reduction for both potato genotypes Riviera and Agria was in T6- 23 mg kg^{-1} $CdCl_2$ (0.26 and 0.59 respectively). For the content of Chlbaffected by Pb^{+2} was found a significant difference between potato genotypes. The potato genotypes Riviera compared to control (0.32 mgg^{-1} FW) was with higher reduction in T3-166.86 mg kg^{-1} $PbCl_2$ on value 0.02 mgg^{-1} FW. While the Agria on value 0.19 mgg^{-1} FW. The differences between genotypes were – 0.17 mgg^{-1} FW.Also, for the Cd^{+2} content, significant changes were determined for both potato genotypes.Thus, the lowest value for content of Chl b for both potato genotypes was found in T6-23 mg kg^{-1} $CdCl_2$ with values of 0.12 mgg^{-1} FW and 0.23 mgg^{-1} FW. The differences between them were statistically significant. Results are presented in *Table 2*. Also, the results for the content of carotenoids depending on the concentration of the content of HM's had different results in relation to the control (1.28 mgg^{-1} FW). The lowest value of the Riviera and Agria genotype for Pb^{+2} and Cd^{+2} content was reached in T3 (0.18 mgg^{-1} FW), while the highest was T4 (0.95 mgg^{-1} FW). While in the Agria genotype, the highest value for Pb^{+2} content was in T1 (1.11 mgg^{-1} FW), while the lowest value was T3 (0.59 mgg^{-1} FW). For Cd^{+2} concentration level content, T4 had a high value (1.01 mgg^{-1} FW) while T6 had a low value (0.84 mgg^{-1} FW). The differences between them were very significant. Results are presented in *Table 2*. Various studies in relation to the presence of heavy metals have shown that the ability of plants to absorb and accumulate metals can vary, because this can depend to a significant extent on genotypes but also between cultivars of the same species. In this direction, significant changes in Cd^{+2} levels were found from one genotype to another and from one part to another of the same plant. Then it was found that some genotypes could accumulate less Cd^{+2} in the tuber, but more in the stem and leaves, results presented according to Dong et al. (2020). But other plants could also show a different change in the presence of heavy metals between different genotypes; for example, rice (Zhou et al., 2015).

For both potato genotypes, Chl a concentration was positively correlated with Chl b,carotenoids, total chlorophyll a+b.There was no correlation of Chla with total RootsandLeaf/ steam. Carotenoids were (next to Chl a) positively correlated with total Chla + b. Total chlorophyll (a and b) was (next to Chl b) positively correlated with Chla and carotenoids but negatively with roots and leaf / steam. The root biomass was (next to already mentioned parameters) positively correlated with the Leaf/steam. Results are presented in *Table 3*.

Table 2. The different concentration of lead (Pb^{2+}), and cadmium (Cd^{2+}) on photosynthetic pigment contents of different potato genotypes

Genotype	Treatments	Chl a (mgg^{-1}FW)	Chl b (mgg^{-1}FW)	Carotenoid (mgg^{-1}FW)	Total Chla+b (mgg^{-1}FW)
Riviera	Control	0.86[a]	0.32[a]	1.28[a]	1.18[a]
	T1	0.29[bc]	0.14[b]	0.41[c]	0.43[b]
	T2	0.08[c]	0.06[c]	0.19[c]	0.14[d]
	T3	0.04[d]	0.02[d]	0.18[d]	0.06[e]
	T4	0.62[b]	0.25[ab]	0.95[ab]	0.87[b]
	T5	0.52[b]	0.20[b]	0.82[bc]	0.72[bc]
	T6	0.20[cd]	0.12[b]	0.79[d]	0.32[c]
Agria	T1	0.65[b]	0.29[ab]	1.11[a]	0.94[b]
	T2	0.33[bc]	0.18[b]	1.01[ab]	0.51[cd]
	T3	0.26[bc]	0.19[b]	0.59[d]	0.45
	T4	0.84[a]	0.34[a]	1.01[ab]	1.18[a]
	T5	0.78[ab]	0.30[ab]	0.95[b]	1.08[ab]
	T6	0.59[b]	0.23[ab]	0.84[bc]	0.82[b]

* – values with in individual marked by at least one equal letter are not significantly different at 0.05 probability level

Table 3. Pearson correlation coefficients for potato genotypes across different treatments

Traits	Chl-a	Chl b	Carotenoids	Total Chl (a+b)	Roots
Chl b	0.96**				
Carotenoids	0.84**	0.88**			
Total Chla+b	0.99**	0.98**	0.86**		
Roots	-0.41	-0.36	-0.44	-0.40	
Leaf/Steam	-0.63	-0.50	-0.52	-0.60	0.69**

Correlation is significant at $p < 0.05$ (*) or $p < 0.01$ (**).

4. Conclusions

The level of different concentrations for heavy metals including Pb^{+2} and Cd^{+2} in the two potato genotypes Agria and Riviera resulted in high effects and differences for the investigated parameters. The content of the quantity of chlorophyll in the control was the highest, while the lowest value of the content of the quantity of chlorophyll in the two genotypes of potato was present in the third (T3) and sixth (T6) treatment with lead and cadmium. From this we can see that the presence of Cd^{+2} and Pb^{+2} had a bad (negative) effect on plant growth, photosynthetic activity, chlorophyll and carotenoid content, and these effects were more apparent when the concentration of two stress factors (Pb^{+2} and Cd^{+2}). We evaluated this effect in laboratory conditions and for future studies we recommend that this stress be evaluated in potato fields in Kosovo

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept:Aliu S., Demirbas S.; Methodology:Aliu S.,Jakupi M.; Formal analysis and investigation:Aliu S., Rusinovci I.,Zeka D.; Statistical Analyses:Aliu S.; Writing: Aliu S., Jakupi M.;Reviewand Editing: Aliu S., Demirbas S.All the authors have read and agreed to the published version of the manuscript.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Yerel Ekmeklik Buğday Genotiplerinin Bazı Kalite Özelliklerinin Belirlenmesi

Determination of Some Quality Traits of Local Bread Wheat Genotypes

Zeki MUT[1], Özge Doğanay ERBAŞ KÖSE[2*], Yusuf Murat KARDEŞ[3]

Öz

Yerel popülasyonlar bölgeye uzun yıllar adapte olan ve ıslah programlarında önemini sürekli koruyan önemli genetik kaynaklardır. Bu çalışma 2019-2020 ve 2020-2021 yetiştirme yıllarında ekmeklik buğday yerel popülasyonlarından seçilen yüz kırk iki saf hat ve iki tescilli ekmeklik buğday çeşidinin bazı kalite özelliklerini belirlemek amacıyla yürütülmüştür. Denemeler 12 × 12 alfa latis deneme deseninde 3 tekrarlamalı olarak yürütülmüştür. İncelenen bütün özellikler bakımından genotipler ve yıllar (nişasta hariç) arasında istatistiksel olarak önemli farklar tespit edilmiştir. Çalışmada iki yılın birleştirilmiş ortalama değerlerine göre bin tane ağırlığının 21.32 ile 45.09 g, hektolitre ağırlığının 75.12 ile 81.09 kg, tane protein oranının % 11.77 ile 16.07, Zeleny sedimantasyon değerinin 24.31 ile 48.50 ml, yaş gluten oranının % 23.96 ile 33.61, tanede kül oranının % 1.23 ile 1.97, yağ oranının % 1.39 ile 2.29, nişasta oranının % 61.45 ile 69.81, asit deterjanda çözünmeyen lif değerinin % 3.29 ile 5.91 ve nötr deterjanda çözünmeyen lif değerinin % 13.75 ile 17.07 arasında değiştiği belirlenmiştir. Biplot grafiğine göre; hektolitre ağırlığı, bin tane ağırlığı ve nişasta oranı arasında; kül oranı ve nötr deterjanda çözünmeyen lif oranı arasında; Zeleny sedimantasyon değeri, yaş gluten oranı ve protein oranı arasında; asit deterjanda çözünmeyen lif değeri ve yağ oranı özellikleri arasında pozitif ilişki belirlenmiştir. Ayrıca bu grafiğe göre; G99, G105, G126 ve G137 numaralı genotiplerin protein, yaş gluten ve Zeleny sedimantasyon değeri bakımından; G53, G67 ve G72 numaralı genotiplerin nişasta değeri bakımından ve G46 ile G53 numaralı genotiplerin bin tane ağırlığı bakımından en yüksek değerlere sahip olduğu belirlenmiştir. Sonuç olarak, araştırmada yer alan popülasyonların birçoğu kalite özellikleri bakımından tescilli çeşitlerden daha iyi sonuçlar göstermiştir. Çalışmada yer alan yerel ekmeklik buğday popülasyonlarının kalite açısından önemli genetik kaynaklar olduğu ve ıslah programlarında kullanım açısından önemli potansiyele sahip oldukları belirlenmiştir.

Anahtar Kelimeler: Buğday, Biplot, Kalite, Protein, Genetik kaynak

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Abstract

Local populations are key genetic resources that have adapted to the region throughout time and are still used in breeding operations. In this study, important quality features of local varieties were determined which was conducted with one hundred forty two local and two registered bread wheat genotypes in the 2019-2020 and 2020-2021 growing years. The trials were set up in a 12 × 12 alpha lattice design with three replications. Significant statistical differences were detected between genotypes and years (except for starch) for all examined traits. According to the two year-average; the thousand grain weight, hectoliter weight, protein ratio, Zeleny sedimentation value, wet gluten ratio, ash ratio, fat ratio, starch ratio, acid detergent fiber and neutral detergent fiber of the genotypes were changed between 21.32 - 45.09 g, 75.12 - 81.09 kg, 11.77 - 16.07%, 24.31 - 48.50 ml, 23.96 - 33.61, 1.23 - 1.97%, 1.39 - 2.29%, 61.45 - 69.81%, 3.29 - 5.51% and 13.75-17.07%, respectively. According to the Biplot graph; positive correlations were found between hectoliter weight, thousand grain weight and starch ratio; between ash ratio and neutral detergent insoluble fiber ratio; between Zeleny sedimentation value, wet gluten ratio and protein ratio; between acid detergent fiber value and oil ratio. Furthermore, according to this graph, genotypes G99, G105, G126, and G137 had the highest protein, wet gluten, and Zeleny sedimentation values; genotypes G53, G67, and G72 had the highest starch value; and genotypes G46 and G53 had the highest thousand grain weight values. As a result, the majority of the populations in the trial outperformed the registered varieties in terms of quality parameters.

Keywords: Wheat, Biplot, Quality, Protein, Genetic resource

1. Giriş

Tek yıllık otsu bir bitki olan buğdayın; ülkemizde yaklaşık 12 bin taksonu, 20'den fazla türü ve ıslah edilmiş 400'den fazla çeşidi bulunmaktadır. Buğday temel gıda maddesi olması ve geniş adaptasyon yeteneği yanında nakliyesi, depolanması, işlenmesi gibi aşamalarının kolaylığı nedenleriyle dünyanın birçok ülkesinde yaygın olarak yetiştirilmektedir (Kün, 1996). Buğday; ekmek, bulgur, makarna, bisküvi, kahvaltılık gevrek gibi birçok gıda ürünü olarak kullanılmasının yanında hayvan beslenmesi ve bazı sanayi ürünlerinin eldesinde çok büyük öneme sahiptir. Buğday, Dünya ve Türkiye'de en fazla yetiştirilen kültür bitkisidir. Dünyada 220 milyon ha alandan 770 milyon ton buğday üretimi yapılmaktadır (Anonim, 2022). Türkiye'de ise yaklaşık 6.9 milyon ha alandan 20.5 milyon ton buğday üretilmektedir. Üretilen bu buğdayın % 80.5 ini ekmeklik buğday oluşturmaktadır (Anonim, 2021). Türkiye Dünya buğday üretiminin % 2.2'sini oluşturmaktadır.

Dünya'da buğday üretiminde kuraklık, hastalık vs. herhangi bir nedenden dolayı meydana gelen azalışlar buğdaydan elde edilen ürünlerin fiyatlarında artışa neden olarak herkesi etkilemektedir. Buğday yetiştiriciliği yapan ülkeler için yeterli miktarda ekim alanı sağlanarak buradan elde edilen ürünlerin bir kısmının stoklanması stratejik bir öneme sahiptir (Süzer, 2019). Dünya nüfusunda meydana gelen sürekli artış ve tarım alanlarının azalması sonucu oluşabilecek gıda açığının kapatılabilmesi için yeni üretim teknikleri ve ıslah edilmiş yeni çeşitlerin kullanımının yaygınlaştırılması gerekmektedir. Ülkemizde de buğday ekim alanlarının son sınırına gelmiş olması sebebiyle birim alandan alınan verimin artırılması çok önemlidir (Mut ve ark., 2005). Buğday yetiştiriciliğinde verim ile birlikte kalitenin de ele alınması gerekmektedir. Buğdayın kalitesi çeşit, iklim faktörleri, toprak özellikleri ve yetiştirme tekniğine bağlı olarak değişmektedir. Buğdayda kalite kavramı buğdayın kullanım yeri ve amacına göre (üretici, sanayi ve tüketici) değişiklik göstermektedir. Günümüzde bu kesimlerin isteklerini karşılayabilen verimi ve kalitesi yüksek, hastalıklara dayanıklı buğday çeşitlerine olan ihtiyaç artarak devam etmektedir (Konak ve ark., 1999). Bir ürünün farklı kullanım amaçlarına uygunluğu kalite olarak ifade edilmektedir. Buğday ıslahında ve ticaretinde protein oranı, hektolitre ağırlığı, 1000 tane ağırlığı, gluten ve sedimantasyon değerleri en önemli kriterlerdir. Protein oranı buğdayda kalitenin belirlenmesinde rol oynayan en önemli faktördür (Sade, 1997). Buğdayın protein kalitesi de kalitenin belirlenmesinde önemli bir faktördür. Bu kriterin belirlenmesinde sedimantasyon değeri ve gluten miktarı kullanılan en önemli yöntemlerdir (Linina ve Ruza, 2015; Mut ve ark., 2017).

Türkiye kültürü yapılan buğday türlerinin ve yabani akrabalarının gen merkezi durumundadır. Bu türler buğdayın gelişimi, farklı koşullara adaptasyonu, yayılması ve modern çeşitlerin geliştirilmesinde önemli rol oynamaktadır. Bitki ıslahçıları ıslah çalışmalarının her evresinde yüksek bir genetik çeşitliliğe ihtiyaç duymaktadırlar. Mevcut materyalde çeşitlilik meydana getirmenin birçok yolu bulunmaktadır, fakat genetik kaynak durumunda olan materyallerde bu çeşitlilik doğal olarak bulunmaktadır. Yerel populasyonlarımız, kültürü yapılan bitkilerin yabani akrabaları ve geçiş formları ile birlikte ıslah çalışmalarında kullanılabilecek bir zenginlik kaynağıdır. Bu özelliklerinden dolayı yerel populasyonlar bitki geliştirme çalışmalarının en önemli kaynakları olarak kullanılmaktadırlar (Karagöz, 2014). Ayrıca, bu genetik materyal kullanılarak ekonomik açıdan üstün özeliklere sahip olanlar belirlenip kullanılabilir.

Başta buğday olmak üzere bütün bitki yerel populasyonları, yetiştiriciler ve ıslahçılar tarafından uzun yıllar boyunca seçilerek kullanılmıştır. Bu yerel genotipler çeşitli biyotik ve abiyotik stres koşullarına dayanıklılık sağlamışlardır. Buğday veriminin artırılması ve kalitesinin korunması yeterli ve güvenli gıda endişesinin arttığı günümüzde önemli bir noktada yer almaktadır. Bu açıdan genetik çeşitlilik barındıran yerel materyallerin toplanması, verim ve kalite özelliklerinin belirlenmesi, çeşitli koşullara adaptasyon kabiliyetlerinin ölçülmesi ve muhafazası çok önemli bir durum haline gelmiştir. Yerel populasyonlarımız verim ve verim özellikleri ile kalite faktörleri açısından büyük önem arz etmektedirler. Bunlar ıslah çalışmalarında ebeveyn olarak ve hatta seleksiyon yöntemiyle saflaştırılarak yeni çeşitler elde edilebilecek potansiyele sahiptir (Dirik ve ark., 2018).

Araştırmada, 104 adet yerel ekmeklik buğday populasyonundan seçilen 142 saf hat ile 2 standart çeşidin (Altındane ve Nusrat) iki yıl boyunca bazı kalite özelliklerinin belirlenmesi amaçlanmıştır.

2. Materyal ve Metot

2.1. Materyal

Çalışmada uzun yıllar önce Karadeniz Bölgesindeki 12 ilden toplanıp Ege Tarımsal Araştırma Enstitüsü Ulusal Gen Bankasında muhafaza edilen 104 adet yerel ekmeklik buğday popülasyonundan kılçık varlığı, başak uzunluğu, başakta başakçık sıklığı, büyüme formu, başaklanma süresi, tane dolum periyodu, bitki boyu, başak uzunluğu, başakta başakçık sayısı, başakta tane sayısı, başakta tane ağırlığı, başak rengi, tek başak verimi, bin tane ağırlığı, tane rengi, biyomas ağırlığı ve hasat indeksi gibi birçok özellik dikkate alınarak tek bitki seleksiyonu yapılmış ve toplam 142 saf hat oluşturulmuştur. Denemede bu 142 saf hat ile birlikte 2 standart çeşit (Altındane ve Nusrat) kullanılmıştır (*Tablo 1*). Bu çeşitlerden 'Altındane' Karadeniz Tarımsal Araştırma Enstitüsü 'Nusrat' ise Sakarya Mısır Araştırma Enstitüsü tarafından tescil ettirilmiştir.

Tablo 1. *Projede kullanılan buğday genotiplerinin kayıt numaraları ve toplandığı yerler*

Table 1. Registration numbers and collected location of wheat genotypes used in the project

No	Kayıt No*	No	Kayıt No	No	Kayıt No	No	Kayıt No
1	TR 54989.ACS1601[a]	37	TR 54986.ACS1601[d]	73	TR 44365.ACS1603[i]	109	TR 87424.ACS1603[j]
2	TR 54989.ACS1602[a]	38	TR 82297.ACS1601[d]	74	TR 44984.ACS1601[i]	110	TR 87425.ACS1601[j]
3	TR 81014.ACS1601[a]	39	TR 82297.ACS1602[d]	75	TR 44984.ACS1602[i]	111	TR 87426.ACS1601[j]
4	TR 81014.ACS1603[a]	40	TR 82297.ACS1603[d]	76	TR 44984.ACS1603[i]	112	TR 87426.ACS1605[j]
5	TR 81014.ACS1604[a]	41	TR 82297.ACS1604[d]	77	TR 44984.ACS1604[i]	113	TR 87426.ACS1606[j]
6	TR 81014.ACS1605[a]	42	TR 82297.ACS1606[d]	78	TR 45098.ACS1601[i]	114	TR 87426.ACS1607[j]
7	TR 81014.ACS1606[a]	43	TR 37585.ACS1601[e]	79	TR 45098.ACS1608[i]	115	TR 87426.ACS1602[j]
8	TR 81014.ACS1607[a]	44	TR 37585.ACS1602[e]	80	TR 45098.ACS1609[i]	116	TR 87426.ACS1604[j]
9	TR 81014.ACS1608[a]	45	TR 37585.ACS1603[e]	81	TR 53874.ACS1601[i]	117	TR 87427.ACS1601[j]
10	TR 81014.ACS1609[a]	46	TR 37585.ACS1612[e]	82	TR 53874.ACS1605[i]	118	TR 87427.ACS1602[j]
11	TR 81014.ACS1611[a]	47	TR 37585.ACS1613[e]	83	TR 53874.ACS1606[i]	119	TR 87429.ACS1602[j]
12	TR 81014.ACS1613[a]	48	TR 37585.ACS1614[e]	84	TR 81468.ACS1603[i]	120	TR 87430.ACS1601[j]
13	TR 82307.ACS1601[a]	49	TR 37585.ACS1615[e]	85	TR 81468.ACS1604[i]	121	TR 87431.ACS1601[j]
14	TR 82307.ACS1602[a]	50	TR 37831.ACS1601[e]	86	TR 81468.ACS1606[i]	122	TR 87431.ACS1605[j]
15	TR 82307.ACS1603[a]	51	TR 37831.ACS1602[e]	87	TR 82329.ACS1601[i]	123	TR 87431.ACS1608[j]
16	TR 39606.ACS1601[b]	52	TR 58073.ACS1602[e]	88	TR 82329.ACS1604[i]	124	TR 87432.ACS1601[j]
17	TR 39606.ACS1602[b]	53	TR 58073.ACS1601[e]	89	TR 37192.ACS1601[i]	125	TR 87432.ACS1605[j]
18	TR 39609.ACS1601[b]	54	TR 14857.ACS1602[f]	90	TR 87012.ACS1601[j]	126	TR 87432.ACS1606[j]
19	TR 39609.ACS1603[b]	55	TR 14857.ACS1603[f]	91	TR 87012.ACS1602[j]	127	TR 87433.ACS1602[j]
20	TR 39609.ACS1604[b]	56	TR 37248.ACS1601[g]	92	TR 87291.ACS1601[j]	128	TR 87433.ACS1606[j]
21	TR 39609.ACS1605[b]	57	TR 37248.ACS1602[g]	93	TR 87417.ACS1601[j]	129	TR 87433.ACS1607[j]
22	TR 45104.ACS1601[b]	58	TR 37248.ACS1603[g]	94	TR 87417.ACS1606[j]	130	TR 87433.ACS1608[j]
23	TR 45104.ACS1602[b]	59	TR 37248.ACS1604[g]	95	TR 87418.ACS1601[j]	131	TR 44388.ACS1601[j]
24	TR 45104.ACS1603[b]	60	TR 37369.ACS1601[g]	96	TR 87418.ACS1607[j]	132	TR 44406.ACS1601[j]
25	TR 81147.ACS1601[c]	61	TR 81420.ACS1601[g]	97	TR 87419.ACS1601[j]	133	TR 44406.ACS1602[k]
26	TR 81147.ACS1602[c]	62	TR 81420.ACS1602[g]	98	TR 87419.ACS1602[j]	134	TR 44431.ACS1604[k]
27	TR 81147.ACS1603[c]	63	TR 81642.ACS1601[g]	99	TR 87420.ACS1601[j]	135	TR 44433.ACS1601[k]
28	TR 37373.ACS1602[d]	64	TR 81646.ACS1602[g]	100	TR 87420.ACS1604[j]	136	TR 44433.ACS1603[k]
29	TR 37373.ACS1603[d]	65	TR 44384.ACS1601[g]	101	TR 87421.ACS1601[j]	137	TR 81263.ACS1601[k]
30	TR 37373.ACS1604[d]	66	TR 44384.ACS1602[h]	102	TR 87421.ACS1605[j]	138	TR 81035.ACS1602[k]
31	TR 37373.ACS1605[d]	67	TR 44400.ACS1601[h]	103	TR 87422.ACS1601[j]	139	TR 39610.ACS1602[k]
32	TR 37383.ACS1604[d]	68	TR 44400.ACS1602[h]	104	TR 87422.ACS1606[j]	140	TR 81265.ACS1603[k]
33	TR 37383.ACS1605[d]	69	TR 44400.ACS1603[h]	105	TR 87422.ACS1607[j]	141	TR 81266.ACS1602[b]
34	TR 53869.ACS1601[d]	70	TR 44400.ACS1606[h]	106	TR 87423.ACS1601[j]	142	TR 44468.ACS1601[l]
35	TR 53869.ACS1603[d]	71	TR 44365.ACS1601[h]	107	TR 87423.ACS1605[j]	143	Altındane (Standart)
36	TR 54984.ACS1603[d]	72	TR 44365.ACS1602[i]	108	TR 87424.ACS1601[j]	144	Nusrat (Standart)

*Kodu ve toplandığı yer (a: Amasya, b: Artvin, c: Bayburt, d:Çorum, e: Giresun, f: Gümüşhane, g:Kastamonu, h: Ordu, i: Samsun, j: Sinop, k: Tokat, l: Trabzon)

2.2. Deneme yıllarına ve yerlerine ait iklim ve toprak özellikleri

Tablo 2'de Bilecik Meteoroloji İl Müdürlüğünden alınan Bilecik ili uzun yıllar (1939-2021), 2019-2020 ve 2020-2021 yetiştirme sezonlarına ait iklim verileri verilmiştir.

Tablo 2. Bilecik ili uzun yıllar, 2019-2020 ve 2020-2021 yetiştirme sezonlarına ait iklim verileri

Table 2. Climate data of Bilecik province during 2019-2020 and 2020-2021 growing seasons

Aylar	Sıcaklık (°C)			Yağış (mm)			Nispi nem (%)		
	UYO	2019-20	2020-21	UYO	2019-20	2020-21	UYO	2019-20	2020-21
Kasım	9.0	12.7	8.3	37.2	27.6	3.6	71.1	63.0	72.0
Aralık	4.5	5.6	7.9	55.9	78.4	9.7	76.0	78.0	71.5
Ocak	2.4	2.4	5.6	50.1	45.4	78.3	76.5	74.0	58.6
Şubat	3.7	5.2	5.7	42.0	65.6	37.7	73.2	72.1	68.0
Mart	6.4	8.6	5.1	47.3	34.1	101.0	69.3	68.8	72.1
Nisan	11.5	10.8	11.4	41.8	36.0	73.0	64.2	61.0	67.0
Mayıs	16.1	16.7	17.5	47.7	55.2	35.0	64.5	62.0	60.1
Haziran	19.9	19.8	19.0	39.3	139.1	62.4	62.0	59.7	68.0
Temmuz	21.7	22.9	23.8	30.9	1.2	35.4	61.0	63.0	60.3
Ortalama	**10.6**	**11.6**	**11.6**				**68.6**	**66.8**	**66.4**
Toplam				**392.2**	**482.6**	**436.1**			

* Bilecik Meteoroloji İl Müdürlüğü (Anonim, 2023), UYO: Uzun yıllar ortalaması

Denemeler her iki yılda da aynı arazide kurulmuştur. Bilecik Şeyh Edebali Üniversitesi Ziraat ve Doğa Bilimleri Fakültesi Toprak Analiz Laboratuvarında yapılan toprak analizi sonuçlarına göre, deneme alanı toprağının killi-tınlı (% 40), pH bakımından hafif alkali (7.78), orta seviyede kireçli (% 6.84), hafif tuzlu (% 0.45), fosfor içeriği fazla (22.16 kg da^{-1}), potasyum içeriği yüksek (66.90 kg da^{-1}) ve organik madde içeriğinin orta (% 2.26) olduğu tespit edilmiştir (Tablo 2).

2.3. Yöntem

Araştırma 2019-2020 ve 2020-2021 yetiştirme sezonlarında Bilecik Şeyh Edebali Üniversitesi Tarımsal Uygulama ve Araştırma Merkezinde yürütülmüştür. Deneme 12 × 12 Alfa-latis deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Ekim işlemi m²'ye 550 tohum gelecek şekilde parsel boyu 2 metre ve 4 sıra olarak birinci yıl 10.11.2019 tarihinde ikinci yıl 16.11.2020 tarihinde yapılmıştır. Dekara 6 kg P_2O_5 ve 10 kg saf azot gelecek şekilde fosforlu gübrenin tamamı ve azotlu gübrenin (2.34 kg da^{-1}) ilk kısmı ekimle birlikte, diğer kalan kısım ise kardeşlenme ve başaklanma öncesi dönemde iki eşit parçaya bölünerek verilmiştir. Yabancı ot mücadelesi için kardeşlenme döneminde herbisit kullanılmıştır. Hasat olgunluğuna gelen bitkilerin hasadı birinci yıl 17.07.2020 ikinci yıl ise 20.07.2021 tarihlerinde elle yapılmış ve hasat edilen materyal bir hafta kadar kurutulduktan sonra parsel harman makinesi ile harmanlanmıştır.

Araştırmadan elde edilen örneklerin yabancı madde temizliği yapıldıktan sonra bin tane ağırlığı, hektolitre ağırlığı, protein oranı, kül oranı, nişasta oranı, yaş gluten oranı, Zeleny sedimantasyon değeri, asit deterjanda çözünmeyen lif (ADF) ve nötr deterjanda çözünmeyen lif (NDF) gibi bazı kalite özellikleri incelenmiştir. Bin tane ağırlığı 4'er kez 100 adet tohum sayma cihazı (Chopin Technologies-Numigral) ile sayılarak ortalaması alınmış ve 10 ile çarpılarak belirlenmiştir. Hektolitre ağırlığı AACC 55-10.01 metoduna göre belirlenmiştir (AACC, 2020). Kimyasal analizler için her parselden alınan örnekler öğütülmüştür. Örnekler analizler için +4 °C'de buzdolabında saklanmış ve her iki yılda da hasattan sonraki üç ay içerisinde analiz edilmiştir. Kül içeriği AACC 08-01.01, ham protein içeriği AACC 46-30.01, nişasta içeriği AACC 76-33.01, yaş gluten içeriği AACC 38-12.02 ve Zeleny sedimantasyon değeri AACC 56-60.01 metotlarına göre yapılmıştır (AACC, 2020). Yağ içeriği Soxhlet yöntemine (Welch, 1977), ADF ve NDF içerikleri Van Soest ve ark. (1991)'nın yöntemine göre ANKOM 220 Fiber cihazı kullanılarak belirlenmiştir.

2.3. İstatistiksel analiz

Araştırmada incelenen tüm özellikler 12×12 Alfa Latis deneme desenine göre (Patterson ve Williams, 1976; Kumar ve ark., 2020) SAS (1998) paket programı kullanılarak analiz edilmiştir. Genotipler ve yıllar arasında farklılık belirlenen özelliklerin ortalamaları arasındaki karşılaştırmalar ise Tukey çoklu karşılaştırma testi ile değerlendirilmiştir. Elde edilen sonuçların ortalama, en düşük, en yüksek değerleri ve kümülatif grafikleri Minitab 15 paket programı kullanılarak oluşturulmuştur. Biplot grafiği JMP (2020) programı kullanılarak çizilmiştir.

3. Araştırma Sonuçları ve Tartışma

Araştırma yerine ait düşen toplam yağış miktarı ilk yılda (482.6 mm) ikinci yıla (436.1mm) göre daha yüksek olmuştur. Yıllar arasında ortalama sıcaklık ve nem bakımından önemli farklılık görülmese de bu değerler aylara göre farklılık göstermiştir (*Tablo 2*). İncelenen özelliklere ait bazı veriler (ortalama, en küçük ve en büyük değerler ile varyasyon katsayısı, kareler ortalaması ve P değeri) Tablo 3'de verilmiştir. İncelenen bütün özellikler bakımından genotipler ve nişasta hariç yıllar arasında önemli farklar bulunmuştur (*Tablo 3* ve *Şekil 1*).

Tablo 3. 144 buğday genotipinin bazı kalite özelliklerine ait yılların birleştirilmiş ortalama, minimum, maksimum ve bazı istatistiksel değerleri.

Table 3. The values of mean, minimum, maximum and some statistical of the some quality traits of the 144 wheat genotypes in the combined data of the years.

Özellikler	Ortalama	En Düşük	En Yüksek	Varyasyon Katsayısı	Kareler Ortalaması	P değeri
Bin tane ağırlığı (g)	35.98	21.32	45.09	12.92	121.40	<.0001
Hektolitre ağırlığı (kg)	78.36	75.12	81.09	1.48	7.72	<.0001
Protein (%)	13.75	11.77	16.07	6.07	109.02	<.0001
Zeleny sedimantasyon (ml)	36.29	24.31	48.50	14.73	154.35	<.0001
Yaş gluten (%)	28.67	23.96	33.61	6.11	16.68	<.0001
Kül (%)	1.61	1.23	1.97	10.18	0.15	<.0001
Yağ (%)	1.82	1.39	2.29	8.17	0.12	<.0001
Nişasta (%)	66.07	61.45	69.81	2.63	16.80	<.0001
Asit deterjanda çözünmeyen lif (%)	4.37	3.29	5.91	10.87	1.17	<.0001
Nötr deterjanda çözünmeyen lif (%)	15.54	13.75	17.07	3.84	2.01	<.0001

Birleştirilmiş varyans analiz sonuçlarına göre, genotiplerin bin tane ve hektolitre ağırlıkları sırasıyla 21.32 (G57) ile 45.09 g (G53) ve 75.12 (G87) ile 81.09 kg (G109) arasında değişmektedir (*Tablo 3*). Hem bin tane ağırlığı hem de hektolitre ağırlığı ilk yıl ikinci yıldan daha yüksek olmuştur (*Şekil 1*). İlk yıl toplam yağışın fazla olmasının bu özellikleri olumlu yönde etkilediği düşünülmektedir. Genotiplerin büyük kısmı bin tane ağırlığı bakımından 35.0-39.0 g sınıfında yer alırken, hektolitre ağırlığı bakımından 77.75-78.25 kg sınıfında yer almıştır (*Şekil 2*). Çalışmada kullanılan Altındane ve Nusrat çeşitlerinin bin tane ağırlığı ve hektolitre ağırlığı sırasıyla % 37.56 g ve 43.37 g, 80.65 kg ve 80.45 kg olarak belirlenmiştir. Çalışmada ele alınan 78 hattın hektolitre ağırlığı 78 kg'ın üzerinde hektolitre ağırlığına sahip olmuştur. Ayrıca G85, G92, G99, G106, G109 ve G111 numaralı genotipler çeşitlerin ortalamasından daha yüksek hektolitre ağırlığına sahip olmuştur. Buğday endüstrisinde bin tane ağırlığı ve hektolitre ağırlığı, un verimini belirleyen temel kalite faktörlerindendir. Buğdayda endosperm büyük ve yoğun tanelerde küçük taneli olanlara göre daha yüksek olduğundan buğday un miktarının tahmininde bin tane ağırlığı iyi bir ölçü olarak kabul edilmektedir (Mut ve ark., 2007). Farklı araştırmacılar ekmeklik buğday genotipleri üzerinde yaptıkları çalışmalarda bin tane ağırlığının genetik, tarımsal uygulamalar ve çevresel faktörlere göre değişiklik gösterdiğini bildirmişlerdir (Kara ve Akman, 2008; Bilgin ve ark., 2016; Mut ve ark., 2017; El Refaey ve ark., 2022; Osekita ve ark., 2022). Hektolitre ağırlığı un veriminin belirlenmesinde kullanılan önemli bir kriterdir. Hektolitre ağırlığı da genotipe, çevre koşullarına, uygulanan tarımsal işlemlere, hastalıklar ve zararlıların etkisine göre değişmektedir (Bilgin ve ark., 2016; Mut ve ark., 2017; Yüce ve ark., 2022). Ayrıca büyüme mevsimi boyunca yağış ve sıcaklık gibi çevresel faktörler hektolitre ağırlığını etkilerken, vejetasyon süresi, tane yoğunluğu, tane boyutu ve şekli, karın boşluğu, tanenin kırışıklığı gibi tanenin yapısal özellikleri de bu özelliği etkilemektedir (Aguirre ve ark., 2002).

Çalışmada genotiplerin protein oranı, Zeleny sedimantasyon değeri ve yaş gluten içeriği sırasıyla % 11.77 (G106) ile 16.07 (137), 24.31 (G106) ile 48.50 ml (G137) ve % 23.96 (G2) ile 33.61 (G13) arasında değişmiştir. Protein oranı ve yaş gluten içeriği ikinci yıl daha yüksek bulunurken sedimantasyon değeri birinci yıl daha yüksek bulunmuştur (*Şekil 1*). Genotiplerin büyük kısmı protein oranı bakımından % 13.00-14.50, Zeleny sedimantasyon değeri bakımından 32.0-40.0 ml ve yaş gluten oranı bakımından ise % 27.25-30.25 olan sınıfta yer almışlardır (*Şekil 2*). Çalışmada kullanılan Altındane ve Nusrat çeşitlerinin protein oranı, Zeleny

sedimantasyon değeri ve yaş gluten oranları sırasıyla % 14.29 ve % 14.01, 41.14 ve 35.32 ml, % 30.32 ve 30.73 olarak belirlenmiştir. Ayrıca protein oranı bakımından 45 genotip, Zeleny sedimantasyon değeri bakımından 24 genotip ve yaş gluten oranı bakımından 11 genotip her iki tescilli çeşitten de yüksek değerlere sahip olmuşlardır. Buğdayda tanenin protein oranı en önemli kalite kriteridir. Bu özellik ekmeğin beslenme ve pişme özelliklerini önemli ölçüde etkilemektedir. Buğdayın protein oranı genotipe, çevre faktörlerine ve tarımsal uygulamalara bağlı olarak değişmektedir (Anjum ve ark., 2014; Özen ve Akman, 2015). Ayrıca Çağlar ve ark. (2006) yağışın az olduğu yıllarda protein oranının arttığını bildirmişlerdir. Bu çalışmada da protein oranın yüksek bulunduğu ikinci yıl daha düşük yağış gerçekleşmiştir. Protein oranı kadar protein kalitesi de buğday için çok önemlidir. Protein kalitesinin belirlenmesinde akla gelen ilk iki özellik sedimantasyon değeri ve gluten içeriğidir. Ekmek ununda gluten proteinleri hamurun şişmesi ve elastikiyeti açısından önemli bileşenlerdir (Egesel ve ark., 2009). Hamurun yoğrulması sırasında ağ benzeri bir yapı oluşturan gluten proteinleri, mayanın oluşturduğu karbondioksitin tutulmasını ve hamurun şişmesini sağlar; dolayısıyla gluten miktarı unun kalitesini belirleyen en önemli özelliklerden biridir. Gluten miktarı sonuçlarının değerlendirilmesinde ≤ %20 (düşük), %20-27 (orta), %28-35 (iyi) ve >%35 (yüksek) değerleri kullanılmaktadır (Özen ve Akman, 2015). Sedimantasyon değeri arttıkça ekmek hacmi de artacağından ekmeklik buğdayda sedimantasyon değerinin yüksek olması istenmektedir (Zeleny ve ark., 1960). Sedimantasyon miktarı sonuçlarının değerlendirilmesinde ≤ 15 (çok kötü), 16-21 (zayıf), 22-27 (orta), 28-33 (iyi) ve > 33 (çok iyi) parametreleri kullanılmaktadır (Şanal ve ark., 2009). Kaliteli ekmeklik buğday ununun yaş gluten içeriğinin ve sedimantasyon değerinin sırasıyla % 28 ve % 25'in üzerinde olması gerektiği rapor edilmiştir (Mutlu ve Taş, 2020). Farklı araştırmacılar yaptıkları çalışmalarda çeşitlere göre sedimantasyon ve yaş gluten değerlerinin farklılık gösterdiğini bildirmişlerdir (Mut ve ark., 2017; Kurt ve Yağdı, 2018; Karaduman ve ark., 2021; Balkan ve ark., 2022).

Figure 1. Mean values and standard deviation for some quality traits of wheat genotypes in 2019-2020 and 2020-2021. Bars not accompanied by the same letter are significantly different at P < 0.05, using Tukey test. (BTA: thousand grain weight, HA: hectolitre weight, PO: protein ratio, SD: Zeleny sedimentation value, YG: wet gluten ratio, KO: ash ratio, YO: fat ratio, ADF: acid detergent fiber, NDF: neutral detergent fiber, od = non significant)

Şekil 1. Buğday genotiplerinin bazı kalite özelliklerine ilişkin 2019-2020 ve 2020-2021 yıllarında ortalama değerler ve standart sapmalar. Farklı harf ile gösterilen çubuklar Tukey testine göre P <0.05 düzeyinde önemli ölçüde farklıdır (BTA: bin tane ağırlığı, HA: hektolitre ağırlığı, PO: protein oranı, SD: Zeleny sedimantasyon değeri, YG: yaş gluten oranı, KO: kül oranı, YO: yağ oranı, ADF: asit deterjanda çözünmeyen lif, NDF: nötr deterjanda çözünmeyen lif, öd= önemli değil)

Figure 2. Histogram graphs of the examined traits of wheat genotypes

Şekil 2. Buğday genotiplerinin incelenen özelliklerine ait histogram grafikleri

Genotiplerin kül, yağ ve nişasta içerikleri sırasıyla % 1.23 (G72) ile 1.97 (G134), % 1.39 (G69) ile 2.29 (G113) ve % 61.45 (G134) ile 69.81 (G67) arasında değişmiştir (*Tablo 3*). Kül ve yağ içeriği ikinci yıl daha yüksek bulunmuş, nişasta oranı bakımından yıllar arasında fark bulunmamıştır (Şekil). Genotiplerin büyük kısmı kül oranı bakımından % 1.45-1.75, yağ oranı bakımından % 1.725- 1.825 ve nişasta oranı bakımından ise %

65.25-67.25 olan sınıfta yer almışlardır (*Şekil 2*). Çalışmada kullanılan Altındane ve Nusrat çeşitlerinin kül, yağ ve nişasta oranları sırasıyla % 1.55 ve % 1.83, % 1.81 ve % 1.76, % 66.81 ve 63.59 olarak belirlenmiştir. Birçok araştırıcı buğdayda kül içeriğinin kullanılan genotipe, yapılan tarımsal uygulamalara, iklim ve toprak faktörlerine bağlı olarak değiştiğini bildirmişlerdir (Egesel ve ark., 2009; Mahla ve ark., 2015; Mut ve ark., 2017; Arduç ve ark., 2020). Küçük ve kırışık taneler büyük ve düz tanelerden daha fazla kül içerir. Küçük taneli buğdaylarda un verimi düşerken kül miktarı artmaktadır. Bu durumda ekmek rengi, hamurun gaz tutma kapasitesi ve ekmek hacmi olumsuz etkileneceğinden yüksek kül içeriği kaliteli bir ekmek üretimi için tercih edilmez (Bulut, 2012; Bilgiçli ve Soylu, 2016). Kül miktarını arttıran çevre faktörleri protein miktarını da arttırır. Çalışmada kullanılan standart çeşitlerin özellik belgelerinde kalite değerlerinin bu çalışmada elde edilen değerlerden daha yüksek olduğu görülmektedir. Kalite özelliklerinin genotiplerin yetiştirildikleri toprak, iklim ve yetiştirme koşullarından da etkilendiği bilinmektedir. Bundan dolayı çalışmada yer alan genotiplerin de kalite değerlerinin daha yüksek olabileceği düşünülmektedir.

Çalışmamıza benzer olarak Çağlar ve ark. (2006) yağışın az olduğu yıllarda genotiplerin kül içeriğinin arttığını ve kül içeriğinin yıllara göre değiştiğini bildirmişlerdir. Birçok araştırıcı yağ oranının genotip, çevre koşulları ve tarımsal uygulamalara bağlı olarak değiştiğini bildirmektedir (Barteczko ve ark., 2009; Mut ve ark., 2017; Arduç ve ark., 2020). Buğdayda tüm tanede yağ oranı genellikle % 2'nin altındadır. Buğdayın en çok yağ içeren kısım embriyo (% 11) kısmıdır ve bu yağ gıda endüstrisinde, kozmetik formülasyonlarda ve biyolojik böcek kontrol ajanlarının hazırlanmasında kullanılmaktadır (Çetinyürek, 2012). Benli ve Koca (2018) buğday çeşitlerinde yağ içeriğinin yıllara ve çeşitlere göre % 1.1 ile 2.4 arasında değiştiğini bildirmişlerdir. Buğdayda nişasta oranı çok önemli bir kalite kriteri olmakla birlikte buğday ununda en yüksek oranda bulunan kimyasal bileşendir. Buğdayda nişasta içeriğinin kullanılan genotipe, yapılan tarımsal uygulamalara, iklim ve toprak faktörlerine bağlı olarak değiştiğini bildirmiştir (Mut ve ark., 2017; Şahin Tenikecier ve Öner, 2018; Arduç ve ark., 2020).

Tablo 3. *İncelenen özellikler bakımından öne çıkan genotipler**

Table 3. Genotypes that promising in terms of the examined traits

İncelenen Özellikler	Öne çıkan genotipler
Bin tane ağırlığı	G2, G10, G22, G24, G26, G29, G46, G47, G53, G67, G71, G72, G88, G98, G109, G111, G120, G128, G127, Nusrat
Hektolitre ağırlığı	G39, G70, G77, G81, G85, G88, G92, G98, G99, G102, G105, G106, G109, G111, G124, G137, G138, G141, Altındane, Nusrat
Protein	G12, G19, G20, G26, G29, G79, G82, G88, G95, G99, G104, G105, G108, G109, G112, G117, G126, G134, G137, G138
Sedimantasyon	G12, G13, G21, G22, G27, G32, G33, G39, G55, G69, G77, G88, G99, G105, G108, G126, G130, G132, G137, G139
Yaş gluten	G8, G12, G13, G20, G26, G77, G79, G82, G95, G99, G104, G105, G108, G109, G112, G117, G126, G134, G137, Nusrat
Kül	G4, G24, G38, G39, G44, G45, G46, G53, G58, G67, G71, G72, G74, G80, G81, G103, G106, G116, G120, G132
Yağ	G28, G47, G57, G59, G67, G76, G83, G85, G92, G104, G109, G110, G111, G112, G113, G114, G116, G117, G119, G139
Nişasta	G4, G24, G34, G38, G39, G45, G46, G53, G58, G67, G71, G72, G73, G74, G80, G81, G86, G103, G116, G120
Asit deterjanda çözünmeyen lif	G6, G13, G14, G18, G21, G22, G26, G35, G39, G61, G63, G77, G79, G87, G95, G126, G129, G130, G142, Nusrat
Nötr deterjanda çözünmeyen lif	G1, G4, G15, G34, G35, G39, G53, G67, G72, G77, G78, G80, G86, G91, G101, G105, G110, G115, G116, Nusrat

*Kül, asit deterjanda çözünmeyen lif ve nötr deterjanda çözünmeyen lif içerikleri bakımından en düşük değerlere sahip 20 genotip, diğer özellikler bakımından en yüksek değerlere sahip 20 genotip ele alınmıştır.

Çalışmada genotiplerin asit deterjanda çözünmeyen lif (ADF) ve nötr deterjanda çözünmeyen lif (NDF) içerikleri sırasıyla % 3.29 (G39) ile 5.91 (G47) ve % 13.75 (G39) ile 17.07 (G11) arasında değişmiştir (*Tablo 3*). Her iki özellikte de ADF ve NDF içerikleri birinci yılda daha yüksek değerlere sahip olmuştur (Şekil 1). Genotiplerin büyük kısmı ADF değeri bakımından % 4.0 ile 4.4 sınıfında yer alırken, NDF değeri bakımından % 15.25- 15.75 sınıfta yer almışlardır (*Şekil 2*). Çalışmada kullanılan Altındane ve Nusrat çeşitlerinin ADF ve NDF

değerleri sırasıyla % 4.07 ve % 3.45, % 15.14 ve % 13.76 olarak belirlenmiştir. Farklı araştırıcılar buğdayda ADF ve NDF içeriklerinin kullanılan genotipe, yıllara, yetiştirme tekniklerine, iklim ve toprak faktörlerine bağlı olarak değiştiğini bildirmiştir (Campell ve ark., 1995; Tilic ve ark., 2012; Mut ve ark., 2017; Arduç ve ark., 2020). Bitki hücre duvarı yapısındaki lignin, selüloz ve çözünmeyen protein miktarını ADF değerini gösterirken, selüloz, hemiselüloz, lignin, kütin ve çözünmeyen protein miktarını NDF değeri göstermektedir. Kutlu (2008) yüksek ADF değerine sahip yemlerin enerji ve sindirilebilirliklerinin daha düşük olduğunu bildirmiştir. Van Soest ve ark. (1991) yemde NDF değeri azaldıkça hayvanın yem alımının arttığını bildirmişlerdir.

Figure 3. Biplot graph showing the relationship between the examined traits and genotypes (BTA: thousand grain weight, HA: hectolitre weight, PO: protein ratio, SD: Zeleny sedimentation value, YG: wet gluten ratio, KO: ash ratio, YO: fat ratio, ADF: acid detergent fiber, NDF: neutral detergent fiber)

Şekil 3. İncelenen özelliklere ile genotipler arasındaki ilişkiyi gösteren Biplot grafiği *(BTA: bin tane ağırlığı, HA: hektolitre ağırlığı, PO: protein oranı, SD: sedimantasyon değeri, YG: yaş gluten oranı, KO: kül oranı, YO: yağ oranı, ADF: asit deterjanda çözünmeyen lif, NDF: nötr deterjanda çözünmeyen lif)*

Biplot grafiği (*Şekil 3*) özellikler ve genotipler arasındaki ilişkilerin görsel olarak değerlendirilmesini, incelenen özellikler arasındaki olumlu veya olumsuz ilişkilerin tanımlanmasını ve başka bir özelliğin dolaylı seçiminde kullanılacak özelliklerin tanımlanmasını sağlamaktadır. Aynı zamanda genotiplerin güçlü ve zayıf özelliklerini de göstermektedir (Yan ve Tinker, 2006). *Şekil 3*'e göre, 144 buğday genotipinin ele alınan özellikler arasındaki ilişkiler ana bileşen 1 ve ana bileşen 2 kullanılarak Biplot grafiği oluşturulmuştur. Ana bileşen 1 toplam varyasyonun % 39.3''ünü, ana bileşen 2 ise %19.0'ını açıklamıştır. Bu iki bileşen toplam varyasyonun yarısından fazlasını (% 58.3) oluşturmuştur. Biplot grafiğinde 90°'den küçük vektör açıları genotip performansının ortalamadan yüksek olduğunu, 90°'den büyük vektör açıları genotip performansının ortalamadan düşük olduğunu ve son olarak 90°'ye eşit veya ona yakın vektör açıları genotip performansının ortalamaya yakın olduğunu göstermektedir (Yan ve Tinker, 2006). Bu grafiğe göre; hektolitre ağırlığı, bin tane ağırlığı ve nişasta oranı arasında dar açı olduğundan bu özellikler birbirleriyle pozitif ilişki göstermişlerdir. Aynı zamanda bu üç özellik birbirleriyle pozitif ilişki gösteren kül oranı ve NDF değeri ile negatif ilişki göstermiştir. Sedimantasyon,

yaş gluten ve protein oranı pozitif ilişki göstermiş, bu özelliklerin ters yönünde kalan ADF ve yağ oranı özellikleri ile negatif ilişki göstermişlerdir. Mut ve ark. (2017)'nın yaptıkları çalışmada da nişasta oranı, hektolitre ağırlığı ve bin tane ağırlığı birbirleriyle pozitif ilişki, aynı çalışmada sedimantasyon, gluten ve protein oranı yine birbirleriyle pozitif ilişki göstermiştir. Genotipler değerlendirildiğinde merkeze daha yakın konumlanan genotipler birden fazla özellik açısından öne çıkmasına rağmen, genel olarak tek bir özellik bakımından öne çıkan genotiplerin değerlerinden daha düşük ortalamalara sahip olmuşlardır. *Şekil 3*'e göre G99, G105, G126 ve G137 numaralı genotipler protein, gluten ve sedimantasyon değerleri; G53, G67 ve G72 numaralı genotipler nişasta oranı; G46 ve G53numaralı genotip bin tane ağırlığı bakımından en yüksek değerlere sahip olmuşlardır. Çalışmada çok fazla genotip kullanıldığından dolayı sadece en yüksek değerlere sahip olanlar yazılmıştır. Fakat Biplot grafiğinin değerlendirilmesinde genotipler hangi özelliklerin vektörleri ile dar açı gösteriyorsa genotipin o özellik bakımından yüksek değerlere sahip olduğu yorumlanabilir.

4. Sonuç

Yerel buğdaylar, tane kalite özellikleri bakımından buğday ıslahı için önemli gen kaynakları olarak kabul edilmektedir. Bu çalışmada, buğday genotipleri arasında kalite özellikleri bakımından önemli farklılıklar olduğu tespit edilmiştir. Biplot grafiğine göre; protein, gluten ve sedimantasyon değerleri bakımından G99, G105, G126 ve G137 numaralı genotipler; nişasta oranı bakımından G53, G67 ve G72 numaralı genotipler; bin tane ağırlığı bakımından ise G46 ve G53 numaralı genotipler en yüksek değerlere sahip olmuşlardır. Farklı özelliklere sahip genotipler arasında belirli son kullanımlar için uygun olanlarının seçilmesi çok önemlidir. Bu açıdan protein değeri önemli bir seleksiyon kriteri olarak değerlendirilebilir. Genotip-özellik biplotu, kalite özellikleri arasındaki korelasyonları görselleştirmek için kullanılan mükemmel bir araçtır ve yüksek kalite özellikleri sunabilen buğday hatlarının güvenilir bir şekilde tanımlanması için tavsiye edilir. Tüm özellikler açısından birçok yerel çeşit standart çeşitlerden daha yüksek değerlere sahip olmuştur. Ayrıca sonuçlar hangi genotiplerin ıslah programlarında kullanılmasının umut verici olduğunu da göstermektedir.

Etik Kurul Onayı

Bu çalışma için etik kuruldan izin alınmasına gerek yoktur.

Çıkar Çatışması Beyanı

Makale yazarları olarak aramızda herhangi bir çıkar çatışması olmadığını beyan ederiz

Yazarlık Katkı Beyanı

Planlama: Mut, Z.; Materyal ve Metot: Mut, Z., Erbaş Köse, Ö.D.; Veri toplama ve İşleme: Mut, Z., Erbaş Köse, Ö.D., Kardeş, Y.M.; İstatistiki Analiz; Mut, Z, Erbaş Köse, Ö.D.; Literatür Tarama: Erbaş Köse, Ö.D.; Makale Yazımı, İnceleme ve Düzenleme: Mut, Z., Erbaş Köse, Ö.D., Kardeş, Y.M.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Investigation of Water Quality of the Karasu River in Bilecik Province in terms of Agricultural Irrigation

Bilecik İli Karasu Irmağı Su Kalitesinin Tarımsal Sulamalar Açısından İncelenmesi

Çayan ALKAN[1*], Ramazan MERAL[2]

Abstract

Together with increasing people's need for water, water needs to be monitored due to the pressure created by factors such as drought and pollution. In this study, water quality was monitored by determining 10 points on the Karasu River in Bilecik Province and it was aimed to examine the seasonal change in water quality. pH, EC, Na, K, Ca, Mg, CO_3, HCO_3, Cl, B, SO_4, Sodium Adsorption Rate (SAR), Residual Sodium Carbonate (RSC) and %Na parameters were determined in water. And then using these parameters, quality classes were determined with the help of water quality classification systems developed by scientists such as Schofield, Wilcox, Thorne, Doneen and Soifer. The study were temporally diveded into 4 periods. They are period 1, period 2 (Spring), period 3 (Summer) and period 4 (Fall). After all, for the period 3, heavy metal pollution, especially Al, attracts attention. Except for the period 2 of point 7 and period 4 of point 9, the class of all periods and points in terms of sulphate was "Very good (class 1)". According to Schofield (1933 and 1935) systems, point 7 is in particularly bad situation in terms of EC. According to Wilcox (1948), point 7 is not suitable for irrigation in the period 2. At point 7, water pollution in period 2 has been identified as common to most classification systems. According to Christiansen et al. (1977), there is intense pollution in terms of EC and Na_2CO_3 parameters. According to Soifer (1987), point 2 is the cleanest point. As a result, Karasu river is not suitable for irrigation in terms of Al and B. It was understood that heavy metal pollution did not cause much of a problem in non-industrialized cities such as Bilecik. Besides, it was understood that the most common element among heavy metals was Pb in Karasu River. The SAR and RSC values of all periods, seasons and points were classified as "Very Good-Safe (C1S1)".

Keywords: Agricultural water quality, Water quality index, Water quality classification systems, Sakarya river, Karasu stream

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Öz

İnsanların suya olan ihtiyaçlarının artmasına ek olarak, kuraklık ve kirlilik gibi etmenlerin yaratacağı baskı sebebiyle suların izlenmesi gerekmektedir. Bu çalışmada; Bilecik ili Karasu ırmağı üzerinde 10 nokta belirlenerek su kalitesi izlenmiş ve mevsimsel olarak su kalitesindeki değişimin incelenmesi amaçlanmıştır. Sudaki; pH, EC, Na, K, Ca, Mg, CO₃, HCO₃, Cl, B, SO₄, Sodyum Adsorpsiyon Oranı (SAR), Kalıntı sodyum karbonat (RSC) ve %Na parametreleri belirlenmiştir. Daha sonra bu parametreler kullanılarak; Schofield, Wilcox, Thorne, Doneen ve Soifer gibi bilim insanlarının geliştirdiği, su kalitesi sınıflandırma sistemleri yardımıyla kalite sınıfları belirlenmiştir. Çalışma, zamansal olarak; 1. dönem, 2. dönem (İlkbahar), 3. dönem (Yaz) ve 4. dönem (Sonbahar) şeklinde 4 döneme ayrılmıştır. Sonuçta; 3. dönem için; Al başta olmak üzere ağır metal kirliliği dikkate çekmektedir. 2. dönemin 7 nolu noktası ve 4. dönemim 9 nolu noktaları haricinde, tüm dönem ve noktalar sülfat açısından "Çok iyi (1. sınıf)" dir. Schofield (1933 ve 1935) sistemlerine göre; 7 nolu nokta, özellikle EC açısından kötü durumdadır. Wilcox (1948)'a göre; 7 nolu nokta, 2. dönemde sulama açısından uygun değildir. 7 nolu noktada, 2. dönemdeki su kirliliği, çoğu sınıflandırma sisteminde ortak olarak tespit edilmiştir. Christiansen ve ark. (1977)'na göre, EC ve Na₂CO₃ parametreleri açısından yoğun bir kirlilik yaşanmaktadır. Soifer (1987)'a göre, 2 nolu nokta en temiz noktadır. Sonuç olarak; Karasu ırmağı, Al ve B açısından sulamaya uygun değildir. Bilecik gibi sanayileşmemiş şehirlerde, ağır metal kirliliğinin çok fazla sorun yaratmadığı anlaşılmıştır. Ayrıca; Karasu Irmağı'ndaki ağır metaller arasında en yaygın bulunan elementin Pb olduğu anlaşılmıştır. Tüm dönem, mevsim ve noktalara ait SAR ve RSC değerleri "Çok İyi-Güvenli (C1S1)" sınıfında yer almaktadır.

Anahtar Kelimeler: Tarımsal su kalitesi, Su kalite indeksi, Su kalitesi sınıflandırma sistemleri, Sakarya nehri, Karasu ırmağı

1. Introduction

Greenhouse gases and pollutants released into the atmosphere by industrializing countries such as Turkey both cause global warming and indirectly pollute water resources (Alaboz et al., 2020). As climate change is excessively experienced on the Mediterrenean watershed, water resources must be managed wisely. Especially in drought country, management of water quality is very essential topic (Gençoğlan et al., 2023).

Nowadays, effective usage and protection of land and water resources and sustainable agriculture are among the priority issues. Owing to the increasing need for water and the pressure on water resources due to factors such as drought and pollution, it becomes necessary to protect water in terms of both quantity and quality. In this context, water monitoring is the primary issue. It forms the basis for the management studies to be implemented. The quality of irrigation water has a significant impact on both crop yield and soil properties. Due to pollution in irrigation water, high osmotic pressures occur in the plant sap and the plant cannot use the available water, which is called physiological drought. The situation means that the plant suffers from a deficiency of both water and plant nutrients. Another side effect is the effect of phytotoxic compounds present in the water on the plant. It is known that this situation is more effective especially during the germination and early development periods. The second effect of irrigation water on plant development is due to its negative effects on the soil. The relationship between water quality and soil causes changes in some physical properties of the soil, such as infiltration rate, soil structure, air and water permeability.

The selection of the irrigation method and the irrigation planning to be applied vary depending on the water quality. Especially in surface irrigation methods where water is applied in larger amounts, the usage of problematic water accelerates the negative processes. In addition, it is possible to consider the need for wash water and carry out reclamation works in order to prevent or eliminate the negative effects that may occur in the soil depending on the water quality, by monitoring the water quality. In general, cation concentrations in freshwater are expected to be Ca>Mg>Na>K. Besides, sulphate deficiency prevents algae growth. Biological Oxygen Demand (BOD) is the amount of oxygen required to decompose organic substances under oxygenated conditions. Chemical Oxygen Demand (COD) shows the amount of oxygen required for the oxidation of all substances in water. For this reason, COD is actually more inclusive. If COD is bigger than 25 mg l^{-1}, it means that the water is polluted (Tepe and Kutlu, 2019).

Kapdı and Aşık (2021) took water samples in Uşak Güllübağ pond in March-May-July and performed physico-chemical analysis. According to SAR, MAR (Magnesium Adsorption Rate), Kelley Index, Permeability Index, Potential Salinity, %Na and EC results, pond water was classified as suitable for irrigation. After all, it was determined that the lake water was of 2nd class quality in terms of Cl and boron, and 1st class quality in terms of sulphate. In terms of irrigation water indices, the results varied in the following ranges: %Na: 52.8-54.6, SAR: 3.1-3.5, MAR: 35.6-37.1, Kelley Index: 0.94-1, Permeability Index: 72.5-75, Potential Salinity: 4.4-5.72 meq l^{-1}. Heavy metal pollution could not be detected in the waters. Karademir et al. (2020) examined the quality of water consumed by farm animals in the Iğdır Aras River. In this context, heavy metals such as Cu, Zn, Mn, Fe and macro ions such as Na, K, Ca, Mg were analyzed. After all, they found lower quality water in Tuzluca district compared to other districts. In general, it has been determined that the water resources in Iğdır are suitable for the usage of farm animals. Ağca and Doğan (2020) determined ions, SAR, MAR, permanent bicarbonate and total hardness values, which are important for agricultural water quality, from 8 points in the Asi River. After all, the order of the amount of cations in the river is Mg>Na>Ca>K and the order of the anions is SO$_4$>HCO$_3$>Cl>CO$_3$. Water quality class of the river is C3S1. In terms of salinity, it is in the "Very salty water" class. Moreover, a strong negative correlation was detected between RSC and Mg (R^2: -0.91). In this situation, the River water should be used carefully for irrigation of crops, which sensitive to Mg deficiency, such as sugar beet, citrus, vineyard, tomato, onion and potato. Topçu and Taş (2020) analyzed the ions that are important for agricultural water quality in the water of 20 wells in the Çanakkale Biga plain and determined that 11 wells had Class 2 water and 9 wells had Class 1 water. They said that the EC values of the wells especially were high around Koruoba and Örtülüce. Tepe and Kutlu (2019) analyzed water samples from depths of 0-4-8 m at 5 stations in the Karkamış Dam Lake in Gaziantep in 2015. Some quality parameters for these 3 depths are respectively (average temperature: 9.5 °C): For pH: 8.5-9-7.8, for EC: 251-332-412 μS cm^{-1}, for Dissolved Oxygen: 9-10-12 mg l^{-1}, for TP: 0.007-0.016-0.026 mg l^{-1}, for TN: 0.72-1.15-1.70 mg l^{-1}. After all, they determined that the lake water had high quality (1st class). Diri (2018) analyzed the water received from 17 points in the Konya watershed and 3 points in the Sakarya watershed.

As a result, while the waters at 3 points were found to be suitable for irrigation, it was understood that the other points were not suitable for irrigation. In addition, although they were suitable in terms of EC and Cl, it was understood that they were not suitable in terms of B and Na. Kar and Leblebici (2020) obtained seasonally 125 samples from 5 points in the Yamula Dam lake in Kayseri and performed a detailed analysis of water quality. After all, they determined that TP, TN, SO₄, Cl, K, Na and pH are effective parameters in evaluating the quality of this lake. Dündar (2008) carried out detailed analysis of many parameters in water and sediment samples in the Lower Sakarya River in 2007. As a result, it was determined that the pollution in the watershed was caused by fertilizers and pesticides in settlements and agricultural areas. Akkan and Mutlu (2022) examined the chromium, manganese, lead, iron, cobalt, nickel, copper, zinc, aluminium and cadmium values in the Artvin Çoruh River watershed. As a result, using the water quality index, they determined that the water quality of the river was in the poor class. After all, they stated that the river water had a high metal load. Osmanoğlu and Özalp (2023) investigated the water quality of Artvin Murgul stream. For this purpose, parameters such as pH, EC, zinc (Zn), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe) and lead (Pb) were examined monthly at 12 points throughout 1 year. As a result, it was determined that heavy metal concentrations were very high at the exit of the mine area.

In this context, monitoring the water quality in the Karasu River in Bilecik Province, it is primarily important in terms of identifying problems and taking precautions against pollution. In addition, the purpose of the study is to select the irrigation method to be applied depending on the existing water quality, to prepare and manage irrigation projects, and to obtain the necessary data for soil reclamation studies.

2. Materials and Methods

2.1. Material (Research area)

The study was carried out on the Karasu river, which is one of the water resources of Bilecik province. The river originates from Bozüyük, includes Dikilitaş, Sorgun, Selöz, Hamsu and Bekdemir streams and flows into the Sakarya River in Vezirhan. Its length is 65 km and its annual average flow rate is 1.4 m³ s⁻¹. In order to monitor water quality, 10 points were selected between the Bozüyük district, which is close to the birth point of the river, and the point where it flows into the Sakarya River. During determining these points, the joining points of the streams and other discharge points were taken into consideration. Near point 3, there is a beer factory. Near point 5, Karasu River flows into the Sorgun River. Near point 5 and 6, there is Bilecik City center. Near point 7, there is a wastewater treatment plant of Bilecik Municipality and the Badun stream flows into the Sorgun (Karasu) River. Point 8 is in Vezirhan Town center (*Figure 1*).

Figure 1. Water sample points

2.2. Method

The sampling process was planned to be carried out four times throughout the year, representing each season. Water samples were taken on 04.07.2022 for period 1, on 03.03.2023 for period 2, 06.07.2023 for period 3,

08.09.2023 for period 4. During determining the sampling points, water samples were taken from 6 points as a preliminary study. In analyses, 12 parameters specified in "Scope 3: Irrigation Water Analysis" in the "Establishment, Authorization and Control Circular of Soil, Plant and Irrigation Water Analysis Laboratories for Agricultural Purposes" of the Ministry of Agriculture and Forestry were taken into consideration.

Elektrical conductivity-EC (dS m^{-1}) and pH: EC was determined by EC meter device, pH was determined by pH glass electrode pH meter.

Ca and Mg (meq l^{-1}): Determined by EDTA titration (Titrimetric) method

CO$_3$ and HCO$_3$ (meq l^{-1}): Determined by sulfuric acid titration (Titrimetric) method

Cl (meq l^{-1}): Determined by silver nitrate titration (Titrimetric) method

SO$_4$ (meq l^{-1}): Determined by anion and cation balance calculation

Na and K (meq l^{-1}): Determined by flame photometer device.

B (mg l^{-1}): Determined by Karmen method (Spectrophotometric).

SAR and RSC: Determined by calculation.

In addition, arsenic, aluminium, iron, chromium, lead and manganese analyzes were carried out through service procurement. However, heavy metal analyzes, which were carried out through service procurement, were only carried out on samples taken during the summer months.

Terms used to express irrigation water quality are as follows (Richards, 1954; Ayyıldız, 1983):

$$\text{Sodium Adsorbtion Ratio (SAR)} = \frac{Na}{\sqrt{\frac{Ca+Mg}{2}}} \tag{Eq. 1}$$

$$\text{Residual sodium carbonate (RSC)} = (CO_3 + HCO_3) - (Ca + Mg) \tag{Eq. 2}$$

$$\%Na = \frac{Na^+}{(Na^+ + K^+ + Ca^{++} + Mg^{++})} \cdot 100 \tag{Eq. 3}$$

Effective salinity: CaCO$_3$ (calcite), MgCO$_3$ (Dolomite) and CaSO$_4$ (gypsum) salts are subtracted from the total salt concentration value, respectively. The remaining amount equals the effective salinity value.

Potential salinity: *PS = Cl + 0.5 SO$_4$* (Eq. 4)

Permeability Coefficient: PC= (Na+ (HCO$_3$)$^{0.5}$) / $\sum$Cation (Eq. 5)

The standards used for irrigation water quality are as follows (Ayers and Westcot, 1989):

1. Schofield (1933) system (EC and %Na)

2. Schofield (1935) system (EC, %Na, Cl, SO$_4$)

3. Wilcox and Magistad (1943) system (EC, %Na, Cl, B)

4. Wilcox (1948) graphic system (EC and %Na)

5. Thorne and Thorne (1951) graphic system (EC and %Na)

6. USA Salinity Laboratory (1954) classification system (EC and SAR)

7. Doneen (1959) Potential Salinity System (Cl and SO$_4$)

8. Doneen (1966) Permeability Indicator System (Sodium, Bicarbonate and Total cation)

9. Christiansen et al. (1977) classification system (EC, %Na, SAR, Na$_2$CO$_3$, Cl, ES (effective salinity) and B)

10. Soifer (1987) graphic system (EC and SAR)

11. "2000/60/EC Water Framework Directive" and the "Surface Water Quality Regulation" harmonized within its scope (Official newspaper in 30th November 2012 and numbered 28483)

3. Results

3.1. Results related to period 1

Water samples taken on 04.07.2022 were analyzed on 18.07.2022 (The analysis performed on 6 samples from this period is a preliminary study). As a result, while Al, Cr, Mn, Fe could not be detected at any point, only Pb ion was found at all points in terms of heavy metals.

3.2. Results related to (Spring) period 2

On 03.03.2023, analyzes were carried out on water samples taken from 10 points. In the spring season, it was determined that K and Na results were quite low at points 1 and 2. In the spring season, while CO_3 values were close to linear, it was seen that HCO_3 results took values similar to a bell curve from upstream to downstream. Besides, SAR and pH values were determined to be suitable for usage in irrigation in the season.

3.3. Results related to (Summer) period 3

On 06.07.2023, analyzes were carried out on water samples taken from 10 points. In summer, the high Na values are noteworthy. In the summer season, the low Pb, Fe and Ar concentration values and the high Al values are noteworthy. Besides, HCO_3 values of some points are quite high. As a result, low SAR and RSC values are an indicator of good water quality in the period 3.

3.4. Results related to (Fall) period 4

On 08.09.2023, analyzes were carried out on water samples taken from 10 points. In the period 4 (Fall), the low B and CO_3 values and the high Na values are noteworthy. Compared to the previous period (Summer), the increase in RSC values in the fall season indicates a deterioration in water quality.

3.5. General Results

The high pH values in the period 4 are noteworthy. Except for the EC value of point 7 in the period 2, the period difference did not have an excessive effect on the change in EC values (*Figure 2*).

Figure 2. pH and EC results graph

The high Na values in the period 4, the low Na values in the period 1, the high K values in the period 2 and the low K values in the period 4 are noteworthy (*Figure 3*).

The high Ca values in the period 2, the low Ca values in the period 4, the high Mg values in the period 4 and the low Mg values in the period 3 are noteworthy (*Figure 4*).

It was seen that CO_3 and HCO_3 concentrations reached high values, especially in the period 2. Besides, Cl value in the point 7 is very high (*Figure 5*).

The high B values in the period 3 and the low B values in the period 4 are noteworthy. In Period 2, there was an extreme increase in the SO_4 value of point 7 (*Figure 6*).

Figure 3. Na and K results graph

Figure 4. Ca and Mg results graph

Figure 5. CO₃, HCO₃ and Cl results graph

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Figure 6. B and SO₄ results graph

Some of the important parameters, which determine the class of water quality are SO_4, SAR and RSC values. In terms of SO_4, except for the period 2 of point 7 and the period 4 of point 9, all SO_4 results were in the "very good (class 1)" category. Period 2 of point 7 is in class 4 (Can be used with caution) with a value of 15.67 meq l^{-1} (752.16 ppm). Besides, the period 4 of point 9 is in class 2 (good) with a value of 6.40 meq l^{-1} (307.2 ppm) (*Figure 6*).

SAR and RSC values of all periods and points were in the "Very Good-Safe (C1S1)" class. In the period 2, except for the SAR value of point 7, the low SAR and RSC values show that the Karasu River is very suitable for agricultural irrigation (*Figure 7*).

Figure 7. SAR and RSC results graph

The high Pb concentrations at points 6 and 9 are noteworthy (Figure 8).

The increased Pb concentration compared to Period 1 indicates serious Pb pollution in the River. In the period 3, especially Cr and Fe concentrations had higher values compared to other heavy metals (*Figure 9*).

In order to see the effect of temperature difference on water quality, index correlations in Summer and Fall periods were determined in this study. While the correlation (R^2: 0.29) between SAR and SO_4 was low in the summer season, the correlation (R^2: 0.72) between RSC and SO_4 was high in the fall season.

Figure 8. Pb result graph in the period 1

Figure 9. Heavy metals results graph in the period 3

3.6. Results related to water quality classification systems

3.6.1. Schofield (1933) system (EC and %Na)

Especially in Period 2, the pollution level of point 7 is very high. EC pollution in the period 4 is remarkable (*Table 1*).

Table 1. Results of water quality class according to Schofield (1933) system

Sampling Point	EC			%Na		
	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4
1	Class 2, good	Class 2, good	Class 3, permissible	Class 1, perfect	Class 1, perfect	Class 1, perfect
2	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect
3	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 2, good	Class 2, good
4	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good
5	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good
6	Class 3, permissible	Class 2, good	Class 3, permissible	Class 2, good	Class 1, perfect	Class 1, perfect
7	Class 5, not suitable	Class 2, good	Class 3, permissible	Class 4, doubtful	Class 1, perfect	Class 2, good
8	Class 3, permissible	Class 2, good	Class 3, permissible	Class 2, good	Class 2, good	Class 2, good
9	Class 3, permissible	Class 2, good	Class 3, permissible	Class 2, good	Class 2, good	Class 2, good
10	Class 3, permissible	Class 3, permissible	Class 3, permissible	Class 2, good	Class 2, good	Class 2, good

3.6.2. Schofield (1935) system (EC, %Na, Cl, Sulphate)

Point 7 is in particularly bad situation in terms of EC. The pollution experienced in terms of EC in the period 4 is remarkable (*Table 2*).

3.6.3. Wilcox and Magistad (1943) system (EC, %Na, Cl, Boron)

According to Wilcox and Magistad (1943), continuous pollution was observed in terms of boron in the period 3 (*Table 3*).

Table 2. Results of water quality class according to Schofield (1935) system

Points	EC			%Na			Cl, meq l^{-1}				SO$_4$, meq.l^{-1}	
	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4
1	Class 2, good	Class 2, good	Class 3, permissible	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
2	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
3	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
4	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
5	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
6	Class 3, permissible	Class 2, good	Class 3, permissible	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
7	Class 5, not suitable	Class 2, good	Class 3, permissible	Class 4, doubtful	Class 1, perfect	Class 2, good	Class 3, permissible	Class 1, perfect	Class 1, perfect	Class 4, doubtful	Class 1, perfect	Class 1, perfect
8	Class 3, permissible	Class 2, good	Class 3, permissible	Class 1, perfect	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect
9	Class 3, permissible	Class 2, good	Class 3, permissible	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 2, good
10	Class 3, permissible	Class 3, permissible	Class 3, permissible	Class 2, good	Class 2, good	Class 2, good	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect	Class 1, perfect

Table 3. Results of water quality class according to Wilcox and Magistad (1943) system

Sampling Points	EC			%Na			Cl, meq l^{-1}				Boron, ppm	
	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4
1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1
2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 2	Class 1
3	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 2	Class 3	Class 1
4	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 2	Class 3	Class 1
5	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 3	Class 1
6	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 2	Class 3	Class 1
7	Class 3	Class 1	Class 1	Class 2	Class 1	Class 1	Class 2	Class 1	Class 1	Class 3	Class 3	Class 1
8	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 3	Class 1
9	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 2	Class 1	Class 1
10	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 2	Class 1	Class 1

3.6.4. Wilcox (1948) Graphic system (EC and %Na)

According to Wilcox (1948), point 7 is not suitable for irrigation in the period 2. Compared to other periods, water quality began to decrease in the period 4 (*Table 4*).

Table 4. Results of water quality class according to Wilcox (1948) system

Sampling point	Intersection of EC and %Na		
	Period 2	Period 3	Period 4
1	Very good-good (class 1)	Very good-good (class 1)	Good-Useable (class 2)
2	Very good-good (class 1)	Very good-good (class 1)	Very good-good (class 1)
3	Very good-good (class 1)	Very good-good (class 1)	Very good-good (class 1)
4	Very good-good (class 1)	Very good-good (class 1)	Very good-good (class 1)
5	Very good-good (class 1)	Very good-good (class 1)	Very good-good (class 1)
6	Good-Useable (class 2)	Very good-good (class 1)	Good-Useable (class 2)
7	Not suitable (class 5)	Very good-good (class 1)	Good-Useable (class 2)
8	Good-Useable (class 2)	Very good-good (class 1)	Good-Useable (class 2)
9	Good-Useable (class 2)	Very good-good (class 1)	Good-Useable (class 2)
10	Good-Useable (class 2)	Good-Useable (class 2)	Good-Useable (class 2)

3.6.5. Thorne and Thorne (1951) graphic system (EC and %Na)

In this system, EC classes are classified between 1 and 5, while %Na values are grouped between A and E. For example, quality class of point 10 in the period 3, while it is in the class 2 in terms of EC, it is in the class 1 in terms of %Na. Especially, there is serious pollution in the period 2 at point 7 (*Table 5*).

Table 5. Results of water quality class according to Thorne and Thorne (1951) system

Sampling point	Intersection of EC and %Na		
	Period 2	Period 3	Period 4
1	1A	1A	2A
2	1A	1A	1A
3	1A	1A	1A
4	1A	1A	1A
5	1A	1A	1A
6	1A	1A	2A
7	4C	1A	2A
8	2A	1A	2A
9	2A	1A	2A
10	2A	2A	2A

3.6.6. Classification system of (USSL) USA salinity laboratory (1954) (EC and SAR)

In parallel with the results in Thorne and Thorne (1951) Graphic System, there is serious pollution especially in the period 2 at point 7. In the period 4, the pollution load increases to downstream (*Table 6*).

Table 6. Results of water quality class according to USSL (1954) system (USSL, 1954)

Sampling point	Intersection of EC and SAR		
	Period 2	Period 3	Period 4
1	C2S1	C2S1	C3S1
2	C2S1	C2S1	C2S1
3	C2S1	C2S1	C2S1
4	C2S1	C2S1	C2S1
5	C2S1	C2S1	C2S1
6	C3S1	C2S1	C3S1
7	C4S2	C2S1	C3S1
8	C3S1	C2S1	C3S1
9	C3S1	C2S1	C3S1
10	C3S1	C3S1	C3S1

3.6.7. Doneen (1959) potential salinity system (Cl and Sulphate)

At point 7, water pollution in period 2 has been identified as common to most classification systems. Water pollution, especially in the period 4, is constantly recurring (*Table 7*).

Table 7. Results of water quality class according to Doneen (1959) classification system

Sampling point	Period 2		Period 3		Period 4	
	Potential salinity, meq l^{-1}	Class	Potential salinity, meq l^{-1}	Class	Potential salinity, meq l^{-1}	Class
1	2.59	1	1.52	1	3.655	2
2	0.52	1	1.76	1	1.64	1
3	1.93	1	2.45	1	2.305	1
4	1.745	1	2.475	1	3.16	2
5	2.425	1	2.245	1	3.2	2
6	3.51	2	2.38	1	3.395	2
7	17.6	3	2.53	1	4.375	2
8	2.735	1	2.795	1	3.69	2
9	3.13	2	2.755	1	3.8	2
10	3.555	2	3.045	2	3.72	2

3.6.8. Doneen (1966) permeability indicator system (Sodium, bicarbonate and total cation)

In common with Doneen (1959) Potential Salinity and Doneen (1966) Permeability Indicator systems, water pollution in the period 4 is constantly recurring (*Table 8*).

3.6.9. Christiansen et al. (1977) classification system (EC, %Na, SAR, Na₂CO₃, Cl and Boron)

There is intense pollution, especially in terms of EC and Na_2CO_3 parameters. In terms of B in the period 3, the pollution load is very high in the middle points of the Karasu River. Except for the period 2 of point 7, there is no pollution in terms of %Na, SAR and Cl (*Table 9*).

Table 8. Results of water quality class according to Doneen (1966) classification system

Sampling point	Period 2		Period 3		Period 4	
	Permeability coefficient	Class	Permeability coefficient	Class	Permeability coefficient	Class
1	0.76	1	0.42	1	0.78	2
2	0.49	2	0.48	1	0.37	2
3	0.83	2	0.80	2	0.84	2
4	0.70	1	0.83	2	0.85	2
5	0.71	1	0.79	2	0.84	2
6	0.83	2	0.74	1	0.84	2
7	0.96	2	0.68	1	0.90	2
8	0.71	1	0.80	2	0.85	2
9	0.76	1	0.85	2	0.85	2
10	0.76	1	0.82	2	0.86	2

3.6.10. Soifer (1987) graphic system (EC and SAR)

According to the graphic system of Soifer (1987), point 2 has very low pollution load and is the cleanest point (*Table 10*).

Table 10. Results of water quality class according to Soifer (1987) graphic classification system

Sampling point	Period 2 Class	Period 3 Class	Period 4 Class
1	III_1	II	III_2
2	I	II	I
3	III_3	III_3	III_3
4	III_3	III_3	III_3
5	III_3	III_3	III_3
6	III_5	III_3	III_4
7	III_5	III_3	III_5
8	III_4	III_4	III_4
9	III_4	III_4	III_4
10	III_4	III_4	III_4

I: Suitable for all crops and soils, II: Suitable for most crops and soils, III: Limited availability, IV: Can be used in certain condition, V: Not suitable

3.6.11. "2000/60/EC Water Framework Directive" and the "Surface Water Quality Regulation" harmonized within its scope (Official newspaper in 30th November 2012 and numbered 28483)

Generally, values above critical thresholds were obtained for Al and B parameters. For this reason, the Karasu river is not suitable for irrigation in terms of Al and B. It is necessary to be careful, especially in agricultural irrigation at points 6 and 7 (*Table 11*).

Table 11. Water quality class results according to surface water quality regulation (Anonymous, 2023)

Parameters	Sampling point									
	1	2	3	4	5	6	7	8	9	10
pH	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
EC	good	good	good	good	good	mean	mean	good	good	good
Manganese	Very good	Very good	Very good	Very good	Very good	Very good	Very good	Very good	Very good	Very good
Aluminium	✘	✘	✘	✘	✘	✘	✘	✘	✘	✘
Arsenic	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Boron	✔	✔	✘	✘	✘	✘	✘	✘	✔	✔
Iron	✔	✔	✔	✔	-	✔	✔	✔	✔	✘
Chromium	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Lead	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

1

Table 9. Results of water quality class according to Christiansen et al. (1977) classification system

Points	EC (dS m^{-1})			%Na			SAR			Na$_2$CO$_3$ (meq l^{-1})			Cl, meq l^{-1}			Boron, ppm		
	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4	Period 2	Period 3	Period 4
1	Class 2	Class 1	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 5	Class 3	Class 6	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1
2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 4	Class 3	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1
3	Class 2	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 6	Class 6	Class 1	Class 1	Class 1	Class 3	Class 4	Class 1
4	Class 2	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 6	Class 6	Class 1	Class 1	Class 1	Class 2	Class 6	Class 1
5	Class 2	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 6	Class 6	Class 1	Class 1	Class 1	Class 1	Class 6	Class 1
6	Class 3	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 5	Class 6	Class 2	Class 1	Class 1	Class 3	Class 6	Class 1
7	Class 5	Class 2	Class 2	Class 3	Class 1	Class 1	Class 3	Class 1	Class 1	Class 6	Class 5	Class 6	Class 3	Class 1	Class 1	Class 4	Class 6	Class 1
8	Class 2	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 6	Class 6	Class 1	Class 1	Class 1	Class 1	Class 6	Class 1
9	Class 2	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 6	Class 6	Class 1	Class 1	Class 1	Class 2	Class 1	Class 1
10	Class 2	Class 2	Class 2	Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	Class 6	Class 6	Class 6	Class 1	Class 1	Class 1	Class 2	Class 1	Class 1

2

4. Discussion and Conclusions

Kapdı and Aşık (2021) determined that SAR varies between 3.1 and 3.5 in the Uşak Güllübağ lake water. Karademir et al. (2020) determined that the water resources in Iğdır are suitable for the usage of farm animals. Topçu and Taş (2020) determined that the Çanakkale Biga plain have class 1 water in the most of wells. Tepe and Kutlu (2019) determined that the Karkamış Dam Lake had high water quality (1st class) in Gaziantep in 2015. In parallel with these results in Bilecik (Karasu), Uşak, Iğdır, Çanakkale and Gaziantep, the presence of high water quality class and low SAR values supports that non-industrialized, low populated cities have high water quality. Ağca and Doğan (2020) determined that Asi river is in the "Very salty water" class. While one water quality parameter is high, another may be low. Therefore, crops suitable for different periods (seasons) and points (areas) should be decided by looking at the ions with obvious deficiency/excess. Diri (2018) found that the water quality of the Konya Watershed and the Sakarya Watershed were not suitable for irrigation in general. Kar and Leblebici (2020) determined that SO_4 and K in the Yamula Dam lake in Kayseri are mainly effective parameters in evaluating the quality of this lake. Dündar (2008) determined that the pollution in the Lower Sakarya River in 2007 was caused by fertilizers in agricultural areas. Therefore, research on pollution caused by SO_4 and K-component fertilizers might be concentrated in agricultural cities such as Konya, Kayseri and Sakarya. Akkan and Mutlu (2022), Osmanoğlu and Özalp (2023) determined that the water quality in the Artvin Çoruh River watershed and Murgul stream were in the bad class. Besides, they stated that the river water had a high heavy metal concentrations at the exit of the mining area. Therefore, if non-industrialized and low-populated cities are to be examined, it is much more important to examine different parameters of water quality in special (sensitive) regions such as mining areas.

When these informations in the literature were compared and evaluated together with the results in this study, it was understood that heavy metal pollution did not cause much of a problem in non-industrialized cities such as Bilecik. Besides, it was understood that the most common element among heavy metals was Pb.

Consequently, the results showed that water quality changed spatially and contamination information occurred between the beginning and the flow into the Sakarya River. According to the results of the period 2 (Spring), especially at points 6 and 7, which are close to the city center, Na, HCO_3 and Cl ions concentrations were very high. When the SAR value is examined, which is an important parameter in controlling suitability for agricultural irrigation, it has been understood that the SAR values of all points are in the lowest class (Water with low sodium) and that they can be used safely in irrigation. Even so, irrigation of stone fruit orchards with this water may reduce fruit yield because low-sodium water with low SAR value can affect stone fruits that are sensitive to alkalinity (Ayyıldız, 1983; Tüzüner, 1990). In particular, it is recommended to use these waters in stone fruit orchards near point 7, provided that sandy and organic soil is available.

For the period 2 (Spring), as a result of water samples taken from 10 points on 03.03.2023, it was understood that the Karasu River was extremely polluted around Vezirhan-Bayırköy before flowing into the Sakarya River. It is thought that this situation is caused by the increase in industrialization in the region. At point 10, which is the last sampling point, an increase in water quality was observed. It is thought that this situation may be caused by mixing with a clean water source in tributary streams flowing into the Karasu River. For the period 3 (Summer), as a result of water samples taken from 9 points on 06.07.2023, it is seen that especially Boron values are high. Besides, heavy metal pollution, especially Al, attracts attention. For the period 3 (Fall), as a result of water samples taken from 10 points on 08.09.2023, it is understood that water quality deteriorates as you observe from upstream to downstream.

As a result of all analyzes being completed and the study completed, the SAR and RSC values of all periods, seasons and points were classified as "Very Good-Safe (C1S1)". When the SO_4 results were examined, the period 2 of point 7 was in class 4 (Can be used with caution) with a value of 15.67 meq l^{-1} (752.16 ppm), and the period 4 of point 9 was in the class 2 (good) with a value of 6.40 meq l^{-1} (307.2 ppm). Except for these, the class of all periods and points in terms of sulphate was "Very good (class 1)". In the period 2 of point 7 (Spring) and in the period 4 of point 9 (Fall), it is recommended that local farmers should be careful when using the water.

The general conclusions reached about water quality classification systems are as follows: According to Schofield (1933 and 1935) systems, point 7 is in particularly bad situation in terms of EC. In addition, the pollution

experienced in terms of EC in the period 4 is remarkable. According to Wilcox and Magistad (1943), continuous pollution was observed in terms of boron in the period 3. According to Wilcox (1948), point 7 is not suitable for irrigation in the period 2. According to Thorne and Thorne (1951) and USSL (1954), the pollution load increases to downstream in the period 4. At point 7, water pollution in period 2 has been identified as common to most classification systems. In common with Doneen (1959 and 1966) systems, water pollution in the period 4 is constantly recurring. According to Christiansen et al. (1977), there is intense pollution in terms of EC and Na_2CO_3 parameters. According to Soifer (1987), point 2 is the cleanest point. The fact that all discharge points of Bilecik Province are collection-spillage points is one of the main reasons for the poor water quality of point 7. As a result, Karasu river is not suitable for irrigation in terms of Al and B.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Alkan, Ç., Meral, R.; Design: Alkan, Ç., Meral, R.; Data Collection or Processing: Alkan, Ç., Meral, R.; Statistical Analyses: Alkan, Ç., Meral, R.; Literature Search: Alkan, Ç., Meral, R.; Writing, Review and Editing: Alkan, Ç., Meral, R.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Antirrhinum majus Bitkisinin Gelişim Parametreleri Üzerine Kısıntılı Sulamanın Etkisi

Effect of Limited Irrigation on Growth Parameters of *Antirrhinum majus* Plants

Işık SEZEN[1*], Ayşe KARAHAN[2], Elif AKPINAR KÜLEKÇİ[3], Sevda YAĞANOĞLU[4]

Öz

2050 yılında dünya nüfusunun büyük bir çoğunluğunun kentlerde yaşayacağı öngörülmektedir. Kentlerdeki hızlı nüfus artışı çevre sorunlarını beraberinde getirmektedir. Tüm bu sorunlar iklimi olumsuz yönde değiştirmektedir. İklim değişikliği ile yağış rejiminin değişmesi, azalması kuraklık sorununa neden olmaktadır. Dünya ortalaması dikkate alındığında kullanılabilir suyun %70'inin sulama amacıyla tüketildiği görülmektedir. Türkiye ortalaması ise %72'dir. Ülkelerin gelişmişlik düzeyi arttıkça, sulama amaçlı su tüketimi azalmaktadır. Sulama amaçlı tüketilen suyun büyük bir miktarının ise kentsel peyzaj çalışmalarında yer alan yeşil alanlarda kullanıldığı bilinmektedir. Bu nedenle gelişmiş ülkeler park ve bahçelerinde kısıntılı sulama, suyun akılcı kullanımı gibi teknikleri uygulamaya başlamışlardır. Bitki suyu ne kadar az tüketiyorsa o kadar fazla alanlarda kullanmaya yönelmek, fazla su tüketen bitkilerin kullanım alanlarını azaltmak, bitkileri su ihtiyaçlarına göre zonlayarak dikim alanlarında kullanmak zorunda kalmışlardır. En fazla su tüketimi ise kentlerdeki yeşil alanların vazgeçilmezi olan çim bitkileri ve mevsimlik çiçeklerdir. En fazla su tüketimi kentsel mekanlardaki süs bitkilerinin kullanıldığı yeşil alanlarda olmasına rağmen süs bitkilerinde su kısıntısı üzerine çok az sayıda akademik çalışma yapıldığı görülmektedir. Bu araştırmanın amacı; kentlerde yeşil alanlarda fazla su tüketimine neden olan mevsimlik çiçeklerden en yaygın olarak kullanılan *Antirrhinum majus* bitkisini kısıntılı sulama uygulamasına tabi tutarak gelişim parametrelerindeki değişimi gözlemlemektir. Bu kapsamda; %25, %50, %75 ve %100 olmak üzere dört farklı oranda sulama uygulaması yapılmıştır. Farklı oranda sulama uygulamalarının etkisini saptamak için 18 gelişim parametresi incelenmiştir. Araştırmanın sonucunda; *Antirrhinum majus* bitkisinde 18 gelişim parametresinin 17'sinde %75 oranında su uygulamasında, %100 oranında su uygulamasından daha fazla gelişim olduğu gözlenmiştir. Bu sonuçlar %75 (%25 kısıntısı) uygulamasının daha avantajlı olduğunu göstermektedir. Kentsel mekanlarda *Antirrhinum majus* bitkisi kullanıldığında %25 oranında su kısıntısı yapılması önerilmektedir. Bu durumda kentsel peyzajda önemli düzeyde su tasarrufu sağlanacaktır. Su kısıntısı uygulandığı halde gelişiminde sıkıntı yaşanmayan *Antirrhinum majus* bitkilerinin kentsel peyzajda sıklıkla kullanımı önerilmektedir.

Anahtar Kelimeler: Mevsimlik çiçekler, Sulama, Kentsel peyzaj, Su tüketimi, Su tasarrufu

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Abstract

It is predicted that the majority of the world's population will live in cities in 2050. Rapid population growth in cities brings environmental problems. All these problems change the climate negatively. Changes and decreases in the precipitation regime due to climate change cause drought problems. Considering the world average, it is seen that 70% of usable water is consumed for irrigation purposes. Türkiye's average is 72%. As the development level of countries increases, water consumption for irrigation purposes decreases. It is known that a large amount of water consumed for irrigation purposes is used in green areas in urban landscaping works. For this reason, developed countries have started to apply techniques such as limited irrigation and rational use of water in their parks and gardens. They had to tend to use the less water consumed by plants in larger areas, reduce the usage areas of plants that consume more water, and use plants in planting areas by zoning them according to their water needs. The highest water consumption is from grass plants and seasonal flowers, which are indispensable for green areas in cities. Although the highest water consumption is in green areas where ornamental plants are used in urban areas, very few academic studies have been conducted on water shortage in ornamental plants. The purpose of this research; The aim of the study is to observe the change in the development parameters of the *Antirrhinum majus* plant, the most commonly used seasonal flower that causes excessive water consumption in green areas in cities, by subjecting it to restricted irrigation. In this context; Four different irrigation rates were applied: 25%, 50%, 75% and 100%. 18 development parameters were examined to determine the effect of different irrigation applications. As a result of the research; It was observed that in *Antirrhinum majus* plant, 17 out of 18 growth parameters showed more development in 75% water application than in 100% water application. These results show that the 75% (25% reduction) application is more advantageous. When using the *Antirrhinum majus* plant in urban areas, it is recommended to reduce water by 25%. In this case, significant water savings will be achieved in the urban landscape. It is recommended that *Antirrhinum majus* plants, whose development is not affected by water shortages, are frequently used in urban landscapes.

Keywords: Seasonal flowers, Irrigation, Urban landscaping, Water consumption, Water saving

1. Giriş

Son yıllarda iklim değişikliğine bağlı su stresi dünyadaki en önemli sorunlardan biridir (Bahar ve ark., 2017). Tüm dünyada olduğu gibi Türkiye'de küresel ısınmanın sonuçlarından etkilenmekte, su kaynakları azalmakta, kuraklık, çölleşme, ekolojik sorunlar yaşamaktadır (Turkes, 1997; Deveci ve ark., 2017). Bitki üretiminde susuzluk önemli bir sorundur. Fakat günümüzde nitelikli su kaynakları azalmaktadır (Cirak ve Esendal, 2006; Deveci ve ark., 2017). Özellikle suyun kıt ve pahalı olduğu yerlerde sulama suyundan tasarrufun sağlanması için su kısıntısı çalışmaları önemli bir avantaj sağlamaktadır (Kaman ve ark., 2023)

Bitkilerin gelişmeleri sürekli olarak kökleri aracılığıyla topraktan su almalarına bağlıdır. Topraktan alınan suyun büyük kısmı terlemeyle atmosfere verilir. Geriye kalan da bitki dokularında depo edilir. Bu nedenle bitkilerin kök bölgesinde yeterli düzeyde nem bulunmalıdır (Aküzüm ve Çakmak, 1992; Bayramoğlu ve ark., 2013). Toprakta bulunan nemin büyük bir miktarı doğal yağışlarla sağlanır. Ancak kurak ve yarı kurak iklimlerde yağışlar yetersiz olduğundan dolayı bitkinin su ihtiyacının karşılanması zor olmaktadır. Bundan dolayı bitki kökündeki nem eksikliği sulama suyu ile karşılanmaktadır (Güngör ve ark., 2002; Bayramoğlu ve ark., 2013). Yarı nemli bölgelerde, bitki su ihtiyacının yağışla karşılanan kısmı daha fazla miktardadır. Ayrıca yarı nemli bölgelerde sulamaya genellikle gerek duyulmaktadır. Nemli bölgelerde bile, kurak geçen dönemlerde sulama yapılmaktadır (Yıldırım, 2008; Bayramoğlu ve ark., 2013). Bu amaçla bitkinin ihtiyacı olan suyun her sulamada hesaplanması gerekmektedir. Bitkilerin cinslerine göre tüketeceği su miktarı farklıdır. Her bitkinin tüketeceği su miktarı farklı olduğundan bitki su tüketimlerinin belirlenmesi gerekmektedir (Jensen, 1968; Selvi, 2012; Bayramoğlu ve ark., 2013).

Bitki su tüketim değerleri doğrudan ölçme yöntemleri veya iklim verileri kullanılarak bitki su tüketimi değerleri hesaplanmaktadır. Peyzaj çalışmalarında kullanılan bitkiler fazla su tüketmektedir. Peyzaj bitkilerinin ideal gelişimi ve homojen görünümü sulama ile sağlanmaktadır (Orta, 2009; Bayramoğlu ve ark., 2013).

Dünyada su kaynaklarının azalmasından dolayı yaşanan güçlükler, suyun etkin kullanımı yönünden yeni çözüm arayışlarına yöneltmiştir. Özellikle park ve bahçeler gibi yeşil alanlarda su tüketiminin büyük boyutlardadır. Bu nedenle peyzaj düzenlemelerinde suyun tasarruflu kullanıldığı yeni peyzaj düzenleme yöntemlerinin geliştirilmesine ihtiyaç duyulmuştur. Bu bağlamda; suyun akılcı kullanımı, az su kullanımı gibi farklı peyzaj düzenleme kavramları ortaya çıkmıştır (Yazgan ve ark., 2017).

Günümüzde iklim değişikliğine bağlı olarak sıcaklığın artışı yağış düzeninin bozulmasına neden olmuştur. Yağış düzeninin bozulması ile susuzluk sorunu öne çıkarak; tasarımcı, planlamacı ve yerel yöneticileri suyun akılcı kullanımı için yeni arayışlara yöneltmiştir. Özellikle bitkisel tasarım çalışmalarında kullanılan süs bitkilerinin yaşamı için su son derece etkili bir faktördür. Park ve bahçe gibi kentsel açık alanlarda su tüketiminin fazla olması peyzaj çalışmalarında, suyu olabildiğince kısıntılı kullanılabilen uygulamalarını gerekli hale getirmiştir (Barış, 2007). Bitkilerin sudan yeterli miktarda yararlanmasının ön şartı, bitkilerin yağışlarla karşılayamadıkları su ihtiyaçlarının sulama ile verilmesi şeklinde ifade edilir. Ancak su kaynaklarının sınırlı olması nedeniyle, kısıntılı sulama uygulamaları yaygınlaşmıştır. Kısıntılı sulama; bitkilerin su stresine sokulmasıyla su maliyetin azaltılması stratejisine dayanan sulama anlayışıdır (English ve Raja, 1996; Bayramoğlu ve ark., 2013).

Kısıntılı sulama metodu, dünyadaki kurak ve kurak çevrelerde kısıntılı ve sınırlı su koşulları altında ürün yetiştirme için yaygın stratejilerden biridir (Foley ve ark., 2020; Katerji ve ark., 2013; Nasseri, 2021).

Gül ve ark. (2012)'na göre de mevsimlik çiçekler kentsel mekanlarda kullanılan çok fazla su tüketen bitkiler arasındadır (Yazıcı ve ark., 2014). Kentsel mekanlarda sulama amaçlı su tüketiminin önüne geçilmesi için mevsimlik çiçeklerin azaltılması önerilmektedir (Çetin ve Mansuroğlu, 2018). Fakat mevsimlik çiçekler kent peyzajının vazgeçilmez bitkileri arasındadır. Alp ve ark. (2011)'nın da belirttiği gibi mevsimlik çiçekler kentsel peyzaj alanlarının düzenlenmesinde kullanılan, renk ve canlılık etkisi oluşturan, monotonluğu gideren, yüzey etkisi oluşturan, mekanı kısa sürede kapatan, her yıl değiştirilebilen, maliyeti düşük, aylarca çiçekli kalan, seralarda kolaylıkla yetiştirilen bitkilerdir.

Bu araştırmada; kentsel peyzaj düzenlemelerinde yaygın olarak kullanılan mevsimlik çiçekler arasında yer alan fazla su tüketen Scrophulariceae familyasından *Antirrhinum majus* bitkisine kısıntılı sulama uygulaması yapılarak gelişim parametrelerindeki değişimi incelemek amaçlanmıştır.

2. Materyal ve Metot

Bu araştırma 2022 yılında Atatürk Üniversitesi'nde sera ortamında gerçekleştirilmiştir. Denemenin kurulduğu seranın temel üstü duvarı 90 cm beton olup, iskelet sistemi olarak çelik malzeme kullanılmıştır. Sera genişliği 7 m, uzunluğu 27 m ve sera yan duvar yüksekliği 2.1 m'dir. Sera örtü malzemesi olarak ısı geçirme katsayısı 6,3 W/m²°C ve kalınlığı 4 mm olan cam kullanılmıştır. Sera çatı eğim açısı 33º olup, beşik çatı tipindedir. Serada herhangi bir ısıtma, soğutma, sisleme, gölgeleme sistemleri bulunmamaktadır. Sera doğal havalandırma sistemine sahip olup havalandırma açıklarının kullanımı elle yapılmaktadır. Araştırmada kullanılan bitkisel materyal *Antirrhinum majus*'dur. *Antirrhinum majus* son yıllarda ticari değeri artan bir bitkidir. *Antirrhinum majus* tohumlarının çimlenme dönemi Aralık-Nisan ayları arasındadır. Soğuk iklime sahip bölgelerde tohum ekimi Mart-Nisan ayları arasında olmalıdır. Denemenin kurulduğu şehir soğuk iklime sahiptir. 1 Mart 2022 tarihinde %90 çimlenme kabiliyetine sahip *Antirrhinum majus* F1 hibrit tohumlarının ekimi yapılmıştır. Tohum ekiminin yapıldığı yetiştirme ortamı %65 torf, %35 topraktan oluşmaktadır. Karışımda %75 organik madde bulunmaktadır. pH değerine 5-7 arasındadır.

Antirrhinum majus tohumlarının ekiminin 9. gününde çimlenme gerçekleşmeye başlamış, 15. gününde çimlenme daha da belirginleşmiştir. Fideler şaşırtılma boyutuna geldiği zaman 20x20 cm boyutunda, 5 litrelik saksılara aktarılmıştır. Saksı içindeki yetiştirme ortamı ise Oral ve Açıkgöz (1991)'ün bahçe çiçeklerinde için önerdiği John Innes Bahçe Bitkileri Enstitüsü tarafından geliştirilmiş 2 kısım tınlı toprak, 1 kısım torf, 1 kısım kumdan oluşmaktadır. Fideler saksıya alındığında tüm uygulamalar için toprak tarla kapasitesine getirilmiştir. Kontrol grubu dışındakilere sırasıyla %75-50-25 seviyelerinde sulama uygulaması yapılmıştır. Sulama aralığı buharlaşma kabından ölçülen buharlaşma miktarının 5-10 mm'ye ulaşması esas alınarak belirlenmiştir. Beklenen buharlaşmaya ulaşıldığında sulama gerçekleştirilmiştir. Kontrol grubuna buharlaşma miktarının tamamı, diğer gruplarda ise buharlaşma miktarının %75-50-25'i ölçülerek tüm saksılara eşit yükseklikten uygulanmıştır. Kontrol grubuna verilen su miktarı Eşitlik 1 (Güngör ve ark., 2010)'e göre hesaplanmıştır.

$$I = kp \times EP \times A \qquad \text{(Eş. 1)}$$

I: sulama suyu (litre x saksı $^{-1}$)

kp: buharlaşma kabı katsayısı (kontrol için 1 diğer uygulamaları için 0,75-0,50-0,25 alınmıştır)

E_p: Küçültülmüş buharlaşma kabından okunan toplam buharlaşma (mm)

A: Saksı alanı (m^2)

Her bir sulama uygulaması için 6 tekerrür bulunmaktadır. %25, %50, %75, %100 oranında sulama uygulamalarının her birinin tekerrür sayısı 6'dır. Saksılar sulanarak tarla kapasitesine getirildikten sonra 60 cm çapında, 25 cm derinliğinde, 15 cm yüksekliğinde ahşap alt destek ünitesi bulunan, 2 mm'lik gri saçtan yapılmış, içi su doldurulmuş buharlaşma ölçüm aletiyle buharlaşan su miktarı milimetrik ahşap cetvelle ölçülmüştür. Sıcaklık-nem ölçüm aletiyle ortalama, maksimum, minimum sıcaklık ve nem ölçülmüştür.

Araştırma boyunca, ortam sıcaklığı 25-30°C'de tutulmuştur. Ortamdaki buharlaşma miktarına bağlı kalarak sulama uygulamaları yapılmıştır. Araştırmada, dört farklı sulama konusu (buharlaşma kabından elde edilen buharlaşan suyu miktarının (mm) saksı hacimle ilişkisine bağlı olarak kullanılabilir su tutma kapasitesinin %100'üne (kontrol), %75'ine, %50'sine ve %25'ine tamamlanması) oluşturulmuştur. Öncelikle her bir saksının saksı kapasitesi (tarla kapasitesi) ve buna bağlı olarak da kullanılabilir su tutma kapasitesi belirlenmiştir (Camoglu, 2013).

Bitkilere verilecek sulama suyu miktarları, saksılardaki nem miktarları taşınabilir bir nemölçer (Delta-T Devices, WET Sensor) kullanılarak hacim esaslı olarak belirlenmiştir. Sulama uygulamalarını gerçekleştirmek için, kullanılan nem ölçerin denemede kullanılan toprak materyaline göre kalibrasyonu yapılmış olup ardından spesifik olarak deneme ortamının tarla kapasitesinde tuttuğu hacimsel nem oranı belirlenmiştir. Saksıların tarla kapasitesi belirlendikten sonra su kısıtı uygulamaları kontrol gurubu dâhil; %100 D0, %75 D1, %50 D2 ve %25 D3 olarak uygulanmış ve sera içi sıcaklık, nem ve buharlaşma değerleri belirlenerek sulama işlemlerine ortalama 2-3 gün ara ile devam edilmiştir. Su kısıtı uygulamaları tohum çıkışından 15 gün sonra saksılara şaşırtılarak yapılmıştır. Sulamalara bitkiler 53 gün sonra hasat edilene kadar devam edilmiştir. 2. Kuru ağırlık belirlenirken

hassas terazi ağırlık ölçümünden önce kurutmanın nasıl yapıldığı varsa ilgili standart yöntem kısmında verilmelidir.

Araştırmada verilmiş olan su içme suyu kalitesinde olup sulama için uygundur. Suyun pH ortalaması 7.4, elektriksel iletkenlik (EC) değeri 0.26 Ds m-1 ve sodyum adsorpsiyon oranı (SAR) 0.5 olup çok iyi su grubundadır. Atatürk Üniversitesi'nin yerleşkesinin şebeke suyudur. Deneme süresince belirli zamanlarda Sezen ve ark. (2023a)'nın *Tagates erecta* bitkisi için belirledikleri gibi çiçeklenme zamanı, çiçeklenme süresi, çiçek sap uzunluğu, yaprak klorofil değeri, yaprak sayısı, yaprak yüzey alanı, bitki kök uzunluğu, bitki kök yaş ağırlığı vb. gibi gelişim parametreleri gözlemlenmiş, ölçümler yapılmış ve sonuçlandırılmıştır.

Bitki boyu, bitki çapı, çiçek sap kalınlığı, çiçek çapı, çiçek boyu dijital kumpasla, yaprak klorofil değerleri SPAD-502 klorofil metre ile ölçülmüştür.

Yaprak alanı ölçününde CID Bioscience CI-202 model yaprak alan ölçeri kullanılmıştır (*Şekil 1*).

Şekil 1. Yaprak alan ölçümü

Figure 1. Leaf area measurement

Deneme sonunda hasadı yapılan bitkilerin taze ağırlıkları alınan yeşil aksam, çiçekler ve kökler etüvde 68°C de 24 saat sabit ağırlığa ulaşıncaya kadar kurutulup, hassas terazi ile tartılarak gram olarak kaydedilmiştir.

Sera içerisine modifiye edilmiş buharlaşma kabından ölçülen kümülatif (yığışım) buharlaşma miktarı mm olarak ölçülmüş ve saksı hacmine oranla ml olarak su verilmiştir. Fide dikim tarihi (08 Nisan 2022)'nden son sulama uygulamasına (31 Mayıs 2022) kadar olan sürede deneme desenindeki kontrol grubu ve diğer sulama gruplarına uygulanan sulama suyunun yığışımlı miktarları ise ml olarak verilmiştir. Sulama uygulamalarına 8 Nisan 2022'de başlanmış, %25 oranında su verilen uygulamanın tüm tekerrürlerinin öldüğü tarih olan 30 Mayıs 2022'de sonlandırılmıştır. 53 günlük vejetasyon dönemi içerisinde buharlaşma kabından toplam 259 mm buharlaşma meydana gelmiştir. Buna göre ortalama buharlaşma miktarı 4.77 mm/gün'dür. Sulama grupları içinde kontrol grubuna (%100 sulama uygulaması) 259 mm sulama suyu uygulanmış, bu değer diğer gruplarda sırasıyla %25 - %50- %75 te 64.75- 129.5-194.25 mm olmuştur. Araştırma süresince uygulanan sulama suyu değerleri ve buharlaşma miktarları *Tablo 1*'de verilmiştir.

Mevsimlik çiçek yetiştiriciliğinde serada günlük buharlaşmanın 5-10 mm'ye ulaştığı zamanlarda sulama yapılmıştır. Buna göre sulama aralığı Tablo 1'de görüldüğü gibi 1-3 günlük periyodlar arasında değişmektedir. En fazla buharlaşma 16 Mayıs 2022 tarihinde gerçekleşmiştir. Bu değer kontrol grubunda 21 mm/gün olarak belirlenmiştir. En az buharlaşma ise 6 Mayıs 2022 tarihinde olmuştur. Bu değer ise kontrol grubunda 3 mm/gün olarak belirlenmiştir.

Araştırmada ölçülen sıcaklık, bağıl nem ve buharlaşma arasındaki ilişkide, 08/04/2022 ve 30/05/2022 tarihleri arasında Nisan ayının 20'sine kadar olan süreçte sıcaklık, nem ve buharlaşmanın aynı kaldığı, 27 Nisan 2022 ve 15 Mayıs 2022 arasında bağıl nemdeki yükselişin ve sıcaklıkla buharlaşmanın sabit kaldığı görülmüştür (*Şekil 2*).

Tablo 1. Araştırma konularındaki sulama suyu (mm) ve buharlaşma miktarları

Table 1. Irrigation water (mm) and evaporation amounts in the research subjects

Tarih	Buharlaşma miktarı(mm)	25%	50%	75%	100%
8.04.2022	10	2.5	5	7.5	10
11.04.2022	15	3.75	7.5	11.25	15
12.04.2022	6	1.5	3	4.5	6
13.04.2022	6	1.5	3	4.5	6
15.04.2022	12	3	6	9	12
18.04.2022	20	5	10	15	20
20.04.2022	10	2.5	5	7.5	10
22.04.2022	14	3.5	7	10.5	14
25.04.2022	16	4	8	12	16
27.04.2022	11	2.75	5.5	8.25	11
29.04.2022	14	3.5	7	10.5	14
2.05.2022	9	2.25	4.5	6.75	9
4.05.2022	6	1.5	3	4.5	6
6.05.2022	3	0.75	1.5	2.25	3
9.05.2022	9	2.25	4.5	6.75	9
11.05.2022	5	1.25	2.5	3.75	5
13.05.2022	10	2.5	5	7.5	10
16.05.2022	21	5.25	10.5	15.75	21
18.05.2022	11	2.75	5.5	8.25	11
20.05.2022	11	2.75	5.5	8.25	11
23.05.2022	15	3.75	7.5	11.25	15
25.05.2022	5	1.25	2.5	3.75	5
28.05.2022	10	2.5	5	7.5	10
30.05.2022	10	2.5	5	7.5	10
Toplam	259	64.75	129.5	194.25	259

Şekil 2. Sıcaklık, nem ve buharlaşma grafiği

Figure 2. Temperature, humidity and evaporation graph

3. Bulgular ve Tartışma

29 Nisan 2022 tarihinde (fidelere su kısıntısı uygulamasının 22. günü) bitki boyu, bitki çapı, çiçek sap kalınlığı, çiçek çapı, çiçek boyu, yaprak klorofil değerleri ölçülmüştür. Yeni tomurcuklanma sayıları belirlenmiştir. Su kısıntısının 34. günde %25, %50, %75, %100 oranında sulama uygulamalarını en iyi temsil eden tekerrürlerin bitki gelişimi karşılaştırılmıştır. *Antirrhinum majus* bitkisinde deneme takvimi *Tablo 2*'de, sulama uygulamalarına göre gelişim farkı ise *Şekil 3*'te verilmiştir.

Şekil 3. Antirrhinum majus bitkisinde sulama uygulamalarına göre gelişim farkı

Figure 3. Growth difference in Antirrhinum majus plant according to irrigation practices

Tablo 2. Antirrhinum majus bitkisinde deneme takvimi

Table 2. Trial schedule on Antirrhinum majus plant

Deneme Takvimi	Tarih	Yapılan İşlemler
Tohum Ekimi	1.03.2022	-
Fidelerin Saksıya Dikimi	08.04.2022	Tohum ekiminin 39. günü, Su kısıntısının başlangıcı
Ölçüm 1	29.04.2022	Tohum ekiminin 60. Günü, Fidelere su kısıntısı uygulamasında 22. gün, Uygulamalarda çiçeklenmelerin gerçekleştiği tarih
Ölçüm 2	30.05.2022	Tohum ekiminin 91. günü, Fidelere su kısıntısı uygulamasında 53. ünü, %25 oranında su verilen uygulamadaki tüm tekerrürlerin kuruduğu tarih
Kuru ağırlık ölçümü	09.06.2022	11 gün boyunca bitkinin çiçek, vejatatif aksam ve kökleri kurutulmuştur.

Tablo 2'de görüldüğü gibi su kısıntısının 53. günü (30 Mayıs 2022) *Antirrhinum majus* bitkisinde %25 oranında su verilen uygulamada bitkilerin kuruduğu gözlenmiştir. *Antirrhinum majus* bitkisinin su kısıntısı uygulaması durdurularak deneme sonlandırılmıştır. %25 oranında su verilen uygulamada, su kısıntısı uygulamasının 53. günü olan 30.05.2022 tarihinden itibaren bitki çiçek, vejatatif aksam ve kök kuru ağırlık ölçümlerin yapılabilmesi için 11 gün boyunca bitkinin çiçek, vejatatif aksam ve kökleri kurutulmuştur. Tüm bitki parçalarının tamamen kuruduğu 09.06.2022 tarihinde hassas terazi ile kuru ağırlık ölçümleri yapılmıştır. %25 oranında su verilen uygulamada tüm tekerrürlerin kuruduğu gün (fidelere su kısıntısının 53. günü) tüm uygulamalarının yaprak alanı miktarı ölçülmüştür.

%25 oranında sulama yapılan uygulamada 6 tekerrürün 2'sinde çiçeklenme olmuş, 4'ünde olmamıştır. %50 oranında sulama yapılan uygulamada tüm tekerrürler fidelere uygulanan su kısıntısından ortalama 27 gün sonra, %75 oranında sulama yapılan uygulamaların tüm tekerrürlerinde 34 gün sonra çiçeklenme olmuştur. %100 oranında sulama yapılan uygulamada 6 tekerrürün 5'inde çiçeklenme olmuştur. %100 oranında sulama yapılan

uygulamaların çiçeklenme olan tekerrürlerinde 36 gün sonra çiçeklenme olmuştur. Bu sonuçlar *Antirrhinum majus* bitkisine %25 oranında su verilmesinin çiçeklenme oranını düşürdüğünü göstermektedir. %75 oranında sulama yapılan uygulamada çiçeklenme, %50 oranında sulama yapılan uygulamadan 3 gün önce, %100 oranında sulama yapılan uygulamadan 2 gün önce çiçeklenme olmuştur. %50, %75 oranında sulamalarda çiçeklenme tüm tekerrürlerde gerçekleşmiştir. En kısa sürede çiçeklenme gerçekleşen uygulama %75 oranında sulama (%25 oranında su kısıntısı)'dır. *Tablo 3*'de *Antirrhinum majus* bitkisinde sulama uygulamalarına göre gelişim parametrelerindeki değişim verilmiştir.

Tablo 3. *Antirrhinum majus* bitkisinde sulama uygulamalarına göre gelişim parametrelerindeki değişim

Table 3. *Change in growth parameters of Antirrhinum majus plant according to irrigation practices*

Su Uygulama Oranı	%25	%50	%75	%100
Çiçek Sayısı (22 gün), adet	0	0	0.16	0
Çiçek Sayısı (53. gün), adet	0.5	2.33	7.16	8
Çiçek Sap Kalınlığı (22. gün), mm	2.13	2.63	2.63	2.78
Çiçek Sap Kalınlığı (53. gün), mm	2.92	3.99	5.05	4.25
Çiçek Çapı (22. gün), mm	0	0	4.95	0
Çiçek Çapı (53. gün), mm	2.96	16.38	23.22	18.84
Çiçek Boyu (22. gün), mm	0	0	6.37	0
Çiçek Boyu (53. gün), mm	3.58	20.51	24.27	21.64
Çiçek Sap Kalınlığı (22. gün), mm	0.07	0.57	3.43	2.64
Çiçek Sap Kalınlığı (53. gün), mm	0.06	0.34	1.01	0.61
Çiçek Çapı (22. gün), mm	41	43.70	40.48	32.78
Çiçek Çapı (53. gün), mm	30.13	39.85	38.95	32.45
Çiçek Boyu (22. gün), mm	8.25	8.90	11.73	10.03
Çiçek Boyu (53. gün), mm	10.33	12.25	15.58	13.66
Çiçek Yaş Ağırlığı (53. gün), gr	8.81	10.58	13.03	9.31
Çiçek Kuru Ağırlığı, gr	10	13.5	18.16	13.5
Yaprak Klorofil Değeri (22. gün)	4.19	9.71	14.64	11.87
Yaprak Klorofil Değeri (53. gün)	1.06	1.98	3.05	2.29
Bitki Boyu (22. gün), cm	9.5	11.83	13.16	11.33
Bitki Boyu (53. gün), cm	91.20	224.39	299.26	261.23
Bitki Çapı (22. gün), cm	2.33	7.83	19.66	10.66
Bitki Çapı (53. gün), cm	15.68	21.00	19.75	15.15
Vejatatif Aksam Yaş Ağırlığı (53. gün), gr	2.13	3.64	4.79	3.07
Vejatatif Aksam Kuru Ağırlığı, gr	0.37	0.45	0.68	0.40
Bitki Sürgün Sayısı (53. gün), adet	9.5	11.83	13.16	11.33
Yaprak Alanı, mm^2	91.20	224.39	299.26	261.23
Tomurcuk Sayısı (53. gün), adet	2.33	7.83	19.66	10.66
Kök Uzunluğu (53. gün), cm	15.68	21.00	19.75	15.15
Kök Yaş Ağırlığı (53. gün), gr	2.13	3.64	4.79	3.07
Kök Kuru Ağırlığı, gr	0.37	0.45	0.68	0.40

Tablo 3'de görüldüğü gibi çiçek sayısı su kısıntısının 22 günü ölçülmüş, %75 oranında su uygulamasında diğerlerine göre daha fazla çiçeklenme olduğu görülmüştür. %75'lik uygulamada çiçeklenme gerçekleşirken, diğer uygulamalarda gerçekleşmemiştir. Ortalama çiçek sayısı su kısıntısının 53. günü ölçülmüş %100 oranında su verilen uygulamada diğerlerine göre fazla çiçeklenme olduğu görülmüştür. Ortalama çiçek sayısı; birinci sırada % 100'lük, ikinci sırada %75'lik, üçüncü sırada %50'lik, dördüncü sırada %25'lik uygulamada olduğu görülmüştür. Su kısıntısının çiçeklenme verimini olumsuz etkilediği görülmüştür. Nitekim Göçmen (2021) de ayçiçeği bitkisi üzerinde uyguladığı farklı sulama uygulamalarında çiçeklenme döneminde su kısıntısından kaçınılması gerektiğini belirtmiştir.

Su kısıntısının 22. gününde yapılan ölçümde %100 oranında su verilen uygulamada çiçek sap kalınlığının diğerlerine göre fazla olduğu görülmüştür. Sırasıyla çiçek sap kalınlığı birinci sırada %100, ikinci sırada %75 ve %50, üçüncü sırada %25'lik uygulamadadır. Bu durum su kısıntısının uygulandığı ilk 22 günde sulama miktarı arttıkça çiçek sap kalınlığının da arttığını göstermektedir. Nitekim Turan (2013), farklı sulama aralıkları ve su miktarlarının *Chrysanthemum morifolium* Ramat (Krizantem) bitkisinin verim ve kalite özelliklerine etkisini belirlemek için yaptığı çalışmasında artan su miktarının çiçek sap kalınlığını artırdığını belirtmiştir (Uçar ve Kazaz, 2016).

Su kısıntısının 53. gününde yapılan ölçümde de *Antirrhinum majus* bitkisine uygulanan su kısıntısı çalışmasında %75 oranında su verilen uygulamada çiçek sap kalınlığı diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla çiçek sap kalınlığı birinci sırada %75, ikinci sırada %100, üçüncü sırada %50, dördüncü sırada %25'lik uygulamadadır.

Su kısıntısının 22. gününde yapılan ölçümde %75 oranında su uygulamasında diğerlerine göre çiçek çapının fazla olduğu görülmüştür. Diğer uygulamalarda çiçek oluşumu gerçekleşmemiştir. Su kısıntısının 53. gününde %75 oranında su uygulamasında diğerlerine göre çiçek çapının fazla olduğu görülmüştür. Sırasıyla çiçek çapı birinci sırada %75, ikinci sırada %50, üçüncü sırada %100, dördüncü sırada %25'lik uygulama olmuştur.

Ortalama çiçek boyu su kısıntısının 22. gününde ölçülmüş %75 oranındaki su uygulamasında ortalama çiçek boyu 6,37 cm olarak ölçülürken, diğer uygulamalarda çiçeklenme gerçekleşmemiştir. 53. Gün yapılan ölçümde ortalama çiçek boyu değerleri %75 oranında su uygulamasında sırasıyla %100, %50 ve %25 oranındaki uygulamalardan fazla olduğu görülmüştür.

53. gün yapılan ölçümde %75 oranında su uygulamasında diğerlerine göre ortalama çiçek yaş ağırlığının fazla olduğu görülmüştür. Sırasıyla ortalama çiçek yaş ağırlığı birinci sırada %75, ikinci sırada %100, üçüncü sırada %50, dördüncü sırada %25'lik uygulamadadır.

Ortalama çiçek kuru ağırlığı %75 oranında su verilen uygulamada diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla ortalama çiçek kuru ağırlığı birinci sırada %75, sonra sırasıyla %100, %50, %25'lik uygulamalarda olmuştur. %75'lik uygulamada ortalama çiçek kuru ağırlığının diğer uygulamalardan yüksek olması, %25 oranında uygulanan su kısıntısının çiçeklenme miktarında daha etkili olduğunu göstermektedir.

Yaprak klorofil değeri, su kısıntısının 22. günü ölçümünde %50 oranındaki uygulamada diğerlerinden yüksek olduğu görülmüştür. Tohum ekiminden 53 gün sonra ikinci kez klorofil değerlerine bakılmış ve %50'lik uygulamada klorofil değerinin yine diğer uygulamalardan yüksek olduğu görülmüştür. 22. gün yapılan ölçümlerde klorofil değerinde en yüksek değer ilk sırada %50, sonra sırasıyla %25, %75, %100'lük uygulamalarda görülmüştür. Tohum ekiminin 53. gününde yapılan ölçümde ise ortalama klorofil değerinde en yüksek değer %50, sonra sırasıyla %75, %100, %25'lük uygulamalarda görülmüştür. Her iki dönemde ölçümde de %50 oranında su verilmesi (%50 oranında su kısıntısı) klorofil değerinde artışa neden olmuştur.

Su kısıntısının 22. gününde yapılan ölçümde *Antirrhinum majus* bitkisine uygulanan su kısıntısı çalışmasında %75 oranında su verilen uygulamada bitki boyunun diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla bitki boyu birinci sırada %75, ikinci sırada %100, üçüncü sırada %50, dördüncü sırada %25'lik uygulamadadır. Su kısıntısının 53. gününde yapılan ölçümde de *Antirrhinum majus* bitkisine uygulanan su kısıntısı çalışmasında %75 oranında su verilen uygulamada bitki boyunun diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla bitki boyu birinci sırada %75, ikinci sırada %100, üçüncü sırada %50, dördüncü sırada %25'lik uygulamadadır.

Su kısıntısının 22. gününde yapılan ölçümde *Antirrhinum majus* bitkisine uygulanan su kısıntısı çalışmasında %75 oranında su verilen uygulamada bitki çapının diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla bitki çapı birinci sırada %75, ikinci sırada %50, üçüncü sırada %100, dördüncü sırada %25'lik uygulamadadır. Su kısıntısının 53. gününde yapılan ölçümde de %75 oranında su verilen uygulamada bitki çapının diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla bitki çapı birinci sırada %75, ikinci sırada %100 ve%50, üçüncü sırada %25'lik uygulamadadır.

Su kısıntısının 53. günü yapılan ölçümde vejatatif aksam yaş ağırlığı ilk sırada %75 oranında uygulamada sonra sırasıyla %75, %100, %50, %25'lik uygulamadadır. %75'lik uygulamada ortalama vejatatif aksam yaş ağırlığı

diğer uygulamalardan yüksek olması, %25 oranında uygulanan su kısıntısının *Antirrhinum majus* bitkisinde vejatatif aksam gelişiminde daha etkili olduğunu göstermektedir.

Ortalama vejatatif aksam kuru ağırlığı %75 oranında su verilen uygulamada diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla ortalama çiçek kuru ağırlığı birinci sırada %75, sonra sırasıyla %100, %50, %25'lik uygulamalardır. %75'lik uygulamada ortalama vejatatif aksam kuru ağırlığının diğer uygulamalardan yüksek olması, %25 oranında uygulanan su kısıntısının vejatatif aksam gelişiminde daha etkili olduğunu göstermektedir. Nitekim Demir ve ark. (2021) da kısıntılı sulamanın mısır çeşitlerinin büyüme parametreleri üzerine etkilerini tespit etmek için yaptıkları çalışmalarında %100 oranında su uygulamasına göre %75 oranında su uygulamasının yetiştiricilik periyodunu uzattığını belirtmişlerdir.

Ortalama bitki sürgün sayısı su kısıntısının 53. günü ölçülmüş en fazla ilk sırada %75 oranında su uygulamasında sonra sırasıyla %50, %100 ve %25 oranında uygulamalarda görülmüştür.

Yaprak alanının %75 oranında su verilen uygulamada diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla ortalama yaprak alanı birinci sırada %75, ikinci sırada %25, üçüncü sırada %100, dördüncü sırada %50'lik uygulamadadır. %75'lik uygulamada (%25 oranında su kısıntısı yapılan uygulama) ortalama yaprak alanının diğer uygulamalardan yüksek olması, %25 oranında uygulanan su kısıntısının *Antirrhinum majus* bitkisinde yaprak alanı gelişiminde daha etkili olduğunu göstermektedir. Nitekim Demir ve ark. (2021) kısıntılı sulamanın hibrit mısır çeşitlerinin bazı büyüme parametreleri üzerine etkisini belirlemek için yaptığı araştırmalarında %75 oranında su verilen uygulamanın diğer su uygulamalarına göre verimi daha fazla artırdığını belirtmiştir.

Su kısıntısının 53. gününde yapılan ölçümde *Antirrhinum majus* bitkisine uygulanan su kısıntısı çalışmasında %75 oranında su verilen uygulamada tomurcuk sayısı diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla ortalama yeni tomurcuk sayısı birinci sırada %75, ikinci sırada %100, üçüncü sırada %50, dördüncü sırada %25'lik uygulamadadır. *Antirrhinum majus* bitkisine uygulanan su kısıntısı çalışmasında %50 oranında su verilen uygulamada kök uzunluğunun diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla kök uzunluğu birinci sırada %50, sonra sırasıyla %75, %25, %100'lik uygulamada olmuştur.

Antirrhinum majus bitkisine uygulanan su kısıntısı çalışmasında %75 oranında su verilen uygulamada kök yaş ağırlığı diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla kök yaş ağırlığı birinci sırada %75, ikinci sırada %50, üçüncü sırada %100, dördüncü sırada %25'lik uygulamadadır.

Antirrhinum majus'un ortalama kök kuru ağırlığı %75 oranında su verilen uygulamada diğer uygulamalardan fazla olduğu görülmüştür. Sırasıyla ortalama çiçek kuru ağırlığı birinci sırada %75, ikinci sırada %50, üçüncü sırada %100, dördüncü sırada %25'lik uygulamadadır. %75'lik uygulamada ortalama vejatatif aksam kuru ağırlığının diğer uygulamalardan yüksek olması, %25 oranında uygulanan su kısıntısının *Antirrhinum majus* bitkisinde kök gelişiminde daha etkili olduğunu göstermektedir. Nitekim Ekinci ve Başbağ (2019) da kısıntılı sulamanın *Gossypium hirsitum* L. (Pamuk) bitkisinin bazı morfolojik özelliklerine etkilerinin belirlenmesi üzerine yaptıkları araştırmalarında su kısıntısının kök gelişimini artırdığını belirtmişlerdir. Nitekim Kaçar (2007)'da pamuk bitkisinin köklerinin kurak koşullarda toprak nemine ulaşabilmek için daha hızlı geliştiğini belirtmiştir (Ekinci ve Başbağ, 2019).

Duncan çoklu karşılaştırma testine göre *Antirrhinum majus* bitkisinde gelişim parametreleri üzerine sulama uygulamaları arasındaki farklılıklar *Tablo 4*'de verilmiştir.

Tablo 4'de Duncan Çoklu Karşılaştırma testinde, 22. gün yapılan ölçümlerde çiçek sayısına göre tüm uygulamalar arası fark önemsiz bulunduğu görülmektedir. 53. gün yapılan ölçümlerde çiçek sayısına göre tüm uygulamalar arası fark önemsiz bulunmuştur. Duncan Çoklu Karşılaştırma testine göre 22. gün yapılan ölçümlerde çiçek sap kalınlığına göre tüm uygulamalar arası fark önemsiz bulunmuştur. 53. gün yapılan ölçümlerde çiçek sap kalınlığına göre uygulamalar arası fark çok önemli bulunmuştur (p<0,01).

22. gün yapılan ölçümlerde çiçek çapına göre tüm uygulamalar arası fark önemsizdir. 53. gün yapılan ölçümlerde %25 oranında sulama uygulaması ile diğer uygulamalar arası fark çok önemli bulunmuştur (p<0,01). 22. gün yapılan ölçümlerde çiçek boyuna göre tüm uygulamalar arasındaki fark önemsiz bulunmuştur. 53. gün

yapılan ölçümlerde %25 oranında su uygulaması ile diğer uygulamalar arasındaki fark çok önemli bulunmuştur (p<0,01).

Tablo 4. Antirrhinum majus bitkisinde gelişim parametreleri üzerine sulama uygulamaları arasındaki farklılıklar

Table 4. Differences between irrigation applications on development parameters of Antirrhinum majus plant

Gelişim Parametreleri	Sulama Uygulamaları	Duncan ± standart sapma
Çiçek Sayısı (22. gün), adet	%25	0.00[a] ±0.00
	%50	0.00[a]±0.00
	%75	0.16[a]±0.41
	%100	0.00[a] ±0.00
Çiçek Sayısı (53. gün), adet	%25	0.50[a]±1.22
	%50	2.33[ab]±1.21
	%75	7.16[b]±5.19
	%100	8.00[b]±8.98
Çiçek Sap Kalınlığı (22. gün)	%25	2.13[a]±0.18
	%50	2.63[ab]±0.62
	%75	2.63[ab]±0.39
	%100	2.78[b]±0.31
Çiçek Sap Kalınlığı (53. gün)	%25	2.92[a]±0.55
	%50	3.99[b]±0.64
	%75	5.05±0.88
	%100	4.25[bc] ±0.93
Çiçek Çapı (22. gün), mm	%25	0.00[a] ±0.00
	%50	0.00[a]±0.00
	%75	4.95[a]±12.14
	%100	0.00[a]±0.00
Çiçek Çapı (53. gün), mm	%25	2.96 [a] ±7.26
	%50	16.38[b]±2.86
	%75	23.22[b]±6.00
	%100	18.84[b] ±15.03
Çiçek Boyu (22. gün), mm	%25	0.00[a]±0.00
	%50	0.00[a]±0.00
	%75	6.37[a]±15.60
	%100	0.00[a]±0.00
Çiçek Boyu (53. gün), mm	%25	3.86[a]±9.47
	%50	22.04[b]±2.81
	%75	30.50[b]±7.32
	%100	23.87[b]±18.58
Çiçek Yaş Ağırlığı (53. gün), gr	%25	0.07[a]±0.18
	%50	0.57[a]±0.62
	%75	3.43[b]±1.30
	%100	2.64[b]±2.61
Çiçek Kuru Ağırlığı, gr	%25	0.06[a]±0.15
	%50	0.34[ab]±0.30
	%75	1.01[c]±0.34
	%100	0.61[bc]±0.54
Klorofil Değeri (22. gün)	%25	41.03[b] ±5.89
	%50	43.70[b]±4.45
	%75	40.48[b]±5.01
	%100	32.78[a] ±3.52
Klorofil Değeri (53. gün)	%25	30.13[a]±6.77
	%50	39.85[b]±4.58
	%75	38.95[b]±4.97
	%100	32.45[ab]±6.97
Bitki Boyu (22. gün), cm	%25	8.25[a]±1.21
	%50	8.90[a]±2.24
	%75	11.73[b]±2.21
	%100	10.33[ab] ±2.71
Bitki Boyu (53. gün), cm	%25	10.33[a]±1.63
	%50	12.25[ab]±1.17
	%75	15.58[c]±1.49
	%100	13.66[bc] ±2.48

Tablo 4. Devamı Table 4. Continued		
Bitki Çapı (22. gün), cm	%25	8.82[a]±1.77
	%50	10.58[ab]±2.11
	%75	13.03[b]±3.02
	%100	9.32[a] ±2.52
Bitki Çapı (53. gün), cm	%25	10.00[a]±2.19
	%50	13.50[b]±1.87
	%75	18.16[c]±1.60
	%100	13.50[b] ±4.37
Vejatatif Aksam Yaş Ağırlığı (53. gün), gr	%25	4.19[a]±0.73
	%50	9.71[b] ±1.83
	%75	14.64[c]±1.87
	%100	11.87[bc]±5.61
Vejatatif Aksam Kuru Ağırlığı, gr	%25	1.06[a]±0.50
	%50	1.98[b]±0,22
	%75	3.05[c]±0.34
	%100	2.29[bc]±0.14
Sürgün Sayısı (53. gün), adet	%25	9.50[a]±2.17
	%50	11.83[ab]1.60
	%75	13.16[b]±2,48
	%100	11.33[ab]±2.07
Yaprak Alanı, mm^2	%25	91.20[a]±4.06
	%50	224.39[b]±47.39
	%75	299.26[b]±45.39
	%100	261.23[b]±51.02
Tomurcuk Sayısı (53. gün), adet	%25	2.33[a]±2.07
	%50	7.83[a]±6.40
	%75	19.6[b]±8.33
	%100	10.6[a]±9.58
Kök Uzunluğu (53. gün), cm	%25	15.68[a] ±5.89
	%50	21.00[a]±9.14
	%75	19.75[a]±4.97
	%100	15.15[a] ±2.75
Kök Yaş Ağırlığı (53 gün), gr	%25	2.13[a] ±0.93
	%50	3.64[bc]±1.40
	%75	4.79[c]±0.73
	%100	3.07[ab] ±1.28
Kök kuru ağırlığı, gr	%25	0.37[a]±0.50
	%50	0.45[a]±0.22
	%75	0.68[a]±0.34
	%100	0.40[a]±0.14

Not: Küçük harfler (a, b, c) farklılığı simgelemektedir.

53. gün yapılan ölçümlerde çiçek yaş ağırlığına göre %25 ve %50 sulama uygulamaları ile %75 ve %100 sulama uygulamaları arasındaki farkın çok önemli olduğu görülmektedir (p<0,01). %75 ile %100 uygulamaları arasındaki fark önemsiz çıkmıştır.

Duncan Çoklu Karşılaştırma testine göre yapılan ölçümlerde çiçek kuru ağırlığına göre tüm gruplar arası çok önemli bir farlılık olduğu görülmüştür (p<0,01).

22. gün yapılan ölçümlerde %25, %50, %75 oranında uygulamalarda klorofil değerine göre uygulamalar arasındaki fark önemsiz bulunmuştur. %100 oranında sulama ile diğer uygulamalar arasındaki fark çok önemli bulunmuştur (p<0,01). 53. gün yapılan ölçümlerde %50 ve %75 oranındaki uygulamalarda yaprak klorofil değerine göre fark önemsiz bulunmuştur. %50 ve %75 oranındaki uygulamalarla diğerleri arasındaki fark önemli bulunmuştur (p<0,05). Nitekim Kırnak ve Doğan (2017) da kavun bitkisinde kısıntılı sulamanın bazı kantitatif parametrelere etkisini belirlemek için yaptıkları çalışmada, farklı sulama suyu seviyelerinin klorofil üzerine etkisinin önemli (p<0.05) görüldüğünü belirtmişlerdir.

22. gün yapılan ölçümlerde bitki boyuna göre tüm uygulamalar arası farkın önemsiz olduğu görülmüştür. Şen ve Erdem (2018) farklı su uygulamalarının badem bitkisinin vejatatif parametreleri üzerine etkisini belirlemek için yaptıkları çalışmalarında, uygulanan sulama suyu miktarlarının bitki boyunu istatistiksel olarak etkilemediği sonucuna varmışlardır. 53. gün yapılan ölçümlerde tüm uygulamalar arası fark çok önemli bulunmuştur (p<0,01).

22. gün yapılan ölçümlerde bitki çap değerine göre tüm uygulamalar arası fark önemsiz bulunmuştur. Nitekim Doğan ve ark. (2020) da *Pelargonium domesticum* bitkisinin gelişim parametreleri üzerine farklı sulama seviyelerinin etkisini belirlemek için yaptıkları çalışmalarında farklı sulama uygulamalarının bitki çapı değerlerinde önemli bir fark oluşturmadığını belirtmiştir. Bitki çap değerine göre %50 ve %100 oranındaki uygulamalar arasındaki fark önemsiz, %50 ve %100 oranındaki uygulamalar ve diğerleri arası fark çok önemli bulunmuştur (p<0,01). Vejatatif aksam yaş ağırlığına göre tüm gruplar arası çok önemli bir farlılık olduğu görülmüştür (p<0,01). Vejatatif aksam kuru ağırlığına göre tüm gruplar arası çok önemli bir farlılık olduğu görülmüştür (p<0,01).

Sürgün sayısına göre %75 sulama uygulaması ile diğer sulama uygulamaları arasındaki fark önemli bulunmuştur (p<0,05). %50 ile %100 sulama uygulamaları arasındaki fark önemsiz çıkmıştır.

Yaprak alanına göre %25 oranındaki su uygulaması ile diğerleri arasında çok önemli bir farlılık olduğu görülmüştür (p<0,01). Tomurcuk sayısına göre tüm %75 oranında su uygulaması ile diğerleri arasındaki fark çok önemli bulunmuştur (p<0,01). Kök uzunluğuna göre tüm uygulamalar arası fark önemsiz bulunmuştur. Kök yaş ağırlığına göre tüm uygulamalar arası fark çok önemli bulunmuştur (p<0,01). Kök kuru ağırlığına göre tüm gruplar arası farlılık olmadığı görülmüştür.

Antirrhinum majus bitkisinde gelişim parametreleri üzerine sulama uygulamaları arasındaki farklılıklar dikkate alındığında %75 oranında su uygulamasının (%25 oranında su kısıntısı) bitki gelişiminde farklılık göstermediği anlaşılmıştır. Nitekim Sezen ve ark. (2023b)'nın *Salvia splendes* L. bitkisinin gelişim parametreleri üzerine kısıntılı sulamanın etkisini belirlemek için yaptıkları çalışmada gelişim parametrelerinin çoğunda %75 oranında su uygulamasının (%25 oranında su kısıntısı) olumlu düzeyde etki göstermesinin yanında, %100 oranında su uygulamasından (%0 oranında su kısıntısı) daha yüksek değerlerde gelişim olduğu sonucuna varılmıştır.

4. Sonuç ve Öneriler

Kentlerde peyzaj düzenlemelerinde kullanılan mevsimlik çiçeklerin çok fazla su tükettiği bilinmektedir. Demirel ve ark. (2018) süs bitkileri üzerinde su kısıntı uygulaması üzerine yapılmış çalışmaların yetersiz olduğunu belirtmişlerdir. Bu boşluğu doldurmak amacıyla gerçekleştirilmiş bu çalışmada bitki gelişimi ile ilgili 18 parametreye göre farklı su uygulamalarının etkileri saptanmaya çalışılmıştır.

Antirrhinum majus peyzaj düzenlemelerinde en yaygın olarak mevsimlik çiçeklerden biridir. *Antirrhinum majus* bitkisinde 18 gelişim parametresinin 17'sinde %75 oranında su uygulamasında (%25 su kısıntısında), %100 oranında su uygulamasından (%0 su kısıntısından) daha fazla gelişim olduğu görülmüştür. *Antirrhinum majus* bitkisinde sadece çiçek sayısı miktarını su kısıntısı azaltmıştır. *Antirrhinum majus* bitkisinde %75 oranında su uygulaması (%25 oranında su kısıntısı) bitki gelişimine olumlu düzeyde etki göstermesinin yanında, %100 oranında su uygulamasından (%0 oranında su kısıntısı) daha yüksek değerlerde gelişim olmaktadır. Sonuç olarak kentsel mekanlarda *Antirrhinum majus* bitkisi kullanıldığında %25 oranında su kısıntısı yapılması önerilmektedir. Bu durumda önemli düzeyde su tasarrufu yapılacaktır. Su tasarrufuna imkan veren *Antirrhinum majus* bitkisi kentsel mekanlarda yaygın olarak kullanılmalıdır.

Etik Kurul Onayı

Bu çalışma için etik kuruldan izin alınmasına gerek yoktur.

Çıkar Çatışması Beyanı

Makale yazarları olarak aramızda herhangi bir çıkar çatışması olmadığını beyan ederiz.

Yazarlık Katkı Beyanı

Planlama: Sezen, I.; Materyal ve Metot: Sezen, I., Karahan, A., Yağanoğlu, S.; Veri toplama ve İşleme: Sezen, I., Karahan, A., Akpınar Külekçi, E., Yağanoğlu, S.; İstatistiki Analiz; Akpınar Külekçi, E., Yağanoğlu, S.; Literatür Tarama: Sezen, I., Karahan, A., Akpınar Külekçi, E.; Makale Yazımı, İnceleme ve Düzenleme: Sezen, I., Karahan, A.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

The Effects of Restricted and Ad Libitum Milk Feeding on Growth and Health of Calves[*]

Sınırlı ve Serbest Süt İçirmenin Buzağıların Gelişimi ve Sağlığı Üzerine Etkisi

İbrahim ÖZTOP[1], Serkan ÖZKAYA[2]

Abstract

In recent years, there has been a growing concern among consumers regarding animal welfare and their requirements, leading to an increased interest in exploring new approaches to calf rearing. Therefore, the aim of this study was to investigate the effects of milk feeding levels on the growth, feed intake, and blood variables of group-raised calves during the suckling period. Ten Holstein calves, 5 in each group, were used in the study when they were five years old. The calves were acclimated to an automated feeder during the initial 19 days of the experiment, and their initial LWs were recorded after weighing them again. The study concluded upon weaning the calves when they were sixty years old, with a total duration of 36 days The calves in the first group (G1) were provided with a total of 4 L/day of milk replacer, divided into 2 L servings in the morning and evening. Conversely, calves in the second group (G2) were allowed a maximum intake of 12 L/day of milk replacer, mimicking the natural sucking behaviour, with feedings in the morning (05:00-08:00), midday (10:00-13:00), and evening (16:00-20:00). Despite no significant differences in the LW, total and daily LW gains, and body measurements averages between of the groups, calves in G1 consumed more starter feed compared to those in G2 (P<0.05). There was a non-significant improvement in feed efficiency in favor of calves in G2. Additionally, significant increases were noted in serum glucose concentrations among the biochemical variables in G2 (P<0.05). Furthermore, hematological blood variables, including haemoglobin, haematocrit, and erythrocyte values, tended to increase in calves in G2. However, there was no significant effect of milk feeding levels observed on oxidative stress, antioxidative defense mechanisms and immune response. In conclusion, the results from this study suggest that the amount of milk feeding does not exert a significant effect on the growth and health of the calves during the milk-feeding period.

Keywords: Calves, Growth, Health, Milk feeding amount

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Öz

Son yıllarda, hayvan refahı ve hayvan gereksinimleri ile ilgili tüketicilerdeki endişeler, buzağı yetiştirmenin yeni yollarını bulma arzusunu arttırmıştır. Bu nedenle çalışmanın amacı, süt içirme miktarının grup halinde yetiştirilen buzağıların süt içme dönemindeki büyüme, yem tüketimi ve kan değişkenleri üzerindeki etkilerinin incelenmesidir. Çalışmada 5 günlük yaşta 10 Holstein buzağı (her grupta 5 buzağı) kullanılmıştır. Denemenin ilk 19 gününde buzağılar otomatik yemleme robotuna alıştırılmıştır. Buzağılar tekrar tartılarak deneme başı canlı ağırlıkları belirlenmiştir. Buzağılar 60 günlük yaşta sütten kesilmişler ve deneme sonlandırılmıştır. Deneme toplam 36 gün sürmüştür. Birinci grupta bulunan (G1) buzağıların, sabah 2 L ve akşam 2 L olmak üzere toplam 4 L/gün süt ikame yemi tüketmelerine müsaade edilmiştir. İkinci grupta bulunan (G2) buzağıların ise doğal emzirme davranışı olan sabah (05:00-08:00), öğlen (10.00-13:00) ve akşam (16:00-20:00) olmak üzere azami 12 L/gün süt ikame yemi tüketmelerine müsaade edilmiştir. Grupların canlı ağırlıkları, toplam ve günlük canlı ağırlık artışları ve vücut ölçüleri ortalamaları arasında önemli farklılık bulunamamıştır. Ancak G1'de bulunan buzağıların, G2'de bulunan buzağılara nazaran daha fazla buzağı başlangıç yemi tükettikleri belirlenmiştir (P<0.05). Yemden yararlanma oranında G2'de bulunan buzağıların lehine önemli olmayan bir iyileşme elde edilmiştir. Biyokimya kan değişkenlerinden serum glikoz konsantrasyonlarında G2'de bulunan buzağılarda önemli artış elde edilmiştir (P<0.05). Hematolojik kan değişkenlerinden Hemoglobin, hematokrit, ve eritrosit değerleri G2'de bulunan buzağılarda artma eğilimi göstermiştir. Ancak, oksidatif stres, antioksidatif savunma mekanizması ve bağışıklık sistemi üzerine süt içirme miktarının önemli bir etkisi gözlenmemiştir. Çalışmada elde edilen sonuçlar, süt içirme miktarının buzağıların süt içme dönemindeki gelişimleri ve sağlıkları üzerine önemli etkisinin olmadığını göstermiştir.

Anahtar Kelimeler: Buzağı, Gelişim, Sağlık, Süt içirme miktarı

1. Introduction

It is widely recognized that a significant portion of revenue in dairy farming, approximately 40%, is derived from calves, with the remaining 60% coming from milk production (Anonymous, 2016). Consequently, the successful rearing of healthy calves is paramount for the future sustainability and profitability of the herd. The primary objective in raising calves is to ensure their optimal health and performance from birth throughout their lifespan. Achieving this goal necessitates the implementation of an effective calf-rearing system characterized by attributes such as high animal performance, cost-effectiveness, and minimal disease incidence and morbidity (Anonymous, 2011).

In modern farming practice, calves are typically separated from their dams shortly after birth and are provided with restricted milk intake via bottle feeding. However, restricted milk feeding can lead to various issues including calf starvation, impaired growth, elevated calf mortality rates, and behavioural abnormalities. While understanding the impact of a*d libitum* milk feeding on calf growth, development, and health before weaning is crucial, it is equally important to consider factors such as hunger, pain and other welfare concerns. The quantity of milk intake, the method of milk delivery, and the overall rearing protocols employed significantly influence calf growth, health, and welfare outcomes. Therefore, careful consideration of these factors is essential for optimizing calf-rearing practices and ensuring the well-being of the animals (Schaff et al., 2016).

Hunger is a subjective sensation characterized by the desire to eat and is regulated by the nervous system. For example, decreased levels of blood glucose prompt the emergence of hunger symptoms, whereas adequate glycaemia indicates a feeling of fullness and the cessation of eating (Stunkard, 1975). Hedonic systems, such as odours and learned cues associated with preferred tastes, also play a role in hunger. For example, the taste of lactose can heighten dairy calves' inclination to suckle milk (de Passille and Rushen, 2006). As a result, hunger is not solely connected to nutrient deficiencies but can also be influenced by various internal and external factors, including neural pathways that are not yet fully understood (Saper et al., 2002).

Growth parameters such as live weight and body measurements provide insights into the general health status of calves (Kozaklı et al., 2022). Calves raised as replacement heifers on dairy farms are generally fed daily with 10% of their live weight, which is about half of the milk they desire (Appleby et al., 2001). These calves usually have free access to starter feed, but their intake of very little starter feed is not enough to compensate for their limited milk intake (Jasper and Weary, 2002). In fact, the traditional practice of feeding young calves limited milk does not account for the energy levels they require for growth (Diaz et al., 2001; Van Amburgh and Drackley, 2005; Bartlett et al., 2006).

Calves that were fed very high quantities of milk (Khan et al., 2007a) or provided milk and milk replacer ad libitum (Jasper and Weary, 2002) demonstrated improved growth rates. Additionally, calves fed ad libitum tended to spend more time resting (de Paula Vieira et al., 2008), implying that calves expend more energy while standing than when lying down (Schrama et al., 1995). However, it is also reported that the increased amount of milk can negatively affect calf health (Quigley et al., 2006). Contrarily, some studies show no significant increase or decrease in disease incidence with higher milk intake (Appleby et al., 2001; Jasper and Weary, 2002; Khan et al., 2007a). Furthermore, it has been reported that calves fed a high percentage of milk or milk replacers have improved live weight gain, reduced starter intake, and no increase in disease incidence (Borderas et al., 2009).

Feeding with a high rate of milk decreases feed intake and increases weight gain. Calves fed with a high rate of milk consume less starter and straw while obtaining more digestible energy, leading to greater live weight gain (de Passille et al., 2011).

When fed with high amounts of milk, calves show more growth (Hartel et al., 2002; Jensen, 2006; Huuskonen and Khalili, 2008). Increased milk intake during the pre-weaning period influenced LW gain, leading to a decreased age of puberty onset and augmented fat-corrected milk yield during the first lactation. However, it does not impact skeletal development (Shamay et al., 2005). Bar-Peled et al. (1997) compared calves suckling from their dams three times a day with restricted milk intake to calves on a different feeding regimen. They reported rapid increases in live weight and wither height in the suckling calves during the first

6 months. Additionally, nutritional status may benefit the immune response and reduce mortality (Drackley, 2005).

As stated above, the difference in milk feeding methods impacts the performance and welfare of the calves. This study contributes to the understanding the effects of the frequency of giving milk replacer to calves using computer-controlled automatic feeders on their growth and health.

The objective of this research is to investigate the effects of milk feeding quantity on the growth, feed intake and health of calves during the suckling period. The study sought to answer the following questions:

How does the amount of milk given to calves fed with a computer-controlled automatic feeder affect their growth and feed intake?

How does this affect the oxidative stress level in calves during the suckling period?

How does this affect the antioxidative defense mechanism and the immune response?

2. Material and Method

In the study, 10 Holstein male calves born at the Faculty of Agriculture, Education, Research and Practice Farm at Isparta University of Applied Sciences, were used.

Commercially available starter and milk replacer used on the farm were utilized in the study.

Calves with similar live weights, at an average of 5 days old, were divided into 2 groups. The calves were adjusted to the automatic feeder in the first 19 days of the experiment. The calves in the groups were weighed again and their initial LW were recorded. The calves were weaned at 60-day-old and the study was terminated. The experiment lasted 36 days. The first group (G1) was given 2 L of milk replacer (Pro Milk, Interchem Limited Dublin, Ireland) in the morning and evening, 4 L/day in total, with a computer-controlled automatic feeder (I-MOM, Itech Robotic Automation Medical Ltd. Isparta, Türkiye). The second group (G2) was fed a maximum of 12 L/day of milk replacer in the morning (05:00-08:00), midday (10:00-13:00), and evening (16:00-20:00) (Odde et al., 1985; Day et al., 1987).

The milk replacer was prepared in the automatic feeder during sucking according to daily limits. 125 g of powder was mixed with 1 L of water at 38-40 °C and fed to the calves.

Live weight and body measurements of the calves were taken at the beginning of the experiment and followed up on a weekly basis. The live weights of the calves were recorded with a 1 g precision scale (Jadever, Coreks Kimya Gıda San. Tic. Ltd. Şti. İstanbul, Türkiye). A measuring stick (Hauptner-Herberholz GmbH & Co. Solingen, Germany) and strip (Adevet Kilometer, Vetaş Veteriner, İstanbul, Türkiye) were used to take body measurements (Yüksel and Karaçuhalılar, 2024) of the calves (*Figure 1*)
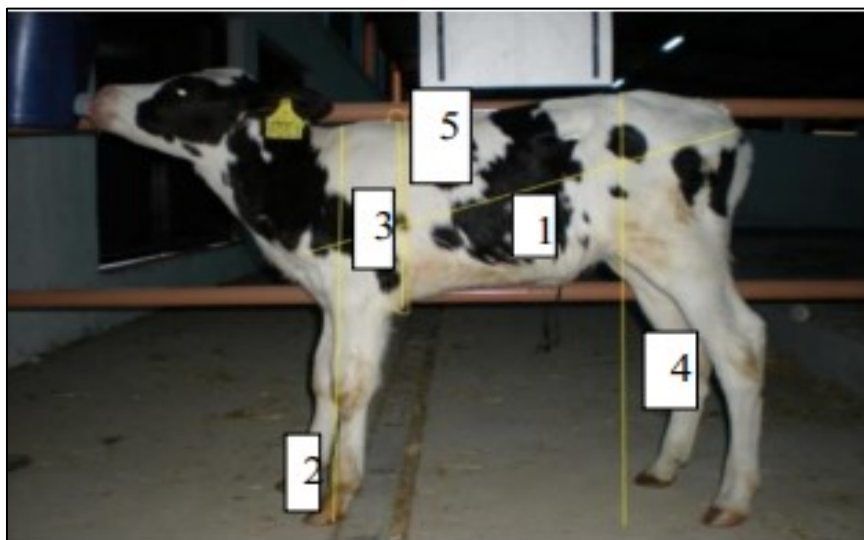

Figure 1. Body measurement parts of the calves.

Body length (BL): Measured from the shoulder to the outside angle of the seat bump using a measuring stick (cm), 2) Wither height (WH): The vertical distance from the top of the withers to the ground, measured by a measuring stick (cm), 3) Body depth (BD): The vertical depth between the highest point of the withers and the sternum measured by a measuring stick (cm), 4) Hip height (HH): The vertical distance from the highest point of the hip to the ground, measured by a measuring stick (cm), 5) Chest girth (CG): The girth (cm) measured with a measuring strip just behind the shoulder blades.

The milk replacer and starter intake of the calves were recorded daily by the computer-controlled automatic feeder's system. The percentage of starter crude protein in the starter was determined using the Kjeldahl method (AOAC, 2005; method 945.18), ether extract was determined using the Soxhlet method (AOAC, 2005; method 920.39), starch content was analyzed using method 996.11 of AOAC (2005), crude cellulose was determined using the Weende method (AOAC, 2005; method 978.01), and moisture content was analyzed using method 930.15 of AOAC (2005). Fat, protein, lactose and dry matter analyses of milk replacer were performed using HasVet Milk Test (HasVet Medikal Yazılım Sağlık Hizmetleri San. Tic. Anonim Şti., Antalya, Türkiye) milk analyser (*Table 1*).

Table 1. Chemical composition of starter, and milk replacer for calves

	Calf starter		Milk replacer
Dry matter, %	90.05	Dry matter, %	12.63
Crude Protein, %	18.17	Protein, %	2.15
Ether extract, %	2.79	Fat, %	2.88
Crude fiber, %	9.41	Fiber, %	-
Moisture, %	9.95	Moisture, %	4.5
Crude ash, %	7.52	Ash, %	0.64
Starch, %	28.25		-
Metabolic energy, kcal/kg*	2751.97		-
	-	Lactose, %	6.90

* The metabolic energy value was calculated according to the Turkish Standards Institution (TSE, 1991).

The starter intake of the calves was measured with a scale integrated into the computer-controlled automatic feeder system with a precision of 1 g. Using chips on the calves' necks and sensors in the manger, the system automatically recorded the amount of starter intake each time a calf entered the manger to feed.

Blood samples were collected from the jugular vein of calves at the beginning and end of the experiment at noon. The samples were collected in EDTA tubes and analyzed for hematological variables using the Mindray BC30 Vet V3D (Mindray Medical Int. Ltd., Shenzhen, China) blood count device.

Liver function tests (Albumin-ALB, Alkaline phosphatase-ALP, Alanine transaminase-ALT, Gamma-glutamyl transferase-GGT, Glucose-GLU, Total bilirubin-TBil, Lactate dehydrogenase-LDH), and kidney function tests (ALB, Blood urea nitrogen-BUN, Calcium-Ca, Creatine-CREA, Inorganic phosphorus-IP, Total protein-TP), as well as triglyceride and total cholesterol variables were analyzed using the Mindray BS120 (Mindray Medical Int. Ltd., Shenzhen, China) blood analyser device. The Globulin variable was calculated using the formula; Globulin = Total protein - Albumin.

Oxidative stress markers (Total oxidative capacity-TOC, Oxidative stress index-OSI, Malondialdehyde-MDA), antioxidative defense mechanism markers (Total antioxidant capacity-TAC, Paraoxonase-1-PON1, Superoxide dismutase-SOD, Glutathione peroxidase-GPx, Catalase-CAT) and immune response markers (ImmunoglobulinA-IgA, IgM and IgG) were analysed using the Mindray BS400 (Mindray Medical Int. Ltd., Shenzhen, China) blood analyser. CAT and MDA were analysed using Rel-Biochem (Rel Assay, Mega Medicine San. Tic. Ltd. Şti., Gaziantep, Türkiye) and IgG was analysed using the Bio-Tek EIX800 (BioTek Ins. Inc. Vermont, USA) Elisa reader with the Bovine Interferon Gamma (E0005BO) Elisa kit (Bioassay Technology Laboratory, Shanghai, China).

The oxidative stress index (OSI) was calculated using the following formula described by Yumru et al. (2009): OSI (Arbitrary unit) = (TOC (μmol H2O2 equivalent/L)) / (TAC (mmol trolox equivalent/L)x1000)x100.

Repeated Measurements ANOVA was employed to analyze the data from the study. Differences between group means were considered significant when P<0.05, and a tendency when 0.06<P≤0.10. The data analysis was conducted using Minitab 20 (Minitab LLC, 2020, Penn State, USA.

3. Results and Discussion

The study did not find significant effects of milk feeding quantity on the live weight, body measurements, and feed efficiency of calves (*Table 2*). However, there was a noteworthy difference in average feed intakes (P<0.05). It is well recognized that the effect of milk feeding on calves' starter intake and growth performance depends significantly on both the quality and quantity of milk provided (Silper et al., 2014). Typically, there is an inverse relationship between milk intake and starter intake among calves (Raeth-Knight et al., 2009; Terre et al., 2009; Gelsinger et al., 2016), a relationship that strengthens with higher milk quantities offered. In this study, the feed intake of G2 was significantly lower compared to G1 (P<0.05; *Table 2*). When calves are fed higher amounts of milk rather than restricted amounts, reduced starter feed intake is observed (Hill et al., 2010; Silper et al., 2014). This reduced starter feed consumption may be attributed to increased satiety resulting from elevated blood glucose and insulin levels and increased gut fill (Khan et al., 2011).

Table 2. The effect of restricted and ad libitum milk feeding growth performance of calves

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
Live weight (LW), kg			
Initial	58.13±8.27	57.50±8.27	0.97
Final	85.30±13.80	85.90±13.80	
Total LW Gain	27.13±6.79	28.38±6.79	
Daily LW Gain	0.75±0.19	0.79±0.19	
Body length (BL), cm			
Initial	77.50±3.70	74.50±3.70	0.76
Final	88.25±4.05	87.75±4.05	
Total BL Gain	10.75±0.83	13.25±0.83	
Daily BL Gain	0.30±0.02	0.37±0.02	
Body depth (BD), cm			
Initial	31.50±1.17	31.38±1.17	
Final	35.88±1.45	35.25±1.45	0.77
Total BD Gain	4.38±0.24	3.88±0.24	
Daily BD Gain	0.12±0.01	0.11±0.01	
Wither height (WH), cm			
Initial	82.00±2.88	80.25±2.88	0.61
Final	90.13±3.15	86.75±3.15	
Total WH Gain	8.13±0.52	6.50±0.52	
Daily WH Gain	0.23±0.01	0.18±0.01	
Hip height (HH), cm			
Initial	83.50±3.05	82.50±3.05	0.76
Final	91.25±3.43	89.75±3.3.43	
Total HH Gain	7.75±1.11	7.25±1.11	
Daily HH Gain	0.22±0.03	0.20±0.03	
Chest girth (CG), cm			
Initial	83.88±3.55	83.00±3.55	
Final	92.63±4.62	94.00±4.62	0.94
Total CG Gain	8.75±1.29	11.00±1.29	
Daily CG Gain	0.24±0.04	0.31±0.04	
Milk replacer and Feed intake and Feed efficiency			
Milk replacer DM intake, kg	13.87±0.36	31.58±1.37	0.00
Starter Feed DM Intake, kg	10.66±0.01	7.28±0.01	0.00
Total DM Intake, kg	24.53±0.35	38.86±1.37	0.00
Daily DM Intake, kg	0.681±0.01	1.08±0.03	0.00
Feed Efficiency	1.30±0.34	1.73±0.49	0.36

a,b Difference between the means in the same row is statistically significant (P<0.05)
Feed Efficiency=Daily DM Intake/Daily LW Gain

During the milk suckling period, non-significant differences in live weights were observed between the groups receiving different amounts of milk (*Table 2*). The live weights of G2 increased insignificantly compared to G1. Calves fed large amounts of milk exhibited higher live weight gains during the milk suckling period due to the intake of more digestible nutrients from milk (Dennis et al., 2018; Orellana Rivas et al., 2020; van Niekerk et al., 2020). Studies have reported non-significant differences in live weights among calves fed different amounts and frequencies of milk replacer meals (MacPherson et al., 2019). Scoley et al. (2020) found that milk feeding frequency did not affect the live weight and live weight gains of the calves. Additionally,

Jafari et al. (2021) noted that calves with higher daily milk intake gained more live weight, though the milk feeding frequency insignificantly affected live weight gain and showed an increasing trend.s

The differences in body measurements between G2 and G1 were not significant (*Table 2*). Similarly, Kmicikewycz et al. (2013) and Jafari et al. (2021) reported that the milk feeding frequency and amount did not significantly affect body measurements of calves. While high milk intake tends to increase live weight, it also tends to linearly increase chest girth (Stamey et al., 2012; Rosenberger et al., 2017). Khan et al. (2011) reported that the body depth of calves fed with restricted milk tended to increase compared to calves consuming high amount of milk.

Feed efficiency remained unaffected (*Table 2*), consistent with previous studies that did not report significant improvements in feed efficiency (Hill et al., 2016; Rosenberger et al., 2017; Dennis et al., 2019; Jafari et al., 2021). While the effect of milk and milk replacer intake on feed efficiency may vary, calves consuming higher amounts of milk have been associated with higher feed efficiency in some studies (Quigley et al., 2018).

3.1. Blood variables

3.1.1. Biochemical blood variables

The biochemical blood results of the groups are presented in *Table 3*. No significant difference was found in biochemical blood variables at the beginning of the experiment, thus these values are not shown in the table. However, a significant increase in GLU concentration was observed at the end of the experiment in G2 ($P<0.05$).

Table 3. The effect of restricted and ad libitum milk feeding on biochemical blood variables of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
ALT, U/L	23.25±2,48	23.75±2.48	0.70
GGT, U/L	15.75±1,32	20.75±1.32	0,31
ALP, U/L	173.50±22,08	217.80±22,08	0.23
Total cholesterol, mg/dL	62.25±4.06	68.50±4.06	0.81
Creatine, mg/dL	0.27±0.46	0.25±0.46	0.65
Calcium, mg/dL	9.43±0.35	9.48±0.35	0.84
Inorganic phosphorus, mg/dL	4.35±0.18	5.02±0.18	0.28
Total Protein, g/dL	5.53±0.15	5.58±0.15	0.77
Albumin, g/dL	2.87±0.13	2.91±0.13	0.80
LDH, U/L	868.50±49.89	967.50±49.89	0.79
Triglyceride, mg/dL	14.00±3.10	14.25±3.10	0.65
Total Bilirubin, mg/dL	0.15±0.06	0.08±0.06	0.59
Glucose, mg/dL	70.60±5.57	98.25±5.57	0.04
Blood urea nitrogen, g/L	6.05±0.06	6.18±0.06	0.48
Globulin, g/dL	2.60±0.11	2.73±0.11	0.53

ALT: Alkaline transaminase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, a,b
Difference between the means in the same row is statistically significant ($P<0.05$)

The feed intake of calves significantly affects GLU concentration. GLU derived from intestinal absorption is the primary energy substrate in calves (Khan et al., 2000b). As solid feed intake, the energy source shifts to volatile fatty acids, particularly propionate, produced through ruminal fermentation, which is converted to GLU (Vi et al., 2004). The increase in the starter intake in calves fed restricted milk may be due to a hyperphagic response triggered by reduced milk volume, leading to decreased blood glucose concentration (Khan et al., 2007b; Khan et al., 2011; Omidi-Mirzaei et al., 2015; de Paula et al., 2017). Elevated GLU concentration in calves fed higher milk amounts are consistent with findings from Terre et al. (2009), MacPherson et al. (2016), Mirzaei et al. (2018) and Jafari et al. (2021). Increased lactose intake likely contributed to higher blood GLU concentration (Palmquist et al., 1992). Conversely, studies have reported that calves fed excessive milk exhibit lower GLU concentrations compared to those fed with restricted amounts (Silper et al., 2014; de Paula et al., 2017), suggesting that lactose from milk replacer alone may not suffice to elevate glycaemia.

3.1.2. Hematological blood variables

There were no significant differences in hematological blood variables at the beginning of the experiment, which are not presented in Table. However, by the end of the experiment, there was a tendency for concentrations of haemoglobin, haematocrit, and erythrocyte to increase in G2 (P≤0.10) (*Table 4*).

Table 4. The effect of restricted and ad libitum of milk feeding on hematological blood variables of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
Leukocyte, 10^9/L	12.50±1.39	9.35±1.39	0.53
Haemoglobin, g/dL	10.13±0.20	11.10±0.20	0.10
Haematocrit, %	24.63±1.44	29.88±1.44	0.08
Platelet, 10^9/L	522.25±42.53	647.50±42.53	0.52
Erythrocyte, 10^{12}/L	6.87±0.40	8.55±0.40	0.06
MCV, fl	36.05±0.89	34.93±0.89	0.19
MCH, pg	15.10±1.15	13.00±0.15	0.17
PDW, fL	6.75±0.03	6.90±0.3	0.38
P-LCR, %	2.45±0.46	1.90±0.46	0.90
PCT, ng/mL	0.32±0.03	0.37±0.03	0.25

MCV: Mean red blood cell volume, MCH: Mean corpuscular haemoglobin, PDW: Platelet distribution width, P-LCR: Large platelet cell ratio, PCT: Procalcitonin, a,b Difference between the means in the same row is statistically significant (P<0.05)

Hematological variables such as leukocyte count, erythrocyte count, and haemoglobin concentration are crucial clinical indicators widely used to assess health and disease status (Kelada et al., 2012). Measurement of these variables helps evaluate the health and physiological conditions of calves (Roland et al., 2014). Although the haemoglobin levels were elevated in G2, they remained within the referenced range (8-15 g/dL) (Plumb, 2005). Similarly, the haematocrit concentrations of both groups were within the reference range (22-47%), as were the erythrocyte counts (5-10x1012/L). The PDW concentrations in both groups also fell within the reference range (12.00-17.50).

3.1.3. Oxidative stress and antioxidative defense mechanism

The differences in oxidative stress and antioxidative defense mechanism markers of calves at the beginning of the experiment were not found to be significant and therefore are not presented in the Table. Similarly, no significant differences were observed between the groups for these markers at the end of the experiment (*Table 5*). The effect of feeding a high amount of milk on oxidative stress and antioxidative defense mechanism was not significant. Wu et al. (2021) also reported that the effect of feeding excess milk on the oxidative stress and antioxidative defense mechanism of calves is not significant.

Table 5. The effect of restricted and ad libitum milk feeding on oxidative stress and antioxidant defense mechanism of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad lbitum) Mean±S. E.	P
TAC, mmol/L	0.60±0.04	0.63±0.04	0.12
TOC, µmol/L	3.58±0.51	3.60±0.51	0.66
OSI	0.61±0.12	0.58±0.12	0.74
PON-1, U/L	332.33±45.47	264.00±45.47	0.38
SOD, U/mL	220.00±97.80	259.33±97.80	0.89
GPx, U/L	361.00±65.43	351.00±65.43	0.81
CAT, U/mL	36.24±3.52	38.84±3.52	0.50
MDA, mmol/L	29.04±10.27	39.80±10.27	0.07

TAC: Total antioxidative capacity, TOC: Total oxidative capacity, OSI: Oxidaitive stress index, PON-1: Paraoxanase-1 enzyme, SOD: Super oxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde

3.1.4. Immune responses

Initially, the difference in immune response between calves was not significant and thus is not shown in the table. Similarly, at the end of the experiment, there was no significant difference observed in the immune

response of the calves (*Table 6*). Wu et al. (2021) also reported that feeding a high amount of milk did not affect the concentration of IgA and IgM in calves, but it significantly increased IgG concentration.

Table 6. The effect of restricted and ad libitum milk feeding on immune response of calves at the end of the experiment

	G1 (restricted) Mean±S. E.	G2 (ad libitum) Mean±S. E.	P
Ig A, mg/dL	21.53±6.76	32.80±6.76	0.16
Ig M, mg/dL	540.37±152.71	445.37±169.00	0.58
Ig G, ug/mL	245.53±22.47	213.85±22.47	0.74

4. Conclusion

This study investigated the effects of varying amounts of milk replacer during the suckling period on growth, antioxidative defense mechanism, and immune response of calves.

The amount of milk replacer given did not affect the growth of the calves. However, calves fed with restricted milk showed an expected increase in feed intake. Conversely, calves fed with excess milk demonstrated a non-significant improvement in feed efficiency.

Biochemical variables were generally unaffected by milk feeding amount, except GGT, IP, and GLU, which significantly decreased in calves fed with restricted milk. Other hematological variables, including haemoglobin, haematocrit, erythrocyte, platelet and PDW concentration, were not significantly influenced by milk amount and remained within the referenced range. However, these concentrations were notably higher in calves fed a higher amount of milk.

Milk feeding amount showed a non-significant effect on oxidative stress and antioxidative defense mechanism. Similarly, there was no significant effect on the immune response of calves.

The study's findings may have been influenced by the decrease in starter consumption alongside the increase in milk intake. It is noteworthy that there was no adverse health issues observed in the calves. However, these results might differ if the health of group-housed calves deteriorates or disease rate increases. Increased starter consumption by calves fed milk replacer twice a day may indicate hunger, underscoring the importance of timely starter feeding for calf welfare.

Ethical Statement

This study was prepared under the permission numbered 002, dated 27.05.2021, from the Ethics Committee of Isparta University of Applied Sciences.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Özkaya, S.; Design: Özkaya, S.; Data Collection or Processing: Öztop, İ.; Statistical Analyses: Özkaya, S., Öztop, İ.; Literature Search: Öztop, I.; Writing, Review and Editing: Özkaya, S.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Local People's View of Non-Benefiting from Murat River Projects: Examples of Bingöl, Elazığ and Muş Basins

Murat Nehri Rehabilitasyon Projelerinden Faydalanmayan Yöre Halkının Görüşleri (Bingöl, Elâzığ ve Muş Mikro Havzaları Örneği)

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Abstract

In Bingöl, Elazığ, and Muş, micro-basin projects have been implemented, but not all local residents have benefited from these initiatives. This study aimed to understand the views and experiences of those locals who did not benefit from the projects, assessing the potential impact on productivity and income. The study was conducted in the provinces of Bingöl, Elazığ, and Muş. Data was gathered between 16-25 August 2021. The primary sources of information were interviews with 168 non-beneficiaries, complemented by focus group discussions and key informant interviews. Findings showed that 75% of participants linked increased productivity to the projects, and all acknowledged an enlarged production area due to project activities. There was no significant difference in production area or income from agricultural sales based on gender or the presence of cash income. However, cash income holders reported higher earnings from both agriculture and livestock. Provincial disparities in income emerged: residents of Bingöl had 2.439 times the income of those in Muş, while Elazığ inhabitants earned 0.882 times less. Men's income surpassed women's by 1.209 times, and those with a history of cash income earned 4.037 times more than those without. Interestingly, owning land or cultivating high-value crops was associated with lesser income. Measures to counteract drought could enhance production areas and, consequently, local incomes. A significant barrier identified was the 20% contribution requirement, preventing some locals from project participation. Recommendations include adjusting project criteria to increase local engagement and benefits. The original value of this article is to reveal the impact of micro catchment projects implemented in Turkey.

Keywords: Bingöl, Elâzığ, Muş, Non-beneficiary local people, Project, Logistic regression analysis

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Öz

Bu araştırma; Bingöl, Elâzığ ve Muş illerinde yer alan mikro havzalarda uygulanan projelerden faydalanmayan yöre halkının görüşlerinin belirlenmesi amacıyla yapılmıştır. Araştırmada; 16-25 Ağustos 2021 tarihleri arasında seçilen köylerde projeden faydalanmayan toplam 168 kişiyle yapılan anket çalışması, odak grup (OG) görüşmeleri ve kilit bilgilendirici (KB) görüşmeler birincil verileri oluşturmuştur. Araştırma sonuçlarına göre; Bireylerin %75'i verim artışının bir proje faaliyetiyle ilgili olduğunu ve bireylerin tamamı üretim alanında artışın bir proje faaliyeti ile ilgili olduğunu ifade etmiştir. Yapılan analizler sonucunda; cinsiyet ile ve nakit gelire sahip olma durumu bakımından üretim alanında istatistiki olarak önemli artış olmadığı sonucuna varılmıştır. Yapılan analiz sonucunda kişilerin cinsiyeti ve yaşadıkları iller itibariyle tarımsal üretim ve hayvansal üretim satışlarından elde ettikleri gelirde istatistiki olarak önemli bir farklılık olmamıştır. Anket yapılan kişilerin nakit geliri olup olmamasına göre tarımsal üretim ve hayvansal üretim satışlarından elde ettikleri gelirde istatistiki olarak önemli farklılık olmuştur. Nakit gelire sahip olan yöre halkının nakit geliri olmayanlara göre tarımsal üretim ve hayvansal üretim satışlarından daha fazla gelir elde ettiği söylenebilir. Bingöl ilinde yaşayanların yüksek gelir elde etmesi, Muş iline oranla 2.439 kat daha yüksektir. Elâzığ ilinde yaşayanların ise yüksek gelir elde etmesi Muş iline nazaran 0.882 kat daha düşüktür. Erkeklerin elde edeceği gelir kadınlardan 1.209 kat daha fazladır. Önceden nakit geliri olanlar olmayanlara göre 4.037 kat daha fazla gelir sağlayacaklardır. Arazisi olanların olmayanlara göre 0.712 kat daha düşük ve yüksek değerli ürün yetiştirenlerin yetiştirmeyenlere oranla 0.422 kat daha düşük gelir kazanacağı sonuçlarına varılmıştır. Sonuç olarak; Kuraklıkla ilgili alınacak tedbirler üretim alanının artmasını sağlayacak ve üretim alanındaki artışla birlikte yöre halkının da geliri artacaktır. Projeden faydalanan bireylerden talep edilen %20'lik katkı payını karşılayamadığı için projeden faydalanamayan bireyler olduğu belirlenmiş bu konuda faydalanıcı olmak isteyen yöre halkına destek verilmelidir.

Anahtar Kelimeler: Bingöl, Elâzığ, Muş, Faydalanıcı olmayan yöre halkı, Proje, Lojistik regresyon analizi

1. Introduction

Watershed Rehabilitation (Improvement) projects are the projects prepared for the improvement of the land, agriculture, forest and pasture resources by watershed-based land modeling, and aim to develop the local people living in the watershed economically and thus to re-establish the corrupted natural balance (Gönenç, 2004; Anonymous, 2012; UHYS, 2015; Kuçukkaya, 2016). In other words, Watershed Integrated Rehabilitation Projects are carried out in order to manage natural resources such as forest, pasture, agriculture, water and soil at a watershed together with the local people of the region and to carry out improvement affairs. That is, Murat River Watershed Rehabilitation Projects (MRHRP); are carried out by OGM (General Directorate of Forestry), ÇEM (General Directorate of Combating Desertification and Erosion), IFAD (International Fund for Agricultural Development). When the MRWRP is examined, the most important reason for the support of this project by IFAD is the fact that it aims to improve water and soil resources of the individuals living in the upper micro-catchments with a sustainable and more intensive agriculture understanding, and in this way to increase the welfare level of the people of the watershed. Stopping the natural resource erosion at the micro-catchments in Elazığ, Bingöl and Muş provinces located in the Murat River precipitation watershed and reducing the poverty of the individuals living in the micro-catchments are among the most important objectives of the project. Thus, enabling improvement and diversification of income sources based on natural resources, supplying effective rehabilitation and sustainable management, ensuring development of human resources, setting a sample and assuring dissemination and investments in natural resources of the people living at the villages of the micro-catchments, and earning income from the maintenance and protection of those can be counted as the aims of the project (Yüksel and Eraslan, 2015; Bilinmiş, 2016; Baydaş et al., 2018; Danış, 2019).

As outsourced projects, Watershed Rehabilitation Projects have been applied in Turkey respectively broadly as follows: Eastern Anatolia Watershed Rehabilitation Project between 1991-2001, Anatolian Watershed Rehabilitation Project completed between 2005-2011, Çoruh River watershed Rehabilitation Project between 2012-2019 and Murat River Watershed Rehabilitation Project between 2012-2018 (Figure 1).

Figure 1. Outsourced watershed rehabilitation projects implemented and being implemented in Turkey

Source: (Turkish Republic Ministry of Forestry and Water Affairs, 2016)

Considering the large river watershed potential, it is planned to establish a monitoring system infrastructure in Turkey having 25 large river watersheds to ensure the effective use of natural resources and sustainable watershed management, and to monitor the data themes determined in coordination with the institutions operating at the watershed. It is aimed that the Watershed Monitoring and Evaluation System, which will be developed on a geographical basis, will reduce the cost of monitoring, take necessary measures by making fast and up-to-date monitoring, ensure the success of investments by effectively monitoring of the projects being carried out in the watershed, and provide significant gains in the balanced use and protection of natural resources. Interesting and important studies were found by using logistic regression analysis on the basis of agriculture and watershed (Neupane et al., 2002; Gobin et al., 2002; Tai-Yang et al., 2011; Mair and El-Kadi, 2013).

This research was conducted to determine the opinions of the local people who did not benefit from the projects implemented in the micro-basins of the provinces of Bingöl, Elazığ and Muş.

2. Materials and Methods

In the research; focus group (FG) interviews and key informative (KI) interviews conducted in selected villages between 16-25 August 2021 consisted the primary data of the survey. The surveys were conducted face-to-face with the heads of the households and interference and effect of them by others have been hindered. In addition, FG meetings and KI meetings were held with the headmen and notables of each village where the survey was conducted. In addition, secondary data (table, map, report, etc.) of micro-catchments were also utilised.

When the evaluation was made for 2021 in the research, although it was determined that the interest of those who did not attend the ask-and-solve meetings and who returned to the village due to the pandemic and other similar conditions was at a high level, it was difficult to reach the non-beneficiaries in almost all of the selected villages. The reason for this has been the fact that almost all of the households in the studied villages benefitting from the project activities, even if being small. Thus, the majority of household heads included in the project have been beneficiaries where project activities not started yet; however, the villagers being aware of the project activities and at the villages with crowded population. A total of 168 non-beneficiaries were surveyed.

The distribution of the answers given by the local people, who did not benefit from the project, to the questions was revealed by creating frequency tables for each question and drawing pie charts for some of them. In addition, "Non-Parametric Statistical Methods" were used to determine whether the opinions on some subjects differed according to gender, province of residence and cash income. For this purpose, Mann-Whitney U and Kruskal-Wallis tests were utilised. The correlations related to the factors affecting the poverty dimension of the local people participating in the survey were examined with the logistic regression model.

Logistic regression analysis (LRA), on the other hand, is an analysis that allows to establish a regression model without requiring assumptions such as normality, continuity, covariance and multivariate normality (Tabachnick and Fidell, 1996). LRA is an analysis in which the predictor variables can be continuous or discrete, continuous discrete predictor variables can coexist, and the predicted variable is discrete. If necessary, a continuous outcome variable can be transformed into a discrete variable in order to establish the LRA model (Tabachnick and Fidell, 1996). LRA shows similarities with some statistical analyses. Multiple linear regression analysis is an analysis similar to LRA in that its purpose is to establish a regression model, discriminant analysis is done with a discrete dependent variable, it is a kind of grouping analysis, cluster analysis is an analysis that divides data into groups. Multiple regression and discriminant analysis basically differ from logistic regression analysis by having assumptions, and cluster analysis differs by the number of groups and their membership not being known (Çokluk et al., 2010). LRA, which offers a more flexible structure compared to similar analyzes, is mathematically based on the logarithm of probability, odds and odds. Probability is simply the ratio of the number of outcomes of a particular type to the total possible outcomes. For example, the probability of rolling a 3 when a dice is rolled is 1/6. As there is only one '3' on the dice and there are six possible outcomes. This ratio can be expressed as 0.167 or 16.70%. In the LRA, odds is defined as the probability of an event occurring divided by the probability of that event not occurring. Odds has no upper limit, but its lower limit is 0. Odds removes the problem of probability estimation being within the range of 0-1, but there is still another problem to be solved, which is how to ensure that the odds ratio does not take a value below zero. This problem is solved by calculating the logit value. Logit is the natural logarithm of the odds ratio. By calculating the logit value, a metric variable that can always be reverted to a probability between 0-1 and can take positive and negative values is obtained (Cokluk et al., 2010; Mertler and Vannatta, 2005). In cases where the dependent variable is expressed on an ordinal scale, the Ordered Logistic Regression (OLR) model is often preferred due to its tendency to yield better results compared to linear and logistic regression models (Köksal, 2009; Şerbetçi and Özçomak, 2013; Özer and Özden, 2016; Terin, 2019; Ağır and Akbay, 2021).

For a response variable Y with two measurement levels (dichotomous) andexplanatory variable X, let:

$$\pi(x) = p(Y = 1 | X = x) = 1 - p(Y = 0 | X = x) \tag{E.q.1}$$

the logistic regression model has linear form for logit of this probability

$$Logit\ [\pi(x)] = log\left(\frac{\pi(x)}{1-\pi(x)}\right) = \alpha + \beta x \qquad \text{(Eq.2)}$$

$$odds = \frac{\pi(x)}{1-\pi(x)} \qquad \text{(Eq.3)}$$

$$odds = \exp(\alpha + \beta x) \qquad \text{(Eq.4)}.$$

and the logarithm of the odds is called logit so,

$$Logit\ [\pi(x)] = log\left(\frac{\pi(x)}{1-\pi(x)}\right) = log[\exp(\alpha + \beta x)] = \alpha + \beta x \qquad \text{(Eq.5)}.$$

The logit has linear approximation relationship, and logit = logarithm of the odds. The sign of β indicates whether curve ascends (β > 0) or descends (β < 0), and the rate of change increases as |β| increases (El-Habil, 2012).

Multiple logistic regressions can be extending to models with multiple independent variables. Let k represents number of predictors for a binary response Y by

$x_1, x_2, ..., x_k$, the model for log odds is

$$Logit\ [P(Y = 1)] = \alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k \qquad \text{(Eq.6)}.$$

and the alternative formula, straightly specifying $\pi(x)$, is

$$\pi(x) = \frac{\exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k)}{1 + \exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k)} \qquad \text{(Eq.7)}.$$

The parameter β_i refers to the effect of x_i on the odds that Y=1, controlling other x_j, to illustrate exp (β_i) is the multiplicative effect on the odds of a oneunit increase in x_i, at fixed levels of other x_j (Chatterjee and Hadi, 2006). A decision was taken in terms of research ethics from T.R. Bingöl University Rectorate Social and Human Sciences Scientific Research and Publication Ethics Board with number 33117789/044/83126 for the study.

3. Results and Discussion

3.1. Living Conditions

It was determined that 62 people who participated in the survey live in Bingöl, 28 people live in Muş and 78 people live in Elazig. Men make up 82.1% of the responders, while women make up 17.9%. 35.14% of those surveyed said they did not have any sources of monetary income, compared to 64.9% who said they had. According to the MRWRP (2018) research, 81.5% of people had a source of cash income, compared to 18.5% who did not. In the MRWRP (2019) survey, 67.2% of the non-beneficiaries had a source of cash income, while the remaining 37.3% did not. The percentage of people who have a source of cash income was 67.2% in the MRWRP (2020) research. People pointed to the Covid-19 epidemic as the cause of the cash income source ratio falling below the figure observed in earlier surveys. 56% (94 participants) of the respondents said their income position was the same as it was last year, 36.3% (61 participants) said it was worse, and 7.1% (12 participants) said it was better. In MRWRP (2018), it was found that 10.6% of people had higher incomes, compared to 28.5% who had lower incomes and 60.9% who had unchanged incomes. In the MRWRP (2019) research, there were 2% more households with rising incomes, 85.7% more homes with stable incomes, and 12% more households with decreasing incomes. In the MRWRP (2020) study, 46.1% of respondents said their income had remained stable, while 39.4% said it had decreased. The Covid-19 epidemic was cited as the cause of this problem by those whose income-decrease rate was higher than that of previous studies. It was discovered that 67 (40.5%) got wages, 33 (19.6%) worked in the production and sale of animals, and 25 (14.9%) of them worked in other occupations. According to the MRWRP (2018) report, 58.7% of respondents cited salaries and earnings as their primary source of income, while 14.7% of them indicated agricultural and animal output as their primary source. In the MRWRP (2019) research, household income was primarily derived from earnings and salaries (51%). Animal products made up 27% of the total. According to a survey done in 2020, the top three sources of revenue for people were salary, animal production, and agricultural output. A total of 141 non-beneficiaries (or 83.9%) and 27 (16.1%) non-beneficiaries declared that they had no other source of income. In the MRWRP (2018) study, it was found that 44.3% of people had additional sources of income. In the MRWRP (2019) research, 45.3% of participants had a secondary source of income, compared to 54.7% who did not. In the MRWRP (2020) study, 81% of respondents said

they had no other source of income than their job. 37% of respondents made money from producing and selling animals, while 55.5% of respondents made money from selling agricultural products. In the MRWRP (2018) study, it was found that 22.7% of people worked in the animal production industry and 31.8% of people worked in the agricultural production industry. In the MRWRP (2019) research, 40.8% of them were involved in agricultural production, while 15% were involved in the production of animals.

3.2. Property Rights

It has been determined that 82 people (48.8%) have agricultural land and 86 people (51.2%) who do not have agricultural land. In the MRWRP (2018) study, it was found that 50.3% of the people had a title deed to agricultural land. According to the MRWRP (2019) research, 67% of them have their own agricultural land, compared to 32% who do not. According to the research done in MRWRP (2020), 53% of people do not have property rights on lands. A maximum of 20 decares were held by 70.7% of respondents, between 21 and 100 decares were held by 28%, and more than 500 decares were held by 1.3% of respondents. In the MRWRP (2018) study, it was found that 28.2% of people had a land between 21 and 100 decares, while 71.8% of people had a land less than 20 decares. According to the MRWRP (2019) study, 21% of it was agricultural land between 20 and 100 decares, and 78% of it was less than 20 decares. In contrast to the 51.2% who said they lacked fertile land, 48.2% of participants claimed to have it. It was discovered in the 2018 assessment that 42.6% of the population lived on fertile land. According to the research done in MRWRP (2019), 71.4% of the population was suitable for farming. In the MRWRP (2020) study, the percentage of people who had fertile land was 63.3%, and the percentage of people who didn't had fertile land was 36.1%. A rate of 28.2% was found for those with irrigated agricultural land under 10 decares, 54.6% for those with irrigated agricultural land between 10 and 40 decares, and 17.2% for those with irrigated agricultural land over 40 decares. In the MRWRP (2018) research, it was found that 20 decares or less of land is owned by 81.5% of those owning fertile irrigated agricultural land. According to a MRWRP (2019) study, 84.5% of the respondents had fertile irrigated agricultural land of 20 decares or fewer. The average area of irrigated agricultural land was found to be 36 decares in the study done in MRWRP (2020). Less than 10 decares of dry agricultural land were owned by 23.5% of people, 10 to 20 decares were owned by 61.7%, and more than 20 decares were owned by 14.8% of people. In the MRWRP (2018) study, it was found that 84.7% of the people possessed land assets for 20 or fewer periods. According to the MRWRP (2019) research, 60.5% of the population owned fertile dry agricultural property measuring 20 decares or fewer. The average dry agricultural land was estimated to be 9 da in the MRWRP (2020) study.

3.3. Agricultural Production and Irrigation

It was discovered that 81 survey respondents (48.2%) only farmed the land for their personal purposes, 79 respondents (47%) did not cultivate the land, 6 respondents farmed the land for both their needs and sale, and 2 respondents farmed the land exclusively for sale. In the survey done in 2020, it was found that 48.3% of people farmed land for their own consumption. 161 individuals (97.5%) voiced unfavourable opinions on the increase in product yield compared to the prior year. 4.5% of respondents only said yes. 3.1% of the people said they had increased their products in the previous year in the 2018 study. In the 2019 study, 4% of respondents said they had increased their products in the previous year. In the 2020 study, 15% of participants claimed that productivity has increased from the previous year. The productivity improvement was attributed to a project activity by 75% of the respondents and not by 25% of the respondents, according to their responses. There was no rise in the production area, according to 164 respondents (97.6%), and there was, according to 4 respondents (2.4%). Everyone cited a project activity as the reason for the expansion of the production area. The primary cause of the extremely small growth in the production area was attributed by the general populace to the drought's effectiveness. In the study that was done in 2018, 6.7% of respondents said that the producing area had grown. In the 2019 study, 11.2% of the respondents said they had expanded their production space in the previous year. In the study conducted in 2020, 10% of respondents claimed to have seen a growth in their production area. The analyses carried out led to the conclusion that there was no statistically significant improvement in production in terms of gender and cash income (*Table 1*). Using the irrigation system, 45.8% of the participants employed drip irrigation, flood irrigation, etc. 2.4% of the interviewees said they have increased the amount of irrigated land. 33.4% of those surveyed claimed that a project activity was responsible for the expansion of the irrigated area. Despite the fact that 54 (32.1%) of the participants were involved in animal husbandry, it was found that 90.7% of cattle breeders and 9.3% of small cattle breeders were involved in livestock breeding. According to the study done in 2020, the majority of people were involved in cattle breeding. It was discovered that 18.6% of the

respondents said there had been an increase in the number of animals during the previous year, and that 50% of people believed that this increase was connected to any project activity. There are various statistical studies in which the production plan is evaluated according to the income of the farmers in the agricultural basins (Everest, 2021; Brown et al., 2014).

Table 1 compares this year's rise in production area by gender, province, and having cash income to last year's increase.

Variables	Number	Average rank
Gender		
Male	137	84.17
Female	30	8322
Mann-Whitney U	2031.500	
Wilcoxon W	2496.500	
Z	-0.370	
Asymp. Sig. (2-tailed)	0.711	
Province		
Bingol	62	84.35
Elazığ	28	86.50
Muş	78	84.35
Kruskal-Wallis H	0.879	
Asymp. Sig.	0.644	
Having cash income		
Yes	109	84.19
No	59	85.08
Mann-Whitney U	3181.500	
Wilcoxon W	9176.500	
Z	-0.428	
Asymp. Sig. (2-tailed)	0.669	

3.4. Access to Markets

While 80.4% claimed they were unable to do so, 19.6% of the participants claimed to make money through selling their livestock and other agricultural products. The revenue generated from the sale of agricultural and animal goods increased by 9%, remained constant by 60.6%, and declined by 30.4% as compared to the prior year. In the study conducted in 2018, it was found that the percentage of people whose income increased in the previous year was 14.1%, the percentage of people whose income declined in the same period was 25.4%, and the income of 60.6% of people did not change. While the income from agriculture and animal production climbed by 6.8% in the study done in 2019, it declined by 7.8% and changed by 85.4%. In the 2020 survey, 50% of the participants said that their income had remained unchanged. 60.6% of the respondents did not have a problem with production marketing, compared to 39.4% of those who did. In the 2018 study, 79.4% of respondents claimed not to have any marketing-related issues. 71% of respondents to the 2020 study said they did not have a marketing problem. The study revealed that there was no statistically significant gender difference in the earnings of those involved in agricultural production and animal production sales. The income of the survey respondents from sales of agricultural produce and animal production by the provinces they reside in has not increased statistically significantly. Depending on whether the respondents had cash income or not, there was a statistically significant difference in the revenue they received from sales of agricultural products and animal production. It can be claimed that locals with cash income generate greater income through sales of agricultural products and livestock than those without (*Table 2*).

3.5. Business Development and Employment

Only 4.8% of the households taking part in the survey run any businesses except farming and pet care. 160 members of the household, or 95.2% of them, work exclusively in agricultural and animal husbandry. According to the 2018 report, 6.1% of the participants owned a business unrelated to agriculture or animal husbandry. In the 2019 study, it was found that 4.1% of the participants owned a business that wasn't related to farming or animal husbandry. 164

respondents to the 2020 study stated that they were solely engaged in agriculture and animal husbandry. A new business for their household or an expansion of their current business was helped by the initiative, according to 62.5% of respondents, compared to 37.5% of people who claimed it had no effect on their ability to start a new business or grow an existing one. In the 2018 study, every participant claimed that the project had no impact on their ability to locate new jobs or improve their working conditions. Not all participants in the 2019 report claimed that the project did not help them find new jobs or improve their working conditions. When compared to other studies, the fact that there are people in the study who think project activities have an impact on starting a new business or growing an existing business might be seen as a positive outcome.

Table 2. shows the association between gender, province, and having cash income and the status of earning revenue from agricultural output and animal production sales.

Variables	Number	Average rank
Gender		
Male	136	88.93
Female	30	82.30
Mann-Whitney U	1877.000	
Wilcoxon W	11193.000	
Z	-0.990	
Asymp. Sig. (2-tailed)	0.322	
Province		
Bingöl	62	89.73
Elazığ	27	78.85
Muş	78	81.23
Kruskal-Wallis H	3.009	
Asymp. Sig.	0.222	
Having cash income		
Yes	109	89.01
No	58	74.59
Mann-Whitney U	2615.000	
Wilcoxon W	4326.000	
Z	-2.661	
Asymp. Sig. (2-tailed)	0.008	

3.6. Access to Natural Resources

While 61.9% claimed they were unable to do so, 38.1% of respondents claimed to have profited from the forest. 5 respondents (8%) said this benefit was infrequent, while 59 people (92%) said it was regular. In the survey that was done in 2018, it was found that 37.8% of the people who said they routinely benefited from the forests did so. 38 participants in the 2020 survey claimed to routinely benefit from the forest. In terms of their ability to benefit from the forest, 50 people (78%) saw an improvement, while 14 people (22%) did not. In the 2018 study, 85% of individuals who benefit from forests said their use has increased. In the 2019 study, 95.3% of respondents claimed that they were using forests more frequently. In the 2020 study, 51% of respondents said that their use of the forest had not changed from the year before. People claimed that they could not go out to the forests much because of the Covid-19 pandemic as the reason why they felt that the possibility to profit from forests has improved, which was significantly lower than the findings of the 2018 and 2019 studies. Regarding the increase in forest productivity over the previous year, 37 persons (58.6%) had negative attitudes and 27 (41.4%) had good ones. According to 79.6% of respondents, project activities have increased forest productivity compared to last year. According to a 2018 poll, 61.9% of respondents believe that forest productivity has increased and that the project has a bearing on this. In the 2019 study, 87.1% of respondents claimed that the project effect boosted forest productivity. It has been shown in both earlier research and this study that project activities have a favourable impact on forest productivity, which is universally acknowledged by people. It was found that 92 (56%) of the people did not benefit from the grazing land, while 74 (44%) of the people did. 38.5% of the people in the research from 2018 were found to have benefited from the pastures. In the 2019 study, 40.8% of respondents said that pastures had been beneficial to them. It has been determined that the majority of those

who gain from the pasture have not changed all that much over time—nearly half. 119 respondents to the study conducted in 2020 claimed they had no advantage from the shared grazing land. In the study, 63 respondents (85%) said the benefit was regular whereas 11 respondents (15%) said it was irregular. In the 2018 survey, 96.7% of the participants who benefited from the pastures said they did so on a regular basis. All of the participants in the 2019 study claimed that the pastures had been beneficial to them. In the 2020 study, 57 participants claimed that grazing land regularly benefits them. The percentage of people who routinely benefit from the pastures was much smaller than that seen in the previous three trials, and those people explained that the Covid-19 epidemic prevented them from spending enough time in the pastures. 61 of the respondents said that the use of the rangeland has improved from the previous year. 13.6% of respondents said there had been no progress. In the 2018 study, 84.6% of the participants said that there are now more opportunities to profit from the pastures than there was the year before. In the 2019 study, 93.4% of respondents said there is now more possibility to gain from pastures. In the 2020 study, 59% of the participants said they had not noticed a change from the prior year in their ability to profit from pastures. People mentioned the Covid-19 epidemic as the reason they could not spend enough time in the pastures as a reason why the rate of people who enhanced their opportunity to profit from the pastures was lower than the findings of the preceding 2018 and 2019 investigations. The production of pastures has increased compared to the previous year, according to 30 respondents (40.5%). In comparison to the prior year, 44 persons (59.5%) claimed that productivity had not increased. 63 of the participants (85.1%) believe that the project activities are to blame for the development, compared to 11 participants (14.9%), who disagree. In the 2018 study, 21.2% of respondents believed that the initiative improved the effectiveness of the rangelands. According to 2019 research, 80.3% of respondents believe that project activities boost pasture productivity. More people than were discovered in the results of the prior study believe that the project's actions have a beneficial impact on pasture productivity.

3.7. Factors Affecting the Local Participants in the Survey's Poverty Dimension

Income from sales of agricultural and animal products is a dependent variable.

Independent variables: Gender, province (where he lives), cash income, land ownership, high value crops are not cultivated.

The impact of characteristics such as land ownership, cash income, gender, place of living, and high-quality product on sales revenue from agricultural and animal production is the goal.

Table 3. Process summary

Unweighted Cases[a]		Number	%
Selected Cases	Included in Analysis	165	98.2
	Missing Cases	3	1.8
	Total	168	100.0
Unselected Cases		0	0
Total		168	100.0

Table 3 shows that 165 data were used for the logistic regression analysis, and 3 data were excluded from the study because they lacked observations. The dependent variable's categories were coded as 1 for yes and 0 for no. *Table 4* lists the categories of categorical independent variables.

Nargelkerke and Cox-Snell R^2 values, which display the model's relevance by demonstrating the ability of the independent variables to account for changes in the dependent variable, in this case R^2, are listed in the model summary table. 15.8% of the change in the dependent variable is thus explained by the independent factors (*Table 5*). According to the Hosmer and Lemeshow test, the model is significant since p=0.413>0.05 (*Table 6*). When the classification table was looked at, it was discovered that the model's accurate classification rate was 82.4% (*Table 7*). The significance of the model's variables Only the cash variable is significant at the 1% significance level when the (p) values are examined. The impacts of the factors on the dependent variable (income) can be understood using the exp(B) values. The relationship between the independent and dependent variables is said to be negative if the Exp(B) value is less than 1, and positive if it is more than 1. The reference variable was the province of Muş. The high standard of living in Bingöl is 2.439 times more than it is in Muş. People in Elazig make 0.882 times less money than those in Muş. The lower value is 1-0.882=0.118 (11.8%). Men make 1.209 times more money than women do. The income generated by people

with prior cash income will be 4,037 times greater than that of those without. It has been found that individuals with land will have incomes that are 0.712 times lower than those without, and those who grow high-value crops would have incomes that are 0.422 times lower than those without (*Table 8*).

Table 4. lists the codes for reference variables and category variables.

		Number	Parameter coding* (1)
Product	0	132	1.000
	1	33	0.000
Elazığ	0	88	1.000
	1	77	0.000
Gender	0	136	1.000
	1	29	0.000
Cash	0	58	1.000
	1	107	0.000
Land	0	84	1.000
	1	81	0.000
Bingöl	0	103	1.000
	1	62	0.000

*0 and 1 are the values of the dependent variable. product 0: no product; product 1: yes product; land 0: no land; land 1: yes land

Table 5. Model summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	147.725[a]	0.100	0.158

Table 6. Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	7.158	7	0.413

Table 7. Classification table

	Observed	Predicted Sales Revenue		
		0	1	Percentage Correct
Step 1	Sales Revenue 0	131	1	99.2
	1	28	5	15.2
	Overall Percentage			82.4

a. The cut value is .500

Table 8. Coefficients of the logistic regression model equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Bingöl (1)	0.892	0.623	2.047	1	0.152	2.439
	Elazığ (1)	-0.126	0.639	0.039	1	0.844	0.882
	Gender (1)	0.190	0.609	0.097	1	0.755	1.209
	Cash (1)	1.395	0.488	8.181	1	0.004	4.037
	Land (1)	-0.339	0.467	0.528	1	0.467	0.712
	Product (1)	-0.862	0.513	2.824	1	0.093	0.422
	Constant	-1.861	0.855	4.738	1	0.029	0.155

Only livestock population and male membership in a local Non-Governmental Organization had a consistently favorable and substantial effect on agroforestry adoption by both project and non-project households in a study on the adoption of agroforestry of independent variables (Neupane et al., 2002). In another study on logistic regression analysis, the percentage to predict agricultural land use determinants of correctly classified fields (95.7%) was highest at a threshold probability of 0.5 in southeastern Nigeria. Four fields were false positive and nine fields were false

negative with the same probability. The model was applied to the validation set of 88 fields, with a threshold probability of 0.5. The percentage of correctly categorized fields was 89.9%, with three false positive and six incorrect negative classifications (Gobin et al., 2002). For each target contaminant and subgroup of contaminants, Mair and Al-Kadi (2013) created a set of multiple-variable Logistic Regression models based on combining hydrogeology, land use, and well geometry and location factors. Overall, the data showed a high correlation with agricultural land use. Tai-Yang et al. (2011) investigated the effect of access on agricultural land loss in a low-lying region in Southeast China. The results of the models utilized in the authors' work revealed that whether the parcel was converted or not was directly related to geographical characteristics such as height, location, and accessibility. Elevation had a detrimental impact on farm land conversion since it increased development costs. In the model, the elevation coefficient was 0.0025. The odds ratio can be determined based on the coefficient using the relationship between the odds ratio and the regression coefficient. In the model, the odds ratio for elevation was 0.9975, which suggests that for every 1 m rise in elevation, the likelihood of being converted decreased 0.9975 times. The estimated odds ratio for an increase of 100 m in height was 0.7788, indicating that the likelihood of being converted reduced 0.78 times for every 100 m increase in elevation. The distance between the road and the town also had a detrimental impact on conversion.

4. Conclusions

Everyone cited a project activity as the reason for the expansion of the production area. The primary cause of the extremely small growth in the production area was attributed by the general populace to the drought's effectiveness. The analyses conducted led to the conclusion that there was no statistically significant rise in the production area for either gender or having a cash income. According to the research, there were no statistically significant differences between people's income from sales of agricultural and animal products by gender or where they resided. Depending on whether the respondents had cash income or not, there was a statistically significant difference in the revenue they received from sales of agricultural products and animal production. It can be claimed that locals with cash income generate more income through sales of agricultural products and livestock than do those without. 95.2 percent of the households who took part in the survey have no businesses except from farming and raising animals. 62.5% of respondents claimed that the programme has assisted them in starting a household company or growing an existing one. When compared to other studies, the fact that there are people in the study who think project activities have an impact on starting a new business or growing an existing business might be seen as a positive outcome. Although it was found that most respondents (61.9%) were unable to benefit from the forest, it was also found that 50 respondents (78%) had improved opportunities to do so this year compared to last year, while 14 respondents (22%) had not. The use of grazing land benefits 44% of people. It has been determined that the majority of those who gain from the pasture have not changed all that much over time—nearly half. The inability to venture out into the forests too often as a result of the Covid-19 pandemic can be demonstrated as one of the reasons why the percentage of people who believe that the potential to gain from forests and pastures has improved is rather low. The model was found to be significant and the independent variables in the model explained 15.8% of the change in the dependent variable as a result of the logistic regression analysis. The model's accurate classification rate was found to be 82.4%. At the 1% significance level, it was found that only the cash variable was significant. The high standard of living in Bingöl was 2.439 times more than it was in Muş. People in Elazig made 0.882 times less money than those in Muş. Men made 1.209 times more money than women do. The income generated by people with prior cash income has been 4,037 times greater than that of those without. It has been concluded that those who have land will earn 0.712 times lower income than those who do not, and those who grow high-value crops will earn 0.422 times lower income than those who do not. In light of these findings, it can be said that the project's implementation led to good developments in the villages and a major contribution to rural development based on the information collected from the participants. Additionally, it has been noted that people have begun to regularly live in the villages and that income-generating activities have developed in the communities with the help of project activities. It was determined that the MRWRP actions resulted in a reduction in the pressure on the union's forest vegetation. It has been determined that the advancements made in the pasture regions significantly benefit animal husbandry. The steps to be done in response to the drought will expand the production area, and as the production area expands, so will the locals' income. Support should be provided to the locals who want to benefit from this issue but who have been identified as persons who are unable to do so because they are unable to provide the 20% payment required of the recipients. According to the available data, it can be concluded that the project is a significant driver of the region's development and that in the years to come, this degree of development will become more apparent.

Ethical Statement

This study was prepared a decision was taken in terms of research ethics from T.R. Bıngöl Unıversıty Rectorate Social and Human Sciences Scientific Research and Publication Ethics Board with number 33117789/044/83126 for the study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: equal author contribution; Design: equal author contribution; Data Collection or Processing: equal author contribution; Statistical Analyses: Şenol Çelik and Ersin Karakaya; Literature Search: equal author contribution; Writing, Review and Editing: Şenol Çelik, Ersin Karakaya, Ahmet Uslu and Semra Çamuka.

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ARAŞTIRMA MAKALESİ   RESEARCH ARTICLE

Environmental Valuation of Wastewater Used in Agricultural Production*

Tarımsal Üretimde Kullanılan Atık Suyun Çevresel Değerlemesi

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Abstract

In recent years, problems related to the adequacy of existing water resources have arisen due to issues such as population growth, rapid urbanization, industrialization, and climate change. Especially in agricultural production where water is used the most, the use of wastewater has become widespread as a solution to this problem. Considering that wastewater may harm the environment due to the substances it contains, it is aimed to make an environmental valuation in this study. In this context, by using the travel cost method used in the valuation of goods without a market, a different approach was brought to this method, as agricultural production is concerned, and an evaluation was made based on producer surplus. In the study, by comparing the agricultural productions with wastewater and well water, 2 hypotheses have been developed about the environmental valuation of wastewater; First, if the producer surplus of agricultural production with wastewater is lower than that of production with well water, there is environmental pollution, and its value is the difference between the two rents. The second hypothesis is that if the producer's surplus with wastewater is high, positive externality will occur as a hypothesis against the negative effects of environmental pollution. By conducting a survey with 125 producers in the research area, the data of 314 parcels in total were included in the analysis, and the producer surplus was calculated by creating the supply function with the regression analysis. As a result of the research, the producer surplus (584.40$) obtained from the agricultural production made by irrigating with wastewater was found to be higher than the producer surplus (553.44$) of the production made by irrigating with well water. In this case, it was concluded that the wastewater irrigated did not affect the environmental pollution, but the positive externality. Although the use of wastewater in agricultural production has made a positive contribution to the economy, it will be beneficial to use it after the necessary treatment processes, considering its harmful effects on human health.

Keywords: Wastewater, Environmental valuation, Agricultural production, Producer surplus

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Öz

Son yıllarda tüm dünyada nüfus artışı, hızlı kentleşme, sanayileşme ve iklim değişikliği gibi konular nedeniyle mevcut su kaynaklarının yeterliliği ile ilgili sorunlar ortaya çıkmıştır. Özellikle suyun en çok kullanıldığı tarımsal üretimde bu soruna çözüm olarak atık su kullanımı yaygınlaşmıştır. Atık suların içeriğinde bulunan maddeler nedeniyle çevreye zarar verebileceği göz önünde bulundurularak bu çalışmada çevresel bir değerleme yapılması amaçlanmıştır. Bu kapsamda, piyasası olmayan malların değerlemesinde kullanılan seyahat maliyeti yöntemi kullanılarak, tarımsal üretim söz konusu olduğundan bu yönteme farklı bir yaklaşım getirilmiş ve üretici rantına dayalı bir değerlendirme yapılmıştır. Çalışmada, atık su ve kuyu suyu ile yapılan tarımsal üretimler karşılaştırılarak, atık suyun çevresel değerlemesi hakkında 2 hipotez geliştirilmiştir; Birinci hipotez atık su ile yapılan tarımsal üretimin üretici rantı kuyu suyu ile yapılan üretimden daha düşük ise çevresel kirlilik vardır ve değeri iki rant arasındaki farktır. İkinci hipotez ise üreticinin atık su ile elde ettiği rantın yüksek olması durumunda, çevre kirliliğinin olumsuz etkilerine karşı bir hipotez olarak pozitif dışsallık oluşacağıdır. Araştırma alanında 125 üretici ile anket yapılarak toplam 314 parselin verileri analize dahil edilmiş ve regresyon analizi ile arz fonksiyonu oluşturularak üretici rantı hesaplanmıştır. Araştırma bulguları sonucunda atık su ile sulama yapılarak gerçekleştirilen tarımsal üretimden elde edilen üretici rantı (584.40$), kuyu suyu ile sulama yapılarak yapılan üretimin üretici rantından (553.44$) daha yüksek bulunmuştur. Bu durumda, sulanan atık suyun çevre kirliliğine değil, pozitif dışsallığa etki ettiği sonucuna varılmıştır. Atık suyun tarımsal üretimde kullanılması ekonomiye olumlu katkı sağlamış olsa da insan sağlığı üzerindeki zararlı etkileri göz önünde bulundurularak gerekli arıtma işlemlerinden sonra kullanılması faydalı olacaktır.

Anahtar Kelimeler: Atık su, Çevresel değerleme, Tarımsal üretim, Üretici rantı

1. Introduction

The needs of humans, animals and all living creatures in the world and all kinds of economic activities necessary to meet these needs; that is, the most basic resource required for the natural cycle of life to function in a certain order is water. The fact that water is the main resource at all stages of the natural cycle shows its importance more and more as the population increases day by day.

The sector in which water resources are used the most in Türkiye and in the world is agriculture. 71% of the water used in the world and 85% in Turkey is used in the agricultural sector (World Bank, 2022). When selecting irrigation systems for agricultural purposes, it is advisable to choose those that conserve water, increase yield and quality per unit area, and reduce the need for labour. Although irrigation systems are preferred for their ability to diversify products and cover large areas of land, it is important to consider the investment cost and economic life of the system (Ağızan and Bayramoğlu, 2021). In this context, due to the decrease in water availability with the effect of climate change experienced in recent years, solutions are tried to be produced regarding water use. One of these solutions is the use of wastewater in agricultural activities.

The safe use of wastewater as an alternative source of irrigation is an accepted strategy for the prevention and efficient use of water pollution with increasing water scarcity (Khan et al., 2013). The use of wastewater in agricultural lands, especially in those close to the central provinces, is economically very important. Because the insufficient underground water in arid lands is replaced by wastewater and the need for natural water resources is reduced; contributes to the formation of a sustainable resource management (Jaramillo and Restrepo, 2017; Direk et al., 2022). The use of wastewater in agriculture not only saves water by reducing the demand for fresh water, but also reduces the fertilizer need of plants thanks to the elements such as nitrogen and phosphorus in its content (Adalı and Kılıç, 2020). Wastewater, even if treated, is rich in organic matter, macronutrients (N, P, K) and has a higher content of numerous micronutrients such as Fe, Zn, Mn and Cu than a synthetic fertilizer (Çakmakcı and Şahin, 2020). USA, Australia and Western European countries consider treated wastewater as an alternative source (Yurtseven et al., 2010). Treatment facilities have been widely used in Turkey and all over the world in order to contribute to sustainable water management by purifying wastewater.

There are risks and advantages that occur or may occur in the use of wastewater as a source of agricultural irrigation. These are given in *Table 1* as follows;

Table 1. Advantages and Risks that May Occur Due to the Use of Wastewater

Advantages	Risks
Due to the nitrogen and phosphorus contained in the wastewater, savings can be made in the use of fertilizers, and it can also benefit both crop and soil fertility.	If the use of wastewater is not controlled, heavy metals, various minerals and organic substances in its content can damage groundwater.
It can be a solution to water scarcity as it is an alternative source.	With the uncontrolled use of wastewater, it can be dangerous to human health, especially in raw vegetables or with pathogens that can be transmitted through wastewater.
Wastewater is continuous because it is the result of a cycle and can be a ready source for irrigation in seasons and periods when water resources are scarce.	Uncontrolled and intensive use of wastewater and the accumulation of its contents in the soil can cause damage to the soil.
If the wastewater is treated in accordance with the requirements apart from agricultural irrigation, it can also provide benefits in different areas as drinking and using.	
As a result of the researches, it was observed that the metabolic activities of beneficial microorganisms in plant cultivation increased with the use of wastewater in irrigation.	

Reference: (Saracoğlu 2014)

The use of treated wastewater in agriculture benefits human health, the environment and the economy. This use has been evaluated as an alternative to the water shortage caused by the need for water due to the growth of the urban population in different regions (Becerra, 2015). The use of wastewater in agriculture is considered beneficial for agricultural and economic reasons, but negative health and environmental effects should be minimized (Alobaidy et al., 2010). For this reason, it is necessary to carry out an effective wastewater management.

There have been many studies showing the advantages and disadvantages of wastewater use. There are studies proving the benefits of wastewater such as increasing in crop yield (Rosemarin, 2004; Keraita et al., 2008) and producer income (Danso et al., 2002; Huibers et al., 2004; Rosemarin, 2004), increased crop diversity (Raschid-Sally et al., 2005), providing balanced nutrition for the plant (Koottatep et al., 2006), and urban livestock production by providing feed production with wastewater (Drechsel et al., 2010). On the other hand, soil salinization (Walker and Lin, 2008), decreased soil porosity (Wang et al., 2003), heavy metal pollution (Ashraf et al., 2013), water impermeability (Wallach et al., 2005) and underground It has been determined that it causes negative effects such as pollution of water (Mahmood and Maqbool, 2006). Wastewater can be beneficial to the environment and human health, as well as be harmful with unconscious and uncontrolled use. Therefore, in this study, the environmental impact of the wastewater used in agricultural production has been made and its economic impact has been revealed.

Wastewater reuse is an important component of sustainable water resources management; Reusing water from various wastewater sources after removing pollutants, nutrients and pathogens offers an alternative for water security (Grant, 2011; Matheyarasu et al., 2016). The current scarcity of resources and the inability to meet the need in terms of quality or the increased pressure on water with unconscious use emphasize the importance of wastewater. Needs will be met with this alternative source, which will provide benefits both environmentally and economically.

Environmental valuation methods are used to determine the benefits and costs associated with the use of environmental products, the improvement of their conditions or the elimination of environmental damage (Lindberg and Lindberg, 1991). Environmental valuation refers to the monetary measurement of the benefit or cost to the life of society of the consequences of environmental improvement interventions and environmental degradation (Christie et al., 2012). In this study, it is aimed to make an environmental valuation for the waste form of water, which is one of the most important natural resources, and to reveal whether it contains benefits or costs for agricultural production.

2. Materials and Methods

In order to obtain the data in the research, 5 neighbourhoods (Acıdort, Çengilti, Divanlar, Göçü, Karakaya) located in the Karatay district of Konya province, which are the research area, and which carry out agricultural irrigation with wastewater, were chosen for the purpose; A total of 742 agricultural enterprises were identified as the main population and the number of samples was determined as 125 according to the stratified random sampling method. However, due to the lack of sufficient producers during the field studies, a total of 114 producers were interviewed.

Konya province is the city with the most agricultural area in Turkey, and Karatay district, which is the research area, is the district with the highest agricultural area in Konya. Karatay district has 178 154.7 ha of agricultural land, 50% of which is dry agricultural land. The fact that Karatay district is the central district of Konya is more exposed to environmental pollution than rural districts, and it is used as an agricultural irrigation alternative due to its proximity to the wastewater source.

There are some environmental valuation methods at the point of determining the pollution with monetary values. Since environmental pollution is associated with externality, it is mostly done by considering methods that have no market value (Gündoğmuş and Kalfa, 2016). Travel cost and contingent valuation methods are mostly used in the studies. In this context, studies in which the travel cost method is used are generally associated with the consumer, and people who benefit from a certain area are used in the calculations. When calculating with the travel cost method for any recreation area, the value of this area, which is valued using consumer surplus, is determined by the number of visitors. Consumer surplus is estimated with the help of the demand function created between the travel cost and the number of visits (Çay et al., 2020). By using travel costs instead of price when

deriving the demand curve, these travel costs are considered to reflect people's propensity to pay for recreation services (Kaya, 2002). In this study, since the effect of agricultural lands and land value on environmental pollution is taken into account, the producer surplus has been calculated over the supply function.

Producers may be willing to sell their product at a price below the equilibrium price in order to sell a good. The difference between the equilibrium price and the price that is below the equilibrium price agreed by the producer is the producer's surplus, and in the study, the producer's surplus is calculated and the income obtained in return for the factors of production used is determined. As a matter of fact, price is equal to cost in a free market. Since the aim of the study is to determine the environmental valuation of the wastewater in the research area, the prices of the production factors are discussed by keeping the other variables constant in the supply function.

$$S = f(Se + Fe + W + P + L + Fu)$$ (Eq.1)

In the function, S = quantity of supply, Se = price of seed, Fe = price of fertilizer, W = price of water, P = price of pesticide, L = price of labour, Fu = price of fuel. Producer surplus was calculated by integrating the function with the following formula (Cinemre et al., 2008).

$$PS = Q.P - \int_0^Q f(Q)dQ$$ (Eq.2)

PS = Producer Surplus, Q = Equilibrium Quantity, P = Equilibrium Price.

It is aimed to compare the difference between the lands irrigated with wastewater and the lands irrigated with well water within the total population by using the producer surplus. Two hypotheses have been developed for this purpose;

- If the producer surplus is high in the lands irrigated with wastewater, positive externality will be seen as a hypothesis against the negative effects of environmental pollution and a different approach will be gained to environmental valuation.

- If the producer surplus is high in the lands irrigated with well water, the difference between the producer's surplus of the lands irrigated with wastewater will be considered as the environmental pollution value.

Regression and t-test analyses were conducted to determine if there is a cost difference between irrigation with wastewater and well water.

3. Results and Discussion

It is seen that the ratios of female and male population in the examined enterprises are at equal levels in all age groups. It has been determined that the rate of 50 years and over is 33.99%; 8.06% of the population aged 0-6; 9.15% between the ages of 7-14; 48.80% between the ages of 15-49. With this result, it is seen that the ratio of the working population, which is the active population, is the highest. Therefore, it is concluded that the workforce potential of the examined enterprises is good. While the age group of 15-49 constitutes 64% of the family workforce, 35.71% is the group aged 50 and over. In the examined enterprises, 12% of the population is illiterate; 51% of them are primary school; 23% of them are secondary school; It was determined that 7.44% were high school graduates and 5.69% were university graduates. It has been observed that the education level with the highest number of people is primary school. In addition, it was determined that 51% of the examined enterprises used wastewater, 23% groundwater and 26% both irrigation sources in agricultural production.

3.1. Calculation of Producer Surplus

Producer surplus is expressed as a producer who is willing to sell her product at a price below the equilibrium price in a perfectly competitive market, making some gains (Dinler, 2016). In this study, a different approach was brought to the consumer surplus in the travel cost method used in the valuation of environmental goods, and it was calculated to determine the rent provided by the producers from the wastewater by adapting it to the agricultural production, which is the subject of the study. The supply function was used to calculate the producer surplus, while keeping the other variables constant, only input costs and production amount were considered and given in *Table 2.*

Table 2. Average Operating Input Costs and Production Quantities of the Products Irrigated with Wastewater ($/decare)

Products	Costs						Production Quantities
	Seed	Fertilizer	Pesticide	Water	Fuel	Labour	
Wheat	5.39	4.88	0.91	-	5.90	7.98	1.533
Barley	7.52	7.59	1.65	-	6.16	8.23	1.140
Sugar Beet	12.48	10.73	5.03	-	6.80	9.73	2.231
Corn	19.52	11.19	3.77	-	5.92	8.81	161
Fodder Corn	46.78	-	3.51	-	4.79	8.55	2.640
Sunflower	11.49	6.08	2.35	-	7.00	7.52	1.524
Alfa alfa	2.46	4.71	8.55	-	3.09	5.05	1.464
Vetch	3.51	-		-	5.93	5.85	600

* 1 $ = 8,55 Turkish Liras at study time (July-2021)

Seeds, fertilizers, pesticides, water, fuel and labour costs, which are the basic expenses in the herbal production process, are minimum in order to be an optimum production process; efficiency should be maximum. At this point, the use of wastewater has a positive effect on production costs. As a matter of fact, the most expense in plant production is irrigation and fertilization costs. Therefore, while there is no cost for water in productions where irrigation is done with wastewater during the production process, it is seen as a result of the research that the use of fertilizers is reduced thanks to many minerals such as nitrogen, phosphorus and salt in the wastewater.

Table 3. Average Operating Input Costs and Production Quantities of the Products Irrigated with Well Water ($/decare)

Products	Costs						Production Quantities
	Seed	Fertilizer	Pesticide	Water	Fuel	Labour	
Wheat	8.33	7.57	1.25	2.51	7.93	9.69	1 354
Barley	7.83	7.16	1.20	17.26	7.19	9.67	1 078
Sugar Beet	11.70	11.74	6.43	57.08	6.69	8.51	2 555
Corn	17.56	14.15	4.39	76.34	6.81	10.47	3 396
Fodder Corn	32.75	11.61	3.51	58.48	7.03	10.53	2 500
Sunflower	14.23	7.33	2.88	48.30	7.02	8.56	1 369
Alfa alfa	4.68	6.81	7.02	23.39	3.42	6.67	1 340
Vetch	4.21	6.43	0.00	21.05	6.46	10.38	800

* 1 $ = 8,55 Turkish Liras at study time (July-2021)

According to the research, the specific input costs of the producers using wastewater and well water were compared, and as seen in *Tables 2* and *3*, it was concluded that the input costs of the producers irrigating with well water in general were higher than the producers irrigating with wastewater. As a matter of fact, there are certain differences in the costs of seeds, fertilizers, pesticides, fuel and labour per product.

Independent t-test was applied to determine whether two different irrigation sources are effective on production cost items in agricultural production activities irrigated with wastewater and well water.

H_0 = There is no difference in production costs between irrigation with wastewater and irrigation with well water.

H_1 = There is a difference in production costs between irrigation with wastewater and irrigation with well water.

According to the purpose of the research, the rejection of the H_0 hypothesis was accepted below the 5% significance level. As a result of the analysis made, it was determined that there was no difference in seed cost (t=1.345), pesticide cost (t=0.331) and fuel cost (t=1.469) according to irrigation source in *Table 4*. It was determined that there was a difference in fertilizer cost (t=5.101), water cost (t=23.215), labour cost (t=5.573) and production amount (t=3.346). The decrease in the use of fertilizers in irrigation with wastewater and the decrease

in the use of labour in irrigation and fertilization activities confirm these analyses. As a result of this analysis, assuming that there will be differences in the rents of the producers according to the two different irrigation sources, the producer's rents were calculated in order to reveal which irrigation source will generate more producer surplus and to determine whether the wastewater and irrigation will cause environmental pollution.

Table 4. Independent T-Test of Production Costs by Irrigation Source

	Code*	N	Average	Standard Deviation	t	Significance Level
Seed	1.00	216	82.9176	51.22802	-1.345	.179
	2.00	98	91.7357	59.14489	-1.275	.204
Fertilizer	1.00	216	43.5935	29.22241	-5.101	.000
	2.00	98	64.1541	40.37807	-4.531	.000
Pesticide	1.00	208	15.0529	20.99625	-0.331	.741
	2.00	98	15.8673	17.95219	-0.350	.727
Water	1.00	216	11.3935	11.52622	-23.215	.000
	2.00	98	305.3061	185.64018	-15.660	.000
Fuel	1.00	216	53.1022	28.39243	-1.469	.143
	2.00	98	57.6949	18.26949	-1.719	.087
Labour	1.00	216	67.0851	17.57427	-5.573	.000
	2.00	98	79.0829	17.90394	-5.534	.000
Production Quantity	1.00	216	618.1065	1022.85750	-3.346	.001
	2.00	98	1173.5000	1912.06478	-2.705	.008

*Code 1.00=Wastewater, Code 2.00=Well water

It is aimed to compare the difference between lands irrigated with wastewater and lands irrigated with well water within the total population by using producer surplus. In order to calculate the producer surplus, firstly, the costs of the factors of production and the supply function were formed by keeping the other variables constant. Since the products irrigated with wastewater will be compared with the products irrigated with irrigation water, two different models have been established: for enterprises irrigating with wastewater and for enterprises irrigating with well water. While the production quantity as the dependent variable and the prices of the factors of production as the independent variable in the supply function, since the purpose of the research is the irrigation cost, the Gross Production Value per decare (GPV) as the dependent variable in the model, the seed cost per decare (S), the fertilizer cost (Fe), pesticide cost (P), water cost (W), fuel cost (Fu), labour cost (L), the price of the product one year ago (Pr) and the production quantity (PQ) are discussed. According to the analysis made in the study, the supply function for agricultural production irrigated with wastewater is as follows;

$$GPV_A = 1238.447 + 0.311S_A + 2.917Fe_A - 2.213Pe_A + 2.517W_A - 1.627Fu_A - 3.727L_A$$

$$+ 285.295PrA + 0.321PQ \tag{Eq.3}$$

Analysis was made by considering the data of 216 parcels that were irrigated with wastewater. Since products that differ from each other in terms of yield and input such as wheat, barley, sugar beet, sunflower, corn, and alfalfa are included in the analysis, when the importance levels of the variables are examined, it is seen that variables such as labour, medicine, fuel are not significant. For the purpose of the research, the importance levels of other variables were not taken into account due to the variety of products included in the analysis, since it was examined whether the water used in production was waste or not.

When the average values of the variables other than water cost are included in the function and the model is reconstructed in terms of production quantity (Q), the equation is obtained.

$$W_A = 0.3973GPVA - 599.0877 \tag{Eq.4}$$

In order to calculate the producer surplus, the function is integrated and subtracted from the product of the equilibrium quantity and price (P.Q) in the research area in 2021, which is the research period. Since GPV is taken as the dependent variable in the model, price is taken as the limitation in the integral.

$$PS = (P)(Q) - \int_0^P (0.3973P - 599.0877) \qquad \text{(Eq.5)}$$

$$PS = (0.23)(1900) - (0.3973P^2 - 599.0877P)$$

PS = **584.40 $**

Table 5. Regression Analysis for Enterprises Irrigation with Wastewater

Variables	Coefficients	Standard Error	t	Significance Level	VIF
Constant	1.238.447	190.773	6.492	.000	-
Seed (S)	.311	.824	.378	.706	1.145
Fertilizer (Fe)	2.917	1.709	1.707	.089	1.600
Pesticide (Pe)	-2.213	1.960	-1.129	.260	1.066
Water (W)	2.517	3.646	.690	.491	1.134
Fuel (Fu)	-1.627	1.474	-1.104	.271	1.124
Labour (L)	-3.727	2.716	-1.372	.171	1.462
Price (Pr)	122.474	36.947	3.315	.001	1.420
Production Quantity (PQ)	.321	.043	7.442	.000	1.357

In the study, the data of 98 parcels that irrigate with well water were analysed, and according to the analysis, the supply function obtained for agricultural production with well water is as follows;

GPVK = 699.170 + 0.104SK + 4.621FeK – 0.596PeK + 2.784WK – 1.052FuK + 0.280LK –

130.135PrK + 0.51PQk (Eq.6)

When the average values of the variables other than water cost are included in the function and the model is reconstructed in terms of production quantity (Q), the equation is obtained.

WK = 0.3592GPVK – 467.5498 (Eq.7)

$$PS = (P)(Q) - \int_0^P (0.3592P - 517.9842) \qquad \text{(Eq.8)}$$

$$PS = (0.23)(1900) - (0.3592P^2 - 517.9842P)$$

PS = **553.44 $**

Table 6. Regression Analysis for Enterprises Irrigation with Well Water

Variables	Coefficients	Standard Error	t	Significance Level	VIF
Constant	699.170	335.081	2.087	.040	-
Seed (S)	.104	1.099	.094	.925	1.432
Fertilizer (Fe)	4.621	1.944	2.377	.020	2.087
Pesticide (Pe)	-.596	3.526	-.169	.866	1.357
Water (W)	2.784	.462	6.019	.000	2.496
Fuel (Fu)	-1.052	3.481	-.302	.763	1.370
Labour (L)	.280	4.316	.065	.948	2.022
Price (Pr)	-130.135	75.628	-1.721	.089	2.129
Production Quantity (PQ)	.051	.046	1.111	.270	2.370

As can be seen in *Table 5* and *Table 6*, producer surplus obtained with irrigation water used in agricultural production were calculated with the analyses made, and it was determined that the producer surplus obtained from irrigation with wastewater was higher. It has been seen that positive externality has been realized as a hypothesis

against the negative effects of environmental pollution due to the high producer surplus in the lands irrigated with wastewater, and a different approach has been brought to environmental valuation. It has been concluded that the wastewater used is not harmful to agricultural production and in this case, it is not possible to talk about environmental pollution. In addition, in the examinations made in the research area, it has been determined that irrigation with wastewater has a positive effect on the yield of the product, as well as reducing the cost of irrigation, fertilization, disinfection and labour costs. Due to the fact that the elements in the wastewater act as fertilizer, it has been determined that the fertilization process is much less than the irrigated lands with well water. The fact that the labour force used for fertilization and irrigation operations is less than irrigation with wells has caused this expense item to be lower. This situation reveals that irrigation with wastewater provides a positive externality to agricultural production.

4. Conclusions

With the effect of rapidly increasing urbanization, industrialization and climate change in parallel with the increase in the world population, the difference between the supply and demand of water is gradually increasing. This situation has revealed the necessity of developing alternative strategies for the evaluation of wastewater within the scope of effective water management. Evaluation of wastewater is of great importance, especially for agricultural activities where water is consumed the most.

In the study, it was aimed to make an environmental valuation of the wastewater used in agricultural production, and by calculating the producer surplus with the travel cost method approach, it was concluded that the wastewater had a positive effect on the producers rather than environmental pollution.

In general, wastewater is perceived as a harmful and negative concept; When other studies in the literature and the result of this research are taken into account, it is concluded that with correct and controlled management, it provides benefits for existing resources and reduces environmental pressure. However, the fact that the use of wastewater without treatment can threaten human health should not be ignored. For this reason, first of all, producers should be aware of this issue. In addition, it is recommended to establish facilities where treated water can be used in the research area. In Turkey, it is possible to benefit from the energy subsidies given by the Ministry of Environment, Urbanization and Climate Change for wastewater treatment establishments. In addition, studies such as reducing the environmental pollution effects of wastewater can be carried out to evaluate it as Agricultural Land Footprint.

Ethical Statement

Since it is an article produced from a master's degree study that used research data before 2022, there is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

There is no conflict of interest between the article authors.

Authorship Contribution Statement

Concept: Karakayacı Z.; Design: Karakayacı Z. X.; Data Collection or Processing: Aydın E.; Statistical Analyses: Karakayacı Z.; Literature Search: Karakayacı Z., Aydın E.; Writing, Review and Editing: Karakayacı Z.

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ARAŞTIRMA MAKALESİ	RESEARCH ARTICLE

Tekirdağ İli Süt Sığırcılığı İşletmelerinde Buzağı Ölümlerinin Araştırılması

Investigation of Calf Deaths in Dairy Cattle Enterprises in Tekirdağ Province

Ahmet Refik ÖNAL[1], Onur MACİT[2]

Öz

Sığırcılık işletmelerinde buzağıların yaşama gücü oranı, işletmedeki buzağı ölümleri hakkında bilgi vermekte olup bir işletmede belli bir döneme kadar yaşayan buzağı sayısının canlı doğan buzağı sayısına bölünmesiyle elde edilmektedir. Yeni doğan buzağıların sağlığını ve yaşama gücünü ırk, besleme, iklim, hastalıklar, barınak, hijyen, doğum tipi, cinsiyet gibi unsurlar etkilemektedir. Tekirdağ ilinde süt sığırcılığı işletmelerinde buzağı ölümleri ile ilgili yapılan bu çalışma, şansa bağlı seçilen 207 adet süt sığırcılığı işletmesinden yüz yüze yapılan anket yolu ile toplanan veriler değerlendirilerek yapılmıştır. Ankette işletmelerin yapısal özellikleri ve işletme içi uygulamalara değinilerek işletmelerin buzağı bakım, besleme ve ölümleri incelenmiştir. İşletmelerde yetiştirilen toplam hayvan sayılarının %15.9'u 0-5 baş, %26.6'sı 6-10 baş, %31.4'ü 11-20 baş, %18.4'ü 21-30 baş, %7.7'si ise 31 ve üzeri baş olarak tespit edilmiştir. İşletme sahiplerinin %71.5'i ilkokul, %14'ü ortaokul, %12.1'i lise, %2.4'ü üniversite mezunu olduğu belirlenmiştir. İşletmelerde gebe hayvanlara septisemi aşısı yaptırma oranı %45.9, doğumdan sonra buzağılara septisemi serumu uygulama oranı %69.1 olarak tespit edilmiştir. Buzağı ölüm oranı %5 olan işletmelerin oranı %33.8, ölen buzağıların %48.3 oranında 1-7 gün arasında yaşam süresi bulunduğu, %18.2 oranında ise ölen buzağıların annesi olan ineğin başka buzağılarının da öldüğü saptanmıştır. Buzağı ölümlerinde ishalin önemli bir yer tuttuğu belirlenmiş olup prebiyotik ve probiyotik kullanımının arttırılması, bakım, besleme, hijyen ve koruyucu sağlık uygulamaları olan aşılamalara daha fazla özen gösterilmesi ile ölüm oranlarının azaltılabileceği söylenebilir. Buzağı kaybı olarak nitelendirilebilecek diğer bir konu ise servis periyodunun uzun olmasıdır. Doğumdan sonra tekrar gebe kalma arasındaki geçen süre olan servis periyodunun uzaması, farklı nedenler sonucu ortaya çıkmaktadır. Servis periyodunun optimum sürelere indirilmesinin ise işletmelerde; teknik personel istihdamı, tekniğine uygun bakım ve besleme uygulamaları, kızgınlık tespit uygulamaları ve gözlem sürelerinin arttırılması, yeterli sayı ve nitelikte personel istihdamı ve suni tohumlama tekniği uygulamalarına bağlı olmaktadır. Yapılan bu çalışmadan elde edilen sonuçlar, süt sığırcılığı alanında faaliyet gösteren işletmelerin buzağı ölüm oranlarını azaltıcı tedbirler almaları ve kayıt tutma ile bu oranları düzenli olarak takip ve kontrol etmeleri gerektiği sonucunu ortaya çıkarmıştır.

Anahtar Kelimeler: Tekirdağ, Süt sığırcılığı, Buzağı ölümleri, Holstein, Çiğ süt

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Abstract

The survival rate of calves in cattle farms provides information about calf mortality in the farm and is obtained by dividing the number of calves that live up to a certain period by the number of live-born calves. Factors such as breed, nutrition, climate, diseases, shelter, hygiene, type of birth, and gender affect the health and survival of newborn calves. In this study conducted on calf mortality in dairy cattle farms in the Tekirdag province, data collected through face-to-face surveys from 207 randomly selected dairy cattle farms were evaluated. The survey examined the structural characteristics of the farms and in-farm practices, focusing on calf care, feeding, and mortality. The total number of animals raised in the farms was found to be 15.9% for 0-5 heads, 26.6% for 6-10 heads, 31.4% for 11-20 heads, 18.4% for 21-30 heads, and 7.7% for 31 or more heads. It was determined that 71.5% of the farm owners were primary school graduates, 14% were secondary school graduates, 12.1% were high school graduates, and 2.4% were university graduates. The rate of septicemia vaccination for pregnant animals in the farms was 45.9%, and the rate of septicemia serum application to calves after birth was 69.1%. It was found that 33.8% of the farms had a calf mortality rate of 5%, with 48.3% of the deceased calves having a lifespan of 1-7 days, and 18.2% of the deceased calves' mothers also having lost other calves. Diarrhea was identified as a significant factor in calf mortality, and it can be suggested that increasing the use of prebiotics and probiotics, paying more attention to care, feeding, hygiene, and preventive health practices such as vaccinations, can reduce mortality rates. Another issue that can be considered as calf loss is the long service period. The extension of the service period, which is the time between giving birth and becoming pregnant again, results from various causes. Reducing the service period to optimal durations in farms depends on employing technical personnel, appropriate care and feeding practices, increasing heat detection applications and observation times, employing sufficient and qualified personnel, and implementing artificial insemination techniques. The results of this study suggest that enterprises involved in dairy cattle farming should take measures to reduce calf mortality rates and systematically monitor and control these rates through record keeping.

Keywords: Tekirdağ, Dairy cattle, Calf deaths, Holstein, Raw milk

1. Giriş

Ülkemizde mevcut büyükbaş hayvan varlığımızın ve verimliliğinin arttırılabilmesi için her bir inekten yılda bir buzağı alınması ve bu buzağının sağlıklı bir şekilde büyütülmesi, buzağı ölümlerinin azaltılması, yaşama gücünün arttırılması önem arz etmektedir. Bütün bunlar içinde buzağı ölüm sebeplerinin incelenip bunlara göre gerekli önlemler alınmalıdır. Buzağılarda yaşama gücü, belli bir döneme kadar (sütten kesime kadar, altı aylık yaşa kadar vb.) yaşayan buzağı sayısının canlı doğan buzağı sayısına bölünmesiyle elde edilir (Akçapınar ve Özbeyaz, 1999). Yeni doğan buzağıların sağlığını ve yaşama gücünü ırk, besleme, iklim, hastalıklar, barınak, hijyen, doğum tipi, cinsiyet gibi unsurlar etkili olmaktadır (Özyürek ve ark., 2013).

Buzağı yaşama gücünün ülkelere göre; İtalya %75, İngiltere %92.58-%97.53, ABD'de ise %93.60 oranında olduğu bildirilmiş, ülkemizdeki yaşama gücünün ise bölge ve işletmelere göre %85-90 arasında olduğu tespit edilmiştir (Anonim, 2017). Buna göre, 2019 yılı TÜİK verilerine göre yılda %10'luk bir buzağı kaybının ülke genelinde yaklaşık 464 bin buzağıyı, Tekirdağ ilinde ise 4.500 baş buzağıyı ifade ettiği ve bu kaybın %5 oranında azaltılması ile ülke genelinde 232 bin baş, Tekirdağ'da ise 2.250 baş buzağının ekonomiye geri kazandırılacağı belirtilmektedir (TÜİK, 2019).

Buzağı ölümleri genel olarak doğum öncesi buzağı kayıpları ve doğum sonrası buzağı kayıpları olarak iki gurupta değerlendirilmektedir. Doğum öncesi buzağı kayıpları döl tutma problemleri ve yavru atmalardan kaynaklanmaktadır. Doğum sonrası buzağı kayıpları ise genel olarak enfeksiyona bağlı (mikrobiyal, paraziter, viral) ve enfeksiyona bağlı olmayan (vitamin-mineral-element eksikliği, anomaliler) olarak iki başlıkta incelenebilir. Buzağı ölüm nedenleri ise gastrointestinal sistem hastalıkları, dolaşım-solunum sistemi hastalıkları, güç doğum, septisemi (kan zehirlenmesi), E vitamini ve selenyum eksiklikleri, yetersiz bakım ve besleme uygulamaları ile çevre koşullarından oluşmaktadır (Atlı ve ark., 2018). Yeni doğan buzağılarda ishal üzerine yapılan bir çalışmada toplamda %30 oranında Rotavirüs, %13 oranında Coronavirus, %17 oranında E. coli, %6 miksenfeksiyon, %33 oranında da diğer nedenlere bağlı buzağı ishalleri tespit edilmiştir (Al ve Balıkçı, 2012).

Yapılan bazı araştırmalarda son yıllarda buzağı ölümlerinde artış olduğu bildirilmiştir (Bleul, 2011; Bayram ve ark., 2016). Yapılan benzer bir çalışmada Şahal ve ark. (2018) Türkiye'de her yıl yaklaşık 900 bin ila 1 milyon buzağının öldüğünün tahmin edildiğini, sütçü ineklerin buzağılarında ölüm oranının dünya genelinde ortalama %10 ve Türkiye'de %15'in üzerinde olduğunu bildirmişlerdir. Günlü (2018), Türkiye'de 2018 yılı içerisinde 5.594.000 baş buzağı doğduğunu ve bu buzağıların yaklaşık %17,6'sının öldüğünü bildirmiştir.

Yapılan bu çalışmada, Tekirdağ ilinde süt sığırcılığı işletmelerinin mevcut yapısal durumlarının ortaya konması ve buzağı ölüm sebepleri ile ölüm düzeylerinin belirlenmesi amaçlanmıştır. Bu amaçla söz konusu işletmelerin yapısal özellikleri ile buzağılara uygulanan bakım besleme pratikleri ve buzağı ölümleri arasındaki ilişkiler değerlendirilmiştir.

2. Materyal ve Metot

Çalışma kapsamında; Tekirdağ ilinde halen üretime devam etmekte olan ve tamamıyla şansa bağlı seçilen 207 adet işletmede yüz yüze anket yapılmış ve elde edilen veriler kullanılmıştır. Özellikler arasındaki ilişkinin belirlenmesinde non-parametrik pearson χ^2 uyum testi yapılmış ve pearson korelasyon katsayısı (r) hesaplanmıştır. Araştırma verilerinin analizinde SPSS 18 paket programı kullanılmıştır (SPSS, 2018).

3. Araştırma Sonuçları ve Tartışma

Tekirdağ ilinde yürütülen anket verileri değerlendirildiğinde sağmal sayısına göre üreticilerin eğitim düzeyleri; ilkokul, ortaokul, lise ve üniversitesi mezuniyet oranlarının sırasıyla %71.5, %14, %12.1 ve %2.4 olduğu gözlenmiştir. Soyak (2006) tarafından Tekirdağ ilinde yapılan benzer bir çalışmada işletme sahiplerinin %59'unun ilkokul, %11'inin ortaokul, %15'inin lise, %14'ünün üniversite mezunu olduğu % 1' inin ise okuma yazma bilmediği belirlenmiştir. Edirne ilinde yapılan bir diğer çalışmada de ise işletme sahiplerinin %75.4 oranında ilkokul, %21.1 oranında ortaokul, %3.5 oranında lise mezunu olduğu belirtilmiştir. Bu çalışmada, işletmelerin sahip oldukları toplam hayvan sayısı değerlendirildiğinde işletmelerin %15.9'u 0-5 baş, %26.6'sı 6-10 baş, %31.4'ü 11-20 baş, %18.4'ü 21-30 baş, %7.7'si ise 30 veya daha fazla hayvan sayısına sahip oldukları tespit edilmiştir. Tekirdağ ilinde yapılmış benzer araştırmada işletmelerin %30'u 1-5 baş, %27'si 6-10 baş, %12'si 11-15 baş, %8'i 16-20 baş, %4'ü 21-25 baş, %4'ü 26-100 ve %1'i 101 baş ve hayvan varlığına sahip oldukları (Soyak, 2006), Edirne ilinde ise %26.3 oranında 11-20 baş, %33.3

oranında 21-30 baş, %28.1 oranında 31-50 baş, %10.5 oranında 51-100 baş hayvan varlığına sahip işletmeler olduğu bildirilmiştir (Önal ve Özder, 2008).

Sağmal sayısına göre gruplar incelendiğinde işletmenin ahır tipi bağlı duraklı olanların oranlarının 0-5 baş, 6-10 baş, 11-20 baş, 21-30 baş ve 31+baş için sırasıyla %96.6, %91.5, %58.2, %0 ve %0 olduğu belirlenmiştir, İşletmenin kullandığı havalandırma sistemine göre; doğal, mekanik ve havalandırma sistemi bulunmayanların oranlarının sırasıyla %86.5, %3.4 ve %10.1 olduğu gözlenmiştir. İşletmenin buzağılarla inekleri barındırma şekli aynı ahırda olanların oranları 0-5 başlık sağmala sahip işletmelerde %93.2 olduğu, 21-30 baş ile 31 ve üzeri baş sağmala sahip işletmelerin tümünde buzağı kulübesi bulunduğu tespit edilmiştir. Beş baş sağmal hayvana sahip işletmelerde döl ve süt verimi kayıtlarını tutma oranı %3.4 olduğu tespit edilmiştir. Yerli ırk, kültür ırkı, kültür melezi ırkı hayvan yetiştirilen işletmelerin oranlarının sırasıyla %0.5, %23.7 ve %75.8 olduğu belirlenmiştir. Kurç ve Kocaman (2016) tarafından Tekirdağ ilinde yapılmış çalışmada işletmelerin %40.32 bağlı duraklı, %35.48 serbest, %24.19 serbest duraklı olduğu belirtilmiştir.

Çalışmada kapsamında değerlendirilen tüm işletmelerde doğan her bir buzağıya ilk altı saat içerisinde kolostrum içerildiği ve işletmelerin %98.6'sında buzağıların mama yerine süt ile beslendiği tespit edilmiştir. İşletmelerin %59.9'unda buzağılar doğumdan iki hafta ve daha sonra yem ile beslenmeye başlandığı, %75.8'inde buzağıların altlıklarının her gün temizlendiği, yem katkı maddesi kullanan işletmelerin oranının %86 ve prebiyotik ve probiyotik kullanan işletmelerin oranının ise %12.6 olduğu belirlenmiştir. Gebelik sürecinde ineklere septisemi aşısı uygulayan işletmelerin oranının %45.9 olduğu tespit edilmiştir. Buzağılara septiserum uygulaması yapılan işletmelerin oranlarının 0-5 baş, 6-10 baş, 11-20 baş, 21-30 baş ve 31 ve üzeri baş sağmala hayvana sahip işletmeler için sırasıyla %48.9, %70.2, %90.9, %100 ve %100 olduğu tespit edilmiştir.

Yapılan çalışmada işletmelerden elde edilen buzağı ölüm oranlarına ait veriler *Tablo 1*'de verilmiştir.

Tablo 1. İşletmelerdeki buzağı ölüm oranları

Table 1. Calf mortality rates in enterprises

Buzağı ölüm oranları										
Sağmal Sayısı		**%5' ten az**	**% 5**	**% 10**	**%15**	**%20**	**% 30+**	**Toplam**	**p**	**r**
0-5 baş	N	21	4	9	23	1	30	88		
	%	*23,9*	*4,5*	*10,2*	*26,1*	*1,1*	*34,1*	*100*		
6-10 baş	N	15	6	3	4	0	19	47		
	%	*31,9*	*12,8*	*6,4*	*8,5*	*0*	*40,4*	*100*		
11-20 baş	N	21	16	4	3	0	11	55	**0.01**	**-0.34****
	%	*38,2*	*29,1*	*7,3*	*5,5*	*0*	*20*	*100*		
21-30 baş	N	3	4	0	0	0	0	7		
	%	*42,9*	*57,1*	*0*	*0*	*0*	*0*	*100*		
31 ve üzeri baş	N	10	0	0	0	0	0	10		
	%	*100*	*0*	*0*	*0*	*0*	*0*	*100*		
Toplam	N	70	30	16	30	1	60	207		
	%	*33,8*	*14,5*	*7,7*	*14,5*	*0.5*	*29*	*100*		

** (p≤0.01), *(p≤0.05)

Tablo 1 incelendiğinde, buzağı ölüm oranı %5'ten az olan işletmelerin oranları; 0-5 baş, 6-10 baş, 11-20 baş, 21-30 baş ve 31 ve üzeri baş için sırasıyla %23.9, %31.9, %38.2, %42.9 ve %100 olduğu gözlenmiştir. İşletmede; yavru atma (abort) vakası gözlenmeyen, bir vaka, iki ve daha fazla sayıda yavru atma vakası gözlenen işletmelerin oranları sırasıyla %93.2, %5.3 ve %1.4 olarak tespit edilmiştir (*Tablo 1*).

İshal hastalığına bağlı olarak bir ile üç adet ölüm vakası görülen işletmelerin oranları; 0-5 baş, 6-10 baş, 11-20 baş, 21-30 baş ile 31 ve üzeri baş için sırasıyla %68.2, %55.3, %58.2, %28.6 ve %30 olarak, diğer sebeplere bağlı olarak bir ile üç adet buzağı ölüm vakası görülen işletmelerin oranları ise 0-5 baş, 6-10 baş, 11-20 baş, 21-30 baş ve 31+baş için sırasıyla %13.6, %19.1, %30.9, %42.9 ve %50 olarak tespit edilmiştir.

Araştırmacılar buzağı ölüm oranlarını; Fransa'da %3.99-4.20 (Raboisson ve ark., 2013), İsveç'te %0.7-2.6 (Olsson ve ark., 1993), Danimarka'da %7 (Nielsen ve ark., 2010), İran'da %6.5 olduğu (Azizzadeh ve ark., 2012),

ABD'de %2.8-8.5 (Oxender ve ark., 1973) ve son yıllarda ABD'de yapılan başka bir çalışmada %4.6 olarak bildirilmiştir (Linden ve ark., 2009).

4. Sonuç

Çalışmada kapsamında, üreticilerin eğitim düzeyleri değerlendirildiğinde üreticilerin önemli düzeyde (%71.5) ilkokul mezuniyet seviyesine sahip oldukları tespit edilmiştir. Günlük üretilen süt verim ortalamasının 20 kg (%48.8) ve işletme tiplerinin yaygın olarak bağlı duraklı işletme tipinde olduğu belirlenmiştir (%77,3). İşletmelerde büyük oranda süt ve döl verim kayıtlarının tutulmadığı (%54.6) ve teknik personel istihdam etme veya danışmanlık hizmeti alan işletmelerin sayısının düşük düzeyde olduğu (%4.3) belirlenmiştir. İşletmelerin önemli bir kısmında yem katkı maddesi kullanıldığı (%86) belirlenmiştir. Çalışmada güç doğum görülen işletmelerin oranı %34.8 olarak belirlenirken, işletmelerin yarısından fazlasında (%59.4) ishale bağlı buzağı ölümlerinin görülmediği ve %1.4' ünde 4-6 baş arasında ölüm görüldüğü tespit edilmiştir. İşletmelerin büyük kısmında (%77.3) diğer sebeplere bağlı buzağı ölümleri görülmediği tespit edilmiştir. Bu çalışma ile Tekirdağ ilinde bulunan işletmelerde genel olarak işletme başına düşen hayvan sayısının düşük olduğu belirlenmiş olup karlı ve bilimsel ilkelere uygun bir hayvansal üretim için işletme başına düşen hayvan sayısının arttırılmasının özendirilmesi gerektiği ortaya çıkmıştır.

Kolostrum, yeni doğan dönemdeki buzağıların yaşamları için en önemli unsurdur. Kolostrum sayesinde antikorların inekten yavruya başarılı olarak aktarılması; yeni doğan buzağının olabildiğince erken, yeterli miktar ve kalitede, immunoglobulin açısından yeterli, düşük patojen içeren kolostruma bağlıdır (Koyuncu ve Karaca, 2018). İşletmede kayıt altına alınan veriler değerlendirilerek ayıklama, seleksiyon daha isabetli kararlar verilebileceği, benzer şekilde işletme içinde hijyen ve aşılama gibi sağlık uygulamalarına önem verilerek istem dışı ayıklamanın önüne geçilebileceği belirtilmiştir (Mundan ve Karabulut, 2008).

Yapılan çalışmada buzağı ölümlerinde ishalin önemli bir yer tuttuğu belirlenmiş olup prebiyotik ve probiyotik kullanımının arttırılması, bakım, besleme, hijyen ve aşılamalara daha fazla özen gösterilmesi ile ölüm oranlarının azaltılabilmesinin mümkün olabileceği belirlenmiştir. Ayrıca solunum sistemi hastalıkları ve anomalilerin de buzağılarda önemli düzeyde ölümlere sebep olan faktörlerden olduğu tespit edilmiştir. Solunum sistemi hastalıklarının önlenmesi amacıyla işletmelerde, ahır içine sürekli temiz hava girişi ve kirli hava çıkışının sağlanması gerekmektedir. Anomalilerin önlenmesinde ise, tohumlamada kullanılan boğaların genetik incelemesinin yapılması ve sağlık problemi olan boğaların damızlıkta kullanılmaması konusuna dikkat edilmesi alınabilecek tedbirlerdendir.

Genel olarak buzağı ölümlerinin azaltılmasında doğum ünitelerinin olması, imkan olmaması halinde doğumun gerçekleşeceği alanın hijyen koşullarına önem verilmesi gerekmektedir. Doğumdan hemen sonra buzağının göbek kordonunun dezenfekte edilmesi, buzağıların ineklerle aynı ahırda bulundurulmaması ve buzağı altlıklarının her gün temizlenerek yenilenmesi alınacak önemli önlemlerdendir. Buzağı kulübelerinin temiz suluk ve yemliklerinin olması ve mümkünse de yemliklerin üstlerinin yağmurdan ıslanıp küflenmemesi için kapalı olması gerekmektedir. Buzağıların parazitlerden korunması için gerekli uygulamaların yapılması, vitamin-mineral eksikliklerine bağlı sorunların oluşmasını önlemek amacıyla yem katkı maddesi kullanılarak rasyonların dengelenmesi sağlık ve büyüme performansı bakımında ayrıca önemlidir. Güç doğumların azaltılması için ineklerin kuruya çıkartılmasına ve kuru dönemde beslenmesine dikkat edilmeli, güç doğumun sık görüldüğü işletmelerde doğuma yardımcı araç ve gereçlerin bulundurulması sağlanmalıdır.

Etik Kurul Onayı

Çalışma Yüksek Lisans Tezinden derlenmiş olup Tez Çalışma Planında Etik Kurul Onayına ihtiyaç duyulmadığına karar verilmiştir.

Çıkar Çatışması Beyanı

Makale yazarları olarak aramızda herhangi bir çıkar çatışması olmadığını beyan ederiz.

Yazarlık Katkı Beyanı

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