International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946

Year: 2025 • Volume: 9 • Issue: 1

INTERNATIONAL JOURNAL OF AGRICULTURE, ENVIRONMENT AND FOOD SCIENCES

e-ISSN: 2618-5946

https://dergipark.org.tr/jaefs https://www.jaefs.com

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Journal Name International Journal of Agriculture, Environment and Food Sciences

Web Page https://dergipark.org.tr/jaefs - http://jaefs.com/

Abbreviation Int. J. Agric. Environ. Food Sci.

Subjects Agriculture, Environment and Food Sciences

e-ISSN 2618-5946 **DOI** 10.31015

Publisher Gültekin Özdemir

Dicle University Faculty of Agriculture Department of Horticulture,

Diyarbakir, Türkiye

E-mail: gozdemir@dicle.edu.tr

Publishing Service Edit Publishing

Dicle Teknokent Yiğit Çavuş Mah. Silvan Yolu Üzeri Kat:2 No:26,

Diyarbakır, Türkiye

WhatsApp: +90 850 309 59 27 E-mail: info@editpublishing.com E-mail: jaefseditor@gmail.com Webpage: https://editpublishing.com/

Language English

Frequency 4 Issues Per Year (March, June, September, December)

Type of Publication International, Scientific, Open Access, Double-blinded peer-review, Widely distributed

periodical.

Aims and Scope JAEFS publishes high-quality original research articles that feature innovative or

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Price Policy Editorial Processing Charge(EPC) are paid by authors or their institution.

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Indexed and Abstracted in

TÜBİTAK ULAKBİM TR Dizin, AGORA (Access to Global Online Research in Agriculture), AGRIS (Agricultural Science and Technology Information), EBSCO, WorldCat, SOBIAD, Scilit, ROAD (Directory of Open Access Scholarly Resources), Neliti, International Citation Index, ROOT Indexing, ResearchBib, Index Copernicus International, ESJI, JournalTOCs, TEELS, ResearchGate, Microsoft Academic,

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Manuscript Submission and Tracking System JAEFS uses the submission system of Tübitak Ulakbim DergiPark Akademik Open

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International Journal of

Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.1

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 1-12

Determination of the effects of different sowing times and densities under II. product conditions on the yield and components of maize varieties belonging to two different maturity groups

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Article History

Received: December 3, 2024 Revised: January 20, 2025 Accepted: January 26, 2025 Published Online: January 30, 2025

Article Info

Article Type: Research Article Article Subject: Agronomy, Cereals and Legumes

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1595602

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Abstract

This study was conducted using maize (Zea mays L.) varieties from two different maturity groups (DKC5747 (FAO 500) and PR31P41 (FAO 650)) under IIproduct conditions. The effects of different sowing times (June 1, June 20, July 10) and planting densities (800, 900, 1000, and 1100 plants per hectare) on these varieties' yield and yield components were investigated. The objective was to determine the optimal maturity group, sowing time, and planting density for achieving the highest yield in maize (Zea mays L.). The features analyzed included plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), single corn grain weight (mg/corn grain), and grain yield per cob (g/cob). The results showed that sowing time significantly influenced all features examined. The highest values for cob length, weight, corn grain number per cob, and grain yield per cob were obtained at the second sowing time (June 20), while the lowest values were recorded at the third sowing time (July 10). The optimal planting density was determined to be 900-1100 plants/ha for the DKC5747 variety and 800 plants/ha for the PR31P41 variety. For the Amik Plain, the most suitable sowing time for second-crop maize cultivation was June 20, with an optimal planting density of 900-1100 plants/da. The most appropriate varieties were those in the short maturity group. In maize cultivation as a second crop under the conditions of the Amik Plain in Hatay, it is crucial to prioritize the selection of maize varieties in the short maturity group (FAO 500 maturity group) to achieve high grain yields. When longer maturity varieties are selected, it should be noted that while the plants may produce higher biomass, their capacity to convert dry matter into grain may be insufficient, potentially leading to reduced

Keywords: Planting time, Planting density, Maturity group

Cite this article as: Kirac, N., Tiryakioglu, M. (2025). Determination of the effects of different sowing times and densities under 11. product conditions on the yield and components of maize varieties belonging to two different maturity groups. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 1-12. https://doi.org/10.31015/2025.1.1

INTRODUCTION

Maize is among the most widely cultivated crops globally in terms of cultivation area, production, usage, and trade. Corn is widely used both as human food and animal feed (silage, green feed, and concentrated feed) because it grows quickly and produces plenty of grain and stalk in a short time (Kara, 2022). Considering the continuous increase in the global population and the reduction in arable land, enhancing the productivity of agricultural lands and utilizing these crops more efficiently is crucial for meeting human food needs and animal feed requirements. As a C4 plant, maize efficiently utilizes solar energy. It produces the highest dry matter per unit area, making it one of the leading crops capable of achieving high yields from shrinking agricultural areas (Yılmaz, 2016).

Maize (Zea mays L.), commonly grown in Turkey and worldwide, belongs to the Maydeae subfamily of the Poaceae (Gramineae) family. Its high adaptability allows it to be cultivated in various regions of the world (Yorgancılar et al., 2019). Maize is a warm-season cereal requiring an 8-10 °C germination temperature, with optimal germination occurring above 18 °C. The ideal temperature range for maize growth is 25–30 °C, while temperatures below 15 °C slow initial growth, resulting in yield losses (Sönmez, 2019).

In maize cultivation, sowing time plays a critical role in realizing the yield potential of a variety, as temperature fluctuations significantly impact maize growth. Variations in sowing time alter climatic factors such as temperature, light intensity, and humidity during the plant's different growth and development stages. These climatic variations affect dry matter assimilation and growth parameters, consequently influencing grain yield. Changes in sowing time can expose plants to harmful high or low temperatures during critical periods of development (e.g., seedling and grain filling stages), leading to yield reductions (Sönmez et al., 2013). For this reason, determining the optimal sowing time based on the maturity group (cobly or late varieties) is vital for achieving the highest yields in maize cultivation.

Plant density, another cultivation technique, significantly affects grain yield by influencing the living space available to each plant. Increased plant density can reduce grain yield due to limited living space per plant. In high-density plantings, plants may not receive sufficient sunlight, notably lower leaves, which remain shaded for extended periods. This shading adversely affects CO₂ assimilation—a key determinant of photosynthesis—and ultimately reduces plant yield (Jia, 2018). Determining the optimal seed rate per unit area ensures that plants efficiently utilize available soil water, nutrients, and sunlight energy (Jia, 2018). Several studies have demonstrated that row spacing significantly impacts maize yield, with excessive density and sparseness limiting yield (Bruns et al., 2012; Eskandarnejad et al., 2013; Jia, 2018).

This study aims to evaluate the combined effects of maturity group, sowing time, and plant density to achieve the highest yields in maize under second cropping conditions.

MATERIALS AND METHODS

This research was conducted in 2017 under the ecological conditions of the Amik Plain, using two dent corn varieties with different maturity durations: DKC5747 (FAO 500) and PR31P41 (FAO 650).

ClimaticCharacteristics

The climatic data for the trial cob and long-term averages are presented in Table 1. According to long-term average data, no rainfall occurred in the trial area during June–September. The precipitation for October in the trial cob and the long-term average were 24.6 mm and 29.5 mm, respectively. The average temperatures for the June–September period were 26.6 °C for the trial cob and 25 °C for the long-term average. Relative humidity for the same period was 57.1% in the trial cob and 62.1% in the long-term average (Table 1).

In the trial cob, average temperatures were consistently higher than the long-term averages except for August. Total relative humidity in October during the trial cob exceeded the long-term average.

Table 1. Climatic data for the trial cob and long-te

Climate data	C-l-			Months		Averages
Climate data	Cobs	June	July	August	September October	
Average temperature (°C)	2016	26.4	30.1	24.4	29.3 22.7	26.6
	long years (1940-2016)	25.2	27.7	25.8	28.8 21.4	25.8
Total humidity (%)	2016	54.4	49.6	61.3	57.1 62.8	57.1
	long years (1940-2016)	63.2	64.0	63.2	61.0 59.2	62.1
Precipitation amount (mm)	2016	0	0	0	0 24.6	
	long years (1940-2016)	1.5	0.1	0.1	9.9 29.5	

^{*} MGM Hatay İl Müdürlüğü, 2016

Soil Characteristics

The soil of the experimental area is essential (pH: 8.22), has a high lime content (23.42%), has a low organic matter content (1.39%), and is clayey (59%).

Establishment of the Study

The study was conducted at the Tel-Kaliş Rescobch and Application Center of the Department of Field Crops, Faculty of Agriculture, Hatay Mustafa Kemal University. It was arranged using a split-split plot design in randomized blocks with three replications. Sowing time was assigned to the main plots, sub-plot varieties, and sub-sub-plot plant densities.

The first sowing date was June 1, followed by subsequent sowings on June 20 and July 10. Each plot consisted of five rows, each 10 meters long. Planting was performed with a planting frame at densities of 800, 900, 1000, and 1100 plants per hectare.

At sowing, 15-15-15 compound fertilizer was applied to provide 8 kg/da of nitrogen (N), phosphorus (P₂O₅), and potassium (K₂O). Thinning was carried out when plants reached the three-leaf stage.

When plants were at the five-leaf stage, urea fertilizer equivalent to 20 kg N/da was applied as a top dressing and incorporated into the soil using a ridging hoe. Harvesting was performed manually by collecting cobs from the middle three rows of each plot.

The maize varieties used in the study were measured for the following features: plant height (cm), cob length (cm), cob weight (g), corn grain number per cob (corn grains/cob), single corn grain weight (mg/corn grain), and grain yield per cob (g/cob).

Table 2. Abbreviations used in the study and their equivalents.

Abbreviations	Meanings	Abbreviations	Meanings
DKC5747	Dekalb	PH	Plant Height
PR31P41	Pioneer	\mathbf{CL}	Cob Length
EZ-1	1. Planting Time (1st June)	CW	Cob Weight
EZ-2	2. Planting Time (20th June)	CGNPC	Corn Grain Number Per Cob
EZ-3	3. Planting Time (10th July)	CCGY	Cob Corn Grain Yield
S1	Plant Density (800 plants per hectare)	SCKW	Single Corn Grain Weight
S2	Plant Density (900 plants per hectare)	DF	Degree of Freedom
S3	Plant Density (1000 plants per hectare)	SV	Source of Variation
S4	Plant Density (1100 plants per hectare)		

Statistical Analysis of Data

The study's data were analyzed using the MSTAT-C statistical program. The analysis of variance (ANOVA) was performed according to the split-plot design in randomized blocks, and mean comparisons were carried out using the Least Significant Difference (LSD) test.

RESULTS

Table 3 presents the combined analysis of variance results for the yield and some yield components of maize (*Zea mays* L.) varieties from different maturity groups under second-crop conditions, based on sowing time.

Table 3. Results of combined variance analyses for yield and some yield components of varieties based on planting time.

Source of Variation	DF	Characteristics					
Source of variation	Dr	PH	CL	CW	CGNPC	CCGY	SCKW
Replication	2	772,3	2,9	3,0	420,3	61,4	0.70
Planting Time (A)	2	4572,75*	28,0*	5965,1***	97548,6***	14760,5**	7344,7***
Error-1	4	1004,7	4,6	23,7	1458,3	396,1	12,5
Variety (B)	1	42,0	0,3	5848,2***	169168,1***	630,1*	4960,1***
AxB	2	636,4	44,8***	1468,7***	4321,5**	2814,3**	431,1***
Error-2	6	659,5	7,3	17,6	809,7	303,5	15,7
Plant Density (C)	3	234,8	33,0***	1092,4***	53970,8***	1654,4*	2008,3***
AxC	6	334,5	68,8***	1416,9***	39802,6***	2049,9	1476,6***
BxC	3	346,3	31,3***	3771,1***	41589,4***	6430,3***	3332.0***
AxBxC	6	201,5	45.8***	998,8***	10108,4	857,5	220,9***
General Error	36	3044,2	37,8	116,3	735,9	5646,7	88,8
SV		5,9	10,0	2,5	8,3	7,8	3,0

^{***):} p≤0,0001; **): p≤0,001 ve *): p≤0,05

When examining the effects of different sowing times, variety, and plant density factors, as well as their interactions, on some plant characteristics (plant height, cob length, cob weight, corn grain number per cob, single corn grain weight, and grain yield per cob), it was found that plant height showed statistically significant results only for sowing time, with no significant statistical differences for other factors. Among all the examined features, cob length showed no significant interaction with the variety factor, and single corn grain weight did not exhibit significant results with the interactions of sowing time x density and sowing time x variety x density. For all other features except the plant's characteristics mentioned above, all factors and their interactions were found to be statistically significant.

In the second-crop conditions, the average values for plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), single corn grain weight (mg/corn grain), and grain yield per cob (g/cob) of maize (*Zea mays* L.) varieties from different maturity groups, under different sowing times and varying plant densities, as well as the groups formed by the Least Significant Difference (LSD) comparison test, are presented in Table 4.

Table 4. Average values of plant height (cm), cob length (cm), cob weight (g/cob), number of corn grains per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) for two different maize varieties from different maturity groups, at different planting times and varying plant densities, and the groups formed according to the LSD comparison test.

Application / Investigated Propert	PH	CL	CW	CGNPC	EKY	SCKW
Application / Investigated Propert	(cm)	(cm)	(g/cob)	(corn grains/	cob) (mg/corn g	rain) (g/cob)
Planting Time (A)						
June 1	167 a	14,1 b	62,7 c	321 b	149 b	48,8 b
June 20	149 b	15,6 a	84,1 a	373 a	181 a	67,2 a
July 10	152 b	14,6 b	67,9 b	283 с	153 b	43,7 c
Variety (B)						
DKC 5747	155	14,7	80,6 a	374 a	164	61,5 a
PR31P41	175	14,8	62,6 b	277 b	158	44,9 b
Plant Density (C)						
800	154	15,9 a	78,2 a	372 a	168 a	62,0 a
900	158	14,6 b	70,7 b	319 b	163 ab	52,6 b
1000	157	14,2 b	68,8 c	312 b	157 b	50,1 c
1100	154	14,3 b	68,6 c	300 b	156 b	48,2 d

Regarding plant height, the tallest plants were found in the first sowing time (June 1) with a height of 167 cm, while the shortest plants were found in the second sowing time (June 20) with a height of 149 cm. When analyzed by variety, the DKC 5747 variety had a plant height of 155 cm, 20 cm shorter than the PR31P41 variety (175 cm); however, this difference was not statistically significant. Regarding plant density, plant heights were found to be 154 cm for 800 plants/hectare, 158 cm for 900 plants/hectare, 157 cm for 1000 plants/hectare, and 154 cm for 1100 plants/hectare, with these differences being statistically insignificant.

For cob length, the plants sown on June 20 (second sowing time) had the longest cobs (15.6 cm), while those sown on July 10 (third sowing time) had intermediate lengths (14.6 cm), and those sown on June 1 (first sowing time) had the shortest cobs (14.1 cm). The cob length for DKC 5747 was 14.7 cm, while for PR31P41, it was 14.8 cm, with both varieties having statistically insignificant and similar values. In terms of plant density, the cob length was longest (15.9 cm) for 800 plants/hectare, while the lengths for 900, 1000, and 1100 plants/hectare were 14.6 cm, 14.2 cm, and 14.3 cm, respectively, and these differences were statistically insignificant.

Cob weight was highest for the plants sown on June 20 (84.1 g) and lowest for those sown on June 1 (62.7 g). The DKC 5747 variety had a cob weight of 80.6 g, significantly higher than the PR31P41 variety, which had a cob weight of 62.6 g. Regarding plant density, the cob weight was highest for 800 plants/hectare (78.2 g), followed by 70.7 g for 900 plants/hectare, and the lowest values of 68.8 g and 68.6 g were found for 1000 and 1100 plants/hectare, respectively. However, the differences between 1000 and 1100 plants/hectare were statistically insignificant.

For corn grain number per cob, the highest value (373 corn grains) was found in the plants sown on June 20 (second sowing time), while the lowest value (283 corn grains) was found in the plants sown on July 10 (third sowing time). The DKC 5747 variety had 374 corn grains per cob, while the PR31P41 variety had 277 corn grains per cob, which is statistically significant. The highest corn grain number was observed in plants sown at 800 plants/hectare (372 corn grains), while plants sown at 900, 1000, and 1100 plants/hectare had 319, 312, and 300 corn grains, respectively, and the differences between these three densities were statistically insignificant.

Regarding single corn grain weight, the highest value (181 mg) was found for plants sown on June 20, while the lowest value (149 mg) was found for those sown on June 1. The single corn grain weight for plants sown on July 10 (153 mg) fell between the two sowing times. The values for DKC 5747 (164 mg) and PR31P41 (158 mg) were very similar, and the difference was not statistically significant. For plant density, the highest single corn grain weight (168 mg) was observed for 800 plants/hectare, with 900 plants/hectare yielding 163 mg, 1000 plants/hectare yielding 157 mg, and 1100 plants/hectare yielding 156 mg.

For grain yield per cob, the highest value (67.2 g) was observed for the plants sown on June 20, while the lowest value (43.7 g) was found for those sown on July 10. The DKC 5747 variety (61.5 g/cob) had a higher grain

yield per cob than the PR31P41 variety (44.9 g/cob). As plant density increased, grain yield per cob decreased regularly, with the highest yield (62 g/cob) observed at 800 plants/hectare, followed by 52.6 g, 50.1 g, and 48.2 g for 900, 1000, and 1100 plants/hectare, respectively.

DISCUSSION

The values of plant height (cm), cob length (cm), cob weight (g/cob), number of corn grains per cob (corn grains/cob), corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) of maize varieties planted under second crop conditions and belonging to different maturity groups, based on different planting times, are presented in Figure 1.

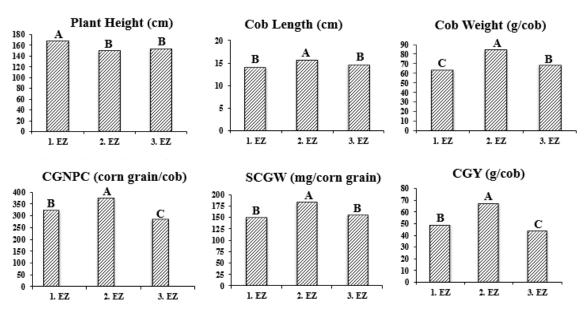


Figure 1. Average values of plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) for two different corn varieties at different sowing times, along with the groups formed according to the LSD comparison test.

Planting time is an essential factor in determining the yield potential of a maize variety. To obtain the highest yield level from the cultivated plants, the planting time should be selected in which the environmental factors such as climate, soil, and temperature required by the plants are ideal. The study also shows that planting time affects plant characteristics (see Table 1). Early or late planting hinders the potential of the plants and reduces yield. Among the plant characteristics examined in this study, plant height reached the best values at the first planting time, while all other characteristics performed best at the second planting time. It is thought that this is because the plants at the first planting time use most of their energy for height growth and thus will have less energy available for cob formation and development, leading to a reduction in cob length, cob weight, corn grain number per cob, corn grain weight, and consequently cob corn grain yield. Güney et al. (2010), Kuşvuran et al. (2015), Seydosoğlu and Saruhan (2017) reported that environmental changes caused by different ecological conditions resulted in significant differences in plant height, similar to the results of our study. Gürses (2010), Kuşvuran et al. (2014), Cakar (2015), Han (2016), Saygı et al. (2017), Doğanlar (2018), and Bueno and Lima (2020) found that, although cob length is a genetic feature, it shows variability depending on environmental factors and the period it is grown (planting time). Additionally, similar to the results of this study, many studies have reported that cob weight changes with planting time (Cesurer, 1995; Sencar et al., 1997; Sönmez et al., 2013; Özlem et al., 2011) and that there is a relationship between planting time and corn grain number per cob (Alan et al., 2011). In addition, while Sönmez et al., (2013) stated that there is a relationship between planting time and yield, contrary to the study results, there are also studies indicating that there is no difference in yield per decare between planting times (Cesurer, 1995; Sencar et al., 1997).

The values of plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) of maize varieties planted under second crop conditions and belonging to different maturity groups, based on different planting densities, are presented in Figure 2.

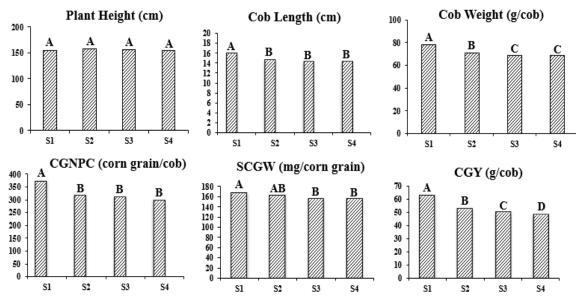


Figure 2. Average values of plant height (cm), cob length (cm), cob weight (g/cob), number of corn grains per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) for two different corn varieties at different planting densities, along with the groups formed according to the LSD comparison test.

Plant density is one of the important factors affecting yield, particularly in reducing or increasing the living space per plant. An increase in plant density reduces the living space per plant, decreasing yield. In dense plantings, the inability of the plants to fully utilize sunlight, especially the extended duration during which the lower leaves remain shaded, negatively affects CO₂ assimilation, one of the critical determinants of photosynthesis, and consequently reduces plant yield (Jia, 2018). Determining the amount of seed to be sown per decare ensures that plants make the most efficient use of favorable water, nutrients, and light energy in the soil. The data from this study support these facts, showing a decrease in all the plant characteristics examined as plant density increased (see Table 2).

An increase in planting density is expected to cause competition among plants and result in taller plants in dense plantings. However, in this study, plant height values did not show statistically significant differences or a consistent trend (see Table 4). This situation is thought to have been caused by the fact that the densest plant plots were directly exposed to the wind, as the density variation in the experimental design changed from east to west while the wind direction was from west to east. During the plant's vegetative period, wind speed ranged from 5 to 10 km/h and continued at similar speeds throughout the day. Many studies have presented similar and different results. Sönmez et. Al., (2013) noted that planting density had no significant effect on plant height, supporting the findings of this study, while Gözübenli et al. (2004), Pagano and Maddonni (2007), Bukhsh et al. (2008), and Yılmaz et al. (2008) reported longer and statistically significant plant heights at higher planting densities. As mentioned earlier, cob length is related to plant height, and changes in plant height affect cob length. Although no statistically significant differences were found in plant heights due to planting densities in this study, values for cob length, cob weight, corn grain number per cob, corn grain weight, and cob corn grain yield showed a consistent decrease in dense plantings (see Table 4). Dense plantings cause plants to shade each other, preventing them from utilizing sunlight effectively, impairing their photosynthetic activity, and negatively affecting cob characteristics, leading to decreased plant productivity. The study's results support these findings, as decreases in all cob characteristics were observed with increased planting density (see Table 2).

At the same time, there are many studies indicating that increases in planting density cause a decrease in ear weight (Şirikçi, 2006), grain number per ear (Öktem et al., 2001; Alıcı, 2005; Yılmaz et al., 2005) and ear grain yield (Andrade et al., 2002; Widdicombe and Thelen, 2002; Alıcı, 2005; Liu et al., 2004; Stahl and Bau, 2009; Bruns et al., 2012; Eskandarnejad et al., 2013).

The values for plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) of maize varieties planted under second crop conditions and belonging to different maturity groups, based on planting time x variety interactions, are presented in Figure 3.

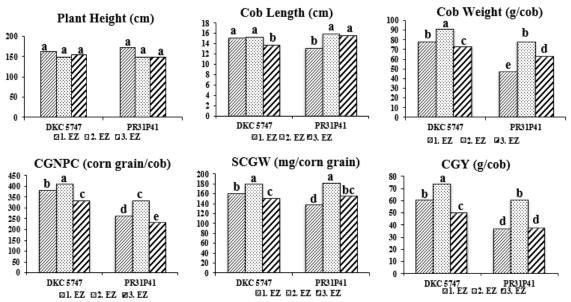


Figure 3. Average values of plant height (cm), cob length (cm), cob weight (g/cob), number of corn grains per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) for two different corn varieties at different planting times x variety interaction, along with the groups formed according to the LSD comparison test.

In second-crop conditions, the maturity groups of maize varieties and planting time are critical. Maturity groups define the speed of maturation and development stages of the plant from planting to harvest. Therefore, in second-crop conditions, varieties that mature and develop faster, particularly under late planting conditions, are preferred. This study used two varieties from both long and short-maturation groups, and their responses to changes in planting time were evaluated. Overall, the highest values were obtained from the DKC5747 variety, which belongs to the short maturity group, and from the second planting time (June 20). The PR31P41 variety, which belongs to the long maturity group, could not adapt to changing environmental factors (temperature, sunlight duration, etc.) during its development and yielded lower results than the variety in the short maturity group. Contrary to expectations, the plant height feature did not show statistically significant differences against the variety x density interaction

In the study, cob lengths, like plant heights, showed values almost close to each other. The DKC 5747 variety in the early maturation group exhibited lower values as the planting time was delayed, and the PR31P41 variety in the long maturation group exhibited lower values at early planting time.

Although the cob length values were quite similar regarding planting time x variety interactions, cob weight, corn grain number per cob, corn grain weight, and cob corn grain yield values were different from cob length and showed similar results. The highest results in cob weight, number of grains on the cob, and cob grain yield values were obtained from the DKC 5747 variety in the early maturity group and from the 2nd planting times. In contrast, unlike the mentioned characteristics, the single-grain weight values were statistically the same and had the highest values in both varieties.

This is because cob length is directly related to corn grain yield in maize. Although the cob length values for varieties were broadly similar according to planting time, it is thought that the PR31P41 variety from the late maturity group was unable to complete its vegetative development during the late planting times, causing the cobs to fail to set corn grains, resulting in lower values for cob weight, corn grain number per cob, and cob corn grain yield. It is thought that the reason why single grain weight reaches the same level of importance, especially at the 2nd sowing time and in both varieties, is this feature. However, it varies depending on the varieties, environmental conditions, and genetic factors, allowing the small number of grains in the cob to access more nutrients and water, thus increasing the single grain weight.

Supporting our findings, many studies have shown that comply-maturing varieties and comply planting times in second-crop conditions have a positive impact on cob length (Jia, 2018), cob weight (Akgün et al., 2017), corn grain number per cob, and cob corn grain yield (Jia, 2018).

The values for plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) of maize varieties planted under second crop conditions and belonging to different maturity groups, based on planting time x density interactions, are presented in Figure 4.

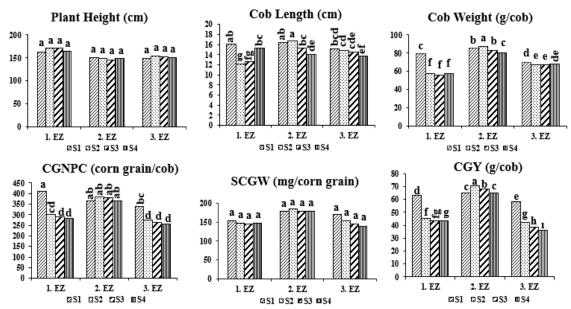


Figure 4. Average values of plant height (cm), cob length (cm), cob weight (g/cob), number of corn grains per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) for two different corn varieties at different planting times x variety interaction, along with the groups formed according to the LSD comparison test.

In the study, except for the corn grain number per cob, the highest values for all the features were obtained from the second planting time and the 800-900 plants per hectare planting densities. The interaction between planting time and density is critical in managing plant competition and determining the optimal planting time. Combining planting time and appropriate planting density is crucial for better plant development and higher yields.

The study observed no significant difference in plant height between the planting time and density treatments. Although many studies (Kara and Kırtok, 2006) have reported significant differences in plant height due to varying planting times and densities, the results of this study align with other research that reports no significant differences in plant height based on these factors (Güler, 2001).

Statistically significant differences were observed in cob length, which varied based on the interaction between planting time and density. Particularly, at the second planting time and with a planting density of 900 plants per hectare, the highest cob length was achieved, while the lowest values were found at the third planting time and with 1100 plants per hectare. These results support the idea that increased competition at higher planting densities reduces nutrient access, negatively impacting cob development.

Ear weight showed significant changes depending on planting time and density, and especially in the second planting time and S1 (900 plants per hectare), higher ear weight was achieved, while in the first planting time and S3 (1000 plants per hectare) and S4 (1100 plants per hectare) planting densities, ear weight generally decreased. These findings align with studies suggesting that the timing and appropriate planting density are critical factors influencing cob weight (Atasever, 2018; Kılınç, 2018).

The corn grain number per cob reached its highest value at the first planting time and the S1 density, but the values for the other densities at this planting time were much lower than the other planting times. No statistical differences were found between planting densities at the second planting time, and the values at the third planting time, except for the S1 density, were the lowest in the study. This situation is consistent with the literature indicating that competition between plants can reduce grain number, and lower densities can increase this parameter (Bozkurt and Karadoğan, 2017). Furthermore, the general decrease in corn grain number as planting time advances emphasizes the importance of planting time on yield.

No significant differences were observed for single corn grain weight based on planting time and density combinations, similar to the results for plant height. Corn grain weight showed similar results across all planting times and densities.

Cob corn grain yield showed significant changes due to the interaction between planting time and density, with the highest values obtained at the second planting time and the S2 density. This result was consistent with the values of cob weight and cob grain number and was similar to studies suggesting that more appropriate planting time and density could increase cob grain yield (Özlem et al., 2011; Sönmez et al., 2013).

The values for plant height (cm), cob length (cm), cob weight (g/cob), corn grain number per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) of maize varieties planted under second-crop conditions and belonging to different maturity groups, based on variety x density interactions, are presented in Figure 5.

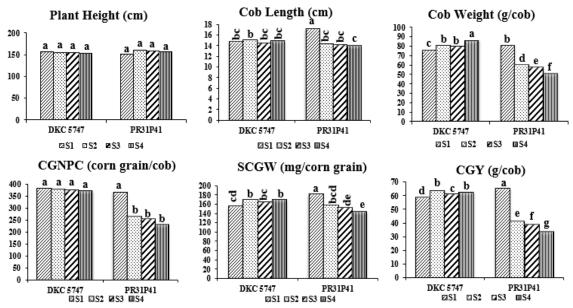


Figure 5. Average values of plant height (cm), cob length (cm), cob weight (g/cob), number of corn grains per cob (corn grains/cob), single corn grain weight (mg/corn grain), and cob corn grain yield (g/cob) for two different corn varieties at different variety x planting density interaction, along with the groups formed according to the LSD comparison test.

In the study, when the variety x density combination values were examined, the highest results were obtained from S1 planting density based on frequency. In contrast, contrary to expectations, the PR31P41 variety, which is in the long maturity group, showed the highest results. The reasons for this situation are,

- Although the PR31P41 variety is in the long maturity group, it may have a genetically high yield potential,
- Since plants generally have a longer growth period in long maturity groups, the photosynthetic activity periods of these varieties are longer. This may result in the PR31P41 variety accumulating more carbon over a longer period and directing it to the grain-filling process,
- The PR31P41 variety may be more resistant to stress by using water and nutrient resources per plant more efficiently under low-density conditions,
- The PR31P41 variety may have adapted better to the environmental conditions (soil structure, climate, water management) under which the study was conducted than the DKC 5747 variety,
- At low density (S1), air circulation and microclimate conditions between plants are improved. This may contribute to the better development of varieties with long growth periods, especially the long maturity group,

Although plant heights did not reveal statistically significant results, cob length, cob weight, number of grains per cob, single grain weight, and cob grain yield values exhibited compatible results.

A regular decrease in cob length was observed with increasing density, especially in the PR31P41 variety, and this was associated with increased competition between plants. Similar to the study results, the literature reports that cob length decreases with increasing density (Özata et al., 2016). While cob grain number, single grain weight, and cob grain yield values also show results compatible with cob length, cob weight showed the highest results in DKC 5747 variety and S4 density. This situation is thought to be because plants can produce heavier cobs with more resource use at lower density levels. At low density, plants have a larger root area and receive more nutrients and water, which can increase cob weight.

CONCLUSION

A study conducted on second-crop maize cultivation in the Amik Plain has thoroughly examined the effects of agricultural practices such as sowing time, planting density, and variety selection on yield, providing significant findings. The study demonstrated that sowing time and planting density created statistically significant differences in traits such as plant height, ear length, ear weight, number of grains per ear, and grain yield. However, the effects of maize varieties on plant height and ear length were statistically limited, whereas they played a significant role in all other traits. This highlights the importance of variety selection, particularly regarding critical production parameters such as grain yield.

According to the findings, using varieties from the short maturity group (FAO 500 maturity group) is a fundamental requirement for achieving high grain yield in second-crop maize production. These varieties converted the produced dry matter into grain more effectively than those with extended maturity periods. In varieties with longer maturity periods, although plants produce high biomass, significant losses in grain yield were

observed due to insufficient biomass conversion into grain. This indicates that short-maturity group varieties are more suitable for second-crop maize cultivation, where the harvest period is limited.

The most suitable sowing date for the region was determined to be around June 20. Sowing at this time ensures that plants benefit from optimal environmental conditions during their growth and development stages, thus achieving high yield potential. Later sowing dates could negatively impact yield due to shortened growth periods and low temperatures encountered during harvest.

Planting density was identified as another critical factor influencing yield. The study concluded that a density of 90,000 to 110,000 plants per hectare, combined with short maturity group varieties, is optimal for achieving high grain yield. Lower or higher planting densities could reduce yield due to increased competition or insufficient growing space.

In conclusion, the following recommendations can be made to producers who aim to cultivate second-crop maize in the Amik Plain:

- Varieties belonging to the short maturity group (FAO 500 maturity group) that are compatible with the region's climate and soil conditions should be preferred.
 - Sowing should be done around June 20 to optimize plant development and yield potential.
 - Planting density should be carefully managed, with a target of 90,000 to 110,000 plants per hectare.

These practices will significantly improve the efficient use of regional resources and productivity, thereby increasing farmers' income levels. This study's findings also serve as a valuable guide for developing region-specific agricultural management strategies.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

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

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.2

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 13-21

Morpho-physiological and water use performance of soybean cultivars under drought stress at early growth stages

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Article History

Received: November 08, 2024 Revised: December 11, 2024 Accepted: December 14, 2024 Published Online: January 30, 2025

Article Info

Article Type: Research Article Article Subject: Industrial Crops

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1581614

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Abstract

Drought is an important environmental stress for soybean (Glycine max (L) Merr.), which frequently occurs under second-crop conditions in the Mediterranean region of Türkiye and negatively affects early plant growth. In this study, we investigated the effects of drought stress (soil water content maintained at a constant 50% field capacity) on the early growth stage (V₃ stage) of different soybean cultivars (Ataem-7, BATEM Erensoy, Göksoy, and Lider). Twentyseven-day-old soybean plants were exposed to drought stress for 20 days. Morphological (plant height, root length, seedling fresh and dry weight, root fresh and dry weight, and leaf area), physiological (leaf temperature, chlorophyll rate (CR), leaf relative water content (RWC), and electrolyte leakage (EL)), and water use (total water consumption (TWC), and water use efficiency (WUE)) traits were assessed. The results revealed a significant decrease in plant height, root length, leaf area, root and shoot fresh and dry weights, and RWC, and an increase in CR under drought stress. Although Lider and BATEM Erensoy exhibited better growth than the other cultivars under control conditions, their root and shoot growth decreased significantly under water stress. Notably, Ataem-7 presented a lower TWC and WUE difference between the drought treatment and the control, and this cultivar efficiently used water for dry matter production in the shoot and root parts. As a result, there were significant genotypic differences in drought susceptibility among the soybean cultivars, and Ataem-7 showed greater tolerance to drought than the other soybean cultivars did during the early growth stage.

Keywords: Glycine max (L.) Merr., Drought, Water use efficiency, Electrolyte leakage

Cite this article as: Ergin, N., Kulan, E.G., Harmanci, P., Kaya, M.D. (2024). Morpho-physiological and water use performance of soybean cultivars under drought stress at early growth stages. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 13-21. https://doi.org/10.31015/2025.1.2

INTRODUCTION

Soybean (Glycine max (L.) Merr.) is the most important legume crop, with high contents of protein (36-45%) and oil (18-24%) in the seeds (Fehr, 1980; Pratab et al., 2012). It accounts for nearly 1/3 of global edible oil and 2/3 of protein sources (Sincik et al., 2008; Ergin et al., 2023). For this reason, it is the most popular oilseed crop in the world and is cultivated on a large area of 134 million hectares (FAO, 2024). The soybean is cultivated on an area of 38,000 hectares and is widely grown in the Mediterranean region of Turkey as the main and second crop after wheat or barley. Approximately 75% of soybeans are produced as a second crop (TUIK, 2024), which means that they can usually be planted in June when the temperature is above 30 °C and the risk of early drought due to low rainfall is high.

Drought stress is one of the most severe abiotic stresses affecting plant growth, especially in arid and semiarid regions of the world, as it suppresses the growth and development of plants during their life cycle. It inhibits various physiological events, such as the rapid degradation of proteins, membrane lipids, and photosynthetic pigments, and increases cell membrane damage due to increased ROS levels (Ahmedizadeh et al., 2011; Basal et al., 2020). These damages are not the same at every stage of plant development. Depending on the plant species, certain stages, such as germination, seedling, or flowering, could be the most critical stages for water stress

(Sinclair et al., 2010). Drought stress can affect soybean from germination to late blooming (Maleki et al., 2013), potentially reducing growth and seed yield by up to 40% (He et al., 2017; Ingwers et al., 2022; Yang et al., 2023), particularly during the flowering stage. The reduction in seed yield due to drought at the reproductive stage was 46-71% (Samarah et al., 2006). However, soybean cultivars may react differently to drought stress, with droughttolerant genotypes retaining elevated traits such as leaf area and chlorophyll content even when exposed to drought stress in the vegetative stage (Yan et al., 2020). Some genotypes can recover from drought-induced injuries upon rewatering, resulting in compensatory effects on growth (Dong et al., 2019). Drought-tolerant genotypes in the early stages of development experience minimal damage and exhibit only slight decreases in yield (Yan et al., 2020; Yang et al., 2023). The screening of genotypes in early growing stages by monitoring certain physiological parameters linked with drought resistance is one strategy to improve selection efficiency (Khan et al., 2016; Guzzo et al., 2021; Simondi et al., 2022). Additionally, understanding the mechanisms of drought tolerance can help in developing cultivars that use water more efficiently and maintain high yields (Yang et al., 2023). Soybean plants develop a variety of mechanisms for drought adaptation. One mechanism is to improve the tolerance of soybean genotypes with relatively high water use efficiency. Another is the decline in whole plant water use during a soil water deficit event. Low leaf epidermal conductance is the third physiological trait that may increase drought tolerance and prolong crop survival during severe water stress (Hufstetler et al., 2007; Sadok and Sinclair, 2011). Therefore, this study aimed to identify the drought response of some soybean cultivars during the first three foliate stages via morphological, physiological, and water use features.

MATERIALS AND METHODS

This study was conducted at the Seed Science and Technology Laboratory of Eskişehir Osmangazi University, Türkiye, in 2023. Four soybean cultivars, Lider registered by Pro Gen Seed Inc. in 2014, and Ataem-7, BATEM Erensoy and Göksoy by Batı Akdeniz Agricultural Research Institute (Antalya) in 2006, 2010, and 2019, respectively, were used.

Plant growth conditions

The seeds of soybean cultivars were pre-germinated in Petri dishes at 20 °C for 48 h on filter paper moistened with distilled water. The seeds with radicle protrusion were transplanted into plastic pots (0.5 L) filled with a mixture of sieved field soil, perlite, and vermiculite (6:1:1 v:v:v). Just after transplanting, the plants were fertilized with the basal macronutrients N, P, and K (8-8-8). Eight plants from each cultivar were grown up to the V_1 stage (the first trifoliate), as reported by Fehr et al. (1971), in a growth chamber at 22 °C/18 °C day/night and 70-75% relative humidity.

Drought treatment

The field-water holding capacity (FC) of the soil mixture was determined before the experiment via the methodology modified by Liyanage et al. (2022). The soil was kept moist at 80% FC until the first leaf fully expanded (V_1 stage, 27 days after the emergence of the plants). The plants were separated into two plots, the control and drought stress plots, which were subjected to 80% and 50% FC for 20 days, respectively. By weighing each pot every other day, the water content of the pots was adjusted to the respective FC.

Assessment of morphological characteristics

The plants were harvested by cutting them from the soil surface and separating the aboveground parts from the underground parts. The leaves were removed as soon as the fresh plant biomass (shoot fresh weight) was weighed. All the leaves were scanned to compute the leaf area via ImageJ software (Cosmulescu et al., 2020). After the roots were washed and cleaned gently, the tap root was measured. After being dried for 24 hours at 80 $^{\circ}$ C in an oven, the samples of the roots and shoot sections were weighed.

Evaluation of physiological characteristics

Leaf temperature was measured via a Trotec BP21 infrared thermometer (Germany) before harvest. The chlorophyll content was estimated as the SPAD value, via a portable chlorophyll meter Konica Minolta SPAD-502 (Japan). Three consecutive readings were collected from distinct positions of fully expanded leaves (specifically, the middle leaflets of the 3rd and 4th leaves). These readings were combined to provide a single value for each duplicate. The third and fourth leaves from the apex were subsequently used for determining the leaf relative water content (RWC) and electrolyte leakage, respectively.

Leaf RWC was determined via the equation (1):

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$$
 (1)

where FW = is the fresh weight of the leaf, DW = is the dry weight of the leaf after drying to a constant weight at $80 \,^{\circ}$ C for 24 h, and TW = is the turgid weight after the leaf samples were immersed in distilled water in a closed Falcon tube for 24 h in the dark at $20 \,^{\circ}$ C (Batool et al., 2020).

Electrolyte leakage (membrane permeability) was determined via the method developed by Hniličková et al. (2019), with a few minor modifications. For each replicate, the third leaf located at the top of each plant was selected and washed with distilled water to remove electrolytes from the leaf surface. Six disks with a diameter of 5 mm were taken from each leaf after a gentle surface-drying process using paper towels. The samples were first weighed and then transferred into 50-mL glass tubes with 20 mL of distilled water before being placed in a dark

incubator at 20 °C for 24 h. After incubation, the electrical conductivity (EC₁) of the bathing solution was measured at 25 °C via a WTW 3.15i EC meter (Germany). The samples were subsequently subjected to incubation inside a thermostatic water bath set at 90 °C for 40 min to eradicate all the cells. The electrical conductivity (EC₂) was measured at 25 °C after the tubes were cooled to room temperature. The electrolyte leakage (EL) was expressed as a percentage of EC₁/EC₂ (Kaya, 2023).

Evaluation of water use efficiency

The pots were weighed every other day and watered according to the determined drought levels. The total amount of water consumed during the study determined the overall water consumption value of the plants. The water use efficiency (WUE) was calculated using the formula (2) (He et al., 2017).

WUE = (shoot dry matter + root dry matter)/total water consumption

Water consumption per shoot and root dry weight was obtained by dividing shoot dry weight by total water consumption.

Statistical analysis

The data were evaluated by a completely randomized design (CRD) with four replications using the MSTAT-C (Michigan State University, v. 2.10) program. The means were grouped by Duncan's Multiple Range Test at the p < 0.05 level. The R program was used to determine the Pearson's correlation coefficients between the characteristics and significance levels (p < 0.01).

RESULTS AND DISCUSSION

This study was conducted to determine the effects of drought stress on soybean cultivars at the early growth stage. A significant difference was determined for the investigated characteristics (p < 0.05) (Table 1).

Table 1. Main effects of drought stress on morphological characteristics of soybean cultivars

	Plant height (cm)	Leaf area (cm ²)	Shoot fresh weight (g plant ⁻¹)	Shoot dry weight (mg plant ⁻¹)
Drought stress (A)				
Drought	9.7 ^b	85.0 ^b	2.21 ^b	462 ^{b↓}
Control	13.3 ^a	161.9 ^a	4.24^{a}	791 ^a
Cultivar(B)				
Ataem-7	12.0 ^a	136.3ª	3.47 ^b	683 ^b
BATEM Erensoy	10.8 ^b	113.6 ^b	2.74 ^c	573°
Göksoy	10.7 ^b	98.9^{c}	2.76^{c}	$504^{\rm d}$
Lider	12.6 ^a	145.1 ^a	3.94^{a}	746 ^a
Analysis of variance				
A	**	**	**	**
B	**	**	**	**
$A \times B$	**	**	**	**

 $[\]downarrow$: Means followed by the same letter(s) are not significant, **: significance level at p < 0.01.

Drought caused a significant decrease in the plant height of the soybean cultivars (Figure 1). Lider was the cultivar most adversely affected by drought, with a reduction of 40.9% (Figure 2A), whereas the reduction rates in plant height of the other cultivars were ranged from 20.0% to 21.7%. The Lider (12.6 cm) and Ataem-7 (12.0 cm) seedlings were longer than the other cultivars were. Drought not only shortened the plant height but also reduced the leaf area of the soybean cultivars.

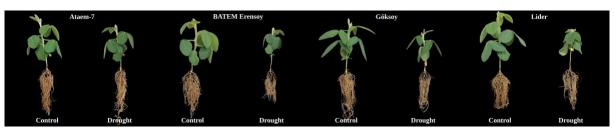


Figure 1. Seedlings of soybean cultivars under drought stress

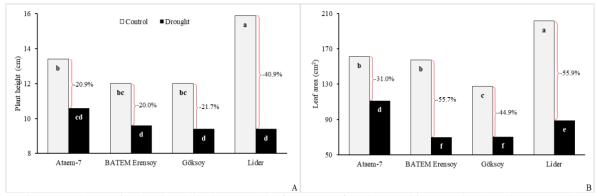


Figure 2. Changes in the plant height (A) and leaf area (B) of soybean cultivars under drought stress. The letter(s) on each column indicate significance at p < 0.05.

Leaf area is one of the most sensitive parameters to drought, and plants slow their leaf growth to protect themselves and continue their lives. The leaf area of the soybean cultivars significantly decreased with drought. The maximum reduction rates were noted in Lider (55.9%) and BATEM Erensoy (55.7%). Compare with the other cultivars, Ataem-7 was less affected by drought, decreasing the leaf area by 31.0% (Figure 2B). In a previous study, Poudel et al. (2023) reported a significant reduction in the leaf area of 10 soybean cultivars under drought stress. Similarly, Aziez (2023) reported that the maximum leaf size was determined at 100% field capacity while the lowest leaf size was at 25% field capacity in soybean. Water deficit inhibited the growth of the soybean cultivars. Miranda et al. (2023) observed a significant reduction in the fresh and dry weights of soybean seedlings as drought severity increased from 45% to 30% field capacity. In addition, the responses of cultivars varied. Similarly, Yan et al. (2020) found that root length varied with genotype, water application, and their interaction. Lumactud et al. (2022) observed that, compared with root, soybean shoots were more susceptible to drought, which led to the rapid suppression of shoot development. They demonstrated lower biomass in roots and shoots under drought stress than did the well-watered control. These results agree with those of the present study.

Table 2. Changes in the root characteristics of soybean cultivars under drought stress

	Root length	Root fresh weight	Root dry weight
	(cm)	(g plant ⁻¹)	(mg plant ⁻¹)
Drought Stress (A)			
Drought	18.8 ^b	2.33 ^b	352 ^b .
Control	25.1 ^a	4.83 ^a	591 ^a
Cultivar(B)			
Ataem-7	21.3 ^{bc}	4.47 ^a	564 ^a
BATEM Erensoy	20.6^{c}	2.77^{d}	410 ^c
Göksoy	21.6 ^b	3.37°	426°
Lider	24.3 ^a	3.72 ^b	487 ^b
Analysis of variance			
A	**	**	**
B	**	**	**
$A \times B$	**	**	**

The shoot fresh and dry weights also decreased by approximately 50% under drought stress and, the greatest reduction in seedling fresh weight was detected in Lider (Table 1). The shoot fresh and dry weights of Lider and Göksoy were lower than those of the other cultivars (Figure 3A and 3B). Lider (12.6 cm) and Ataem-7 (12.0 cm) plants had longer shoots than did the other cultivars. Drought caused a reduction in soybean growth. Our results align with the findings of Fatema et al. (2023), who determined shorter plants in soybean under water stress, and the findings of Desclaux et al. (2000), who reported the inhibitory effects of drought on the vegetative growth of soybean plants during the early growth stage.

Drought significantly impeded root development, but the cultivars responded differently (Table 2). As expected, the root length and fresh and dry weights of the soybean cultivars were substantially lower under drought stress, and the longest root length (24.3 cm) was measured in Lider. Under drought stress, the root length decreased by 37.6% in BATEM Erensoy, 35.6% in Lider, and 21.5% in Göksoy. The heaviest fresh (4.47 g plant⁻¹) and dry weights (564 mg plant⁻¹) of the roots were recorded in Ataem-7. It can be inferred that the root characteristics of Ataem-7 were the most stable, as minimal changes in root growth were observed between drought-exposed and control plants (Figure 3). The decrease in fresh root weight of the soybean cultivars due to drought stress ranged

from 36.1% to 60.5% (Figure 3C). Significant reductions in root dry weight were also observed, with the greatest reductions in Lider (48.7%) and Göksoy (47.2%) (Figure 3D).

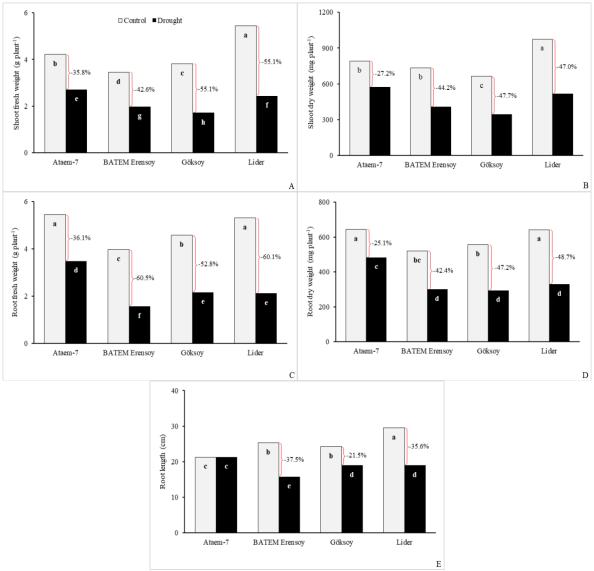


Figure 3. Changes in shoot fresh weight (A), shoot dry weight (B), root fresh weight (C), root dry weight (D), and root length (E) of soybean cultivars under drought stress. The letter(s) in each column indicate significance at p < 0.05.

Although the leaf temperatures did not vary with drought, significant differences were noted among the soybean cultivars. Of soybean cultivars, a relatively higher mean leaf temperature was recorded in Ataem-7, whereas the lower was recorded in Göksoy (Table 3). The chlorophyll content was higher in plants subjected to drought than in control plants. Lider had the lowest chlorophyll rate among the soybean cultivars (37.4 SPAD). Increased chlorophyll content due to drought stress was more pronounced in Lider and Göksoy (Figure 4A). The RWC of the soybean cultivars decreased in a similar manner due to drought, and no significant differences among the soybean cultivars was detected. On the other hand, drought caused a reduction in the RWC of BATEM Erensoy by 18.6%, followed by Göksoy (17.9%), Lider (15.6%), and Ataem-7 (10.4%) (Figure 4B). Compared with control plants, drought-stressed plants leaked significantly more electrolytes. Among the cultivars, the highest leakage was recorded in Lider and Ataem-7 (Table 3). Our results are in agreement with the findings of Tiwari et al. (2023), who reported that electrolyte leakage increased under drought stress in chickpea. The relative water content was reduced in soybean plants exposed to drought, but this reduction varied among the soybean cultivars. Under drought stress, the lowest reduction in leaf water content was detected in Ataem-7, and the highest was recorded in BATEM Erensoy and Göksoy. Zegaoui et al. (2017) stated that plants can regulate water use when exposed to water stress; therefore, the relative water content of leaves could be used to determine their resistance to drought stress. Mishra and Patidar (2023) also found that drought-tolerant genotypes of several crops presented relatively

high leaf-relative water content under water stress and revealed significant differences in RWC among soybean cultivars. This difference might be explained by genotypic variations that vary in their capacity to regulate stomatamediated water loss during transpiration or their ability to absorb water from the soil. Moreover, Delevar et al. (2023) reported that drought stress damaged the membrane system and chlorophyll content of soybean leaves.

Table 3. Main effects of drought stress on physiological characteristics of soybean cultivars

	Leaf	temperature	Chlorophyll rate	Relative	water	Electrolyte leakage
	(°C)		(SPAD)	content (%)		(%)
Drought Stress (A)						
Drought	26.7		41.8 ^a	75.7 ^b		18.4 ^{b↓}
Control	26.6		35.3 ^b	89.8 ^a		20.3^{a}
Cultivar(B)						
Ataem-7	26.8a		38.4 ^{ab}	83.0		19.9 ^{ab}
BATEM Erensoy	26.7^{ab}		39.8a	81.4		18.6 ^b
Göksoy	26.3°		38.7^{ab}	83.4		18.7 ^b
Lider	26.6^{b}		37.4 ^b	83.3		20.2^{a}
Analysis of variance						
A	ns		**	**		**
B	**		*	ns		*
$A \times B$	ns		*	*		ns

 \downarrow : Means followed by the same letter(s) are not significant, *, **: significance level at p < 0.05 and p < 0.01, respectively; ns: not significant.

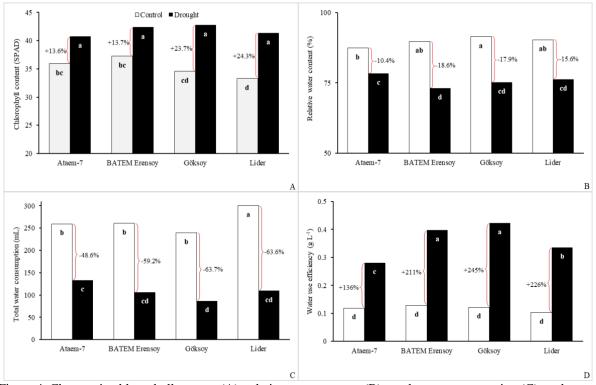


Figure 4. Changes in chlorophyll content (A), relative water content (B), total water consumption (C), and water use efficiency (D). The letter(s) in each column indicate significance at p < 0.05

Plant water consumption changes when water availability is limited. Among the soybean cultivars, Ataem-7 had the highest water use efficiency, whereas Göksoy (63.7%) and Lider (63.6%) had the highest water consumption (Figure 4C). Water use efficiency (WUE) reflects the water production of a plant under various irrigation water availability and soil moisture conditions. Drought-tolerant plants can maintain their physiological progress while also adjusting their water consumption. In general, the WUE of plants decreases under water deficit conditions, and vice versa, plants fail to generate optimal yields, and a greater transpiration rate results in a lower WUE under drought stress (Farajollahi et al., 2023). In this study, the WUEs of soybean cultivars were similar to each other under unstressed conditions, but they responded differently to drought. Göksoy achieved the highest WUE, followed by Batem Erensoy. Compared with the other cultivars, Ataem-7 presented the minimum increase

in WUE due to drought (Figure 4D). This result is in line with the findings of Guo et al. (2023), who reported that, compared with the control, drought stress considerably increased the photosynthetic water-use efficiency in maize cultivars.

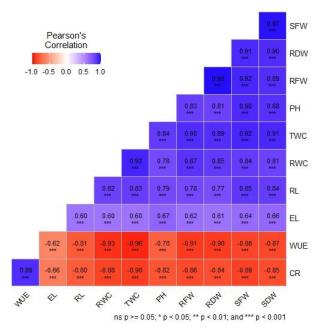


Figure 5. Pearson's correlation coefficients between the investigated characteristics. PH: Plant height, EL: Electrolyte leakage, RL: Root length, RWC: Relative water content, CR: Chlorophyll content, TWC: Total water consumption, WUE: Water use efficiency, RFW: Root fresh weight, RDW: Root dry weight, SFW: Shoot fresh weight, SDW: Shoot dry weight

The correlation coefficients between the examined characteristics and significance levels are given in Figure 5. The total water consumption (TWC) was highly correlated with shoot fresh weight (SFW) (r = 0.92***). Additionally, the relationship between TWC and RFW was significantly positive (r = 0.90***). WUE was correlated with CR (r = 0.89***), suggesting that increased WUE may stimulate CR. As expected, a negative and significant correlation was detected between TWC and WUE (r = -0.96***). In addition, TWC was significantly related to RFW (r = 0.90***) and SFW (r = 0.92****). Recent studies demonstrated that the wue had significant relationships with root fresh and dry weight under long-term drought stress and that root dry weight should be a useful selection criterion for high WUE (Puangbut et al., 2009; Wijewardana et al., 2019; Gebre and Earl, 2021).

CONCLUSION

This study demonstrated that drought stress resulted in a decreased leaf area, relative water content, seedling fresh weight, seedling dry weight, root length, root fresh weight, and root dry weight, while soybean cultivars showed different responses to drought. Higher total water consumption and lower water use efficiency were obtained from the plants subjected to drought in terms of the characteristics mentioned above. Among the soybean cultivars, Ataem-7 appeared more tolerant to drought stress because it had the lowest percent reduction in both morphological and physiological traits. Drought tolerance stems from improved root growth and total water use under drought, and these traits should be considered for promising breeding traits in soybean.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

NE, MDK and EGK designed the study. NE and PH executed the experiments and analyzed the data. All the authors interpreted the data, critically revised the manuscript for important intellectual content and approved the final version.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors declare that there are no conflicts of interest related to this article.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.3

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 22-26

Determination of aphid (Hemiptera: Aphidoidea) species on vegetable and fruit fields in central districts of Konya province

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Article History

Received: September 19, 2024 Revised: January 30, 2025 Accepted: February 3, 2025 Published Online: February 24, 2025

Article Info

Article Type: Research Article Article Subject: Entomology in Agriculture

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https://dergipark.org.tr/jaefs/issue/90253/1552664

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Abstract

This study was carried out to determine the aphid species found on fruit and vegetable fields in central districts (Karatay, Meram, and Selçuklu) of Konya province, a total of 218 samples were examined between 2022-2023 years and 20 aphid species belonging to Aphidinae subfamily of Aphidoidea superfamily were identified. These species are: Aphis craccivora Koch, Aphis fabae Scopoli, Aphis gossypii Glover, Aphis nasturtii Kaltenbach, Aulacorthum solani (Kaltenbach), Brachycaudus (Thuleaphis) amygdalinus (Schouteden), **Brachycaudus** (Prunaphis) cardui (L.), Brachycaudus helichrysi (Kaltenbach), Brachycaudus (Scrophulaphis) persicae (Passerini), Brevicoryne brassicae (Linnaeus), Dysaphis (Pomaphis) plantaginea (Passerini), Dysaphis (Pomaphis) pyri (Boyer de Fonscolombe), Hyalopterus pruni (Geoffroy), Macrosiphum euphorbiae (Thomas), Myzus cerasi (Fabricius), Myzus lythri (Schrank), Myzus (Nectarosiphon) persicae (Sulzer), Nasonovia Rhopalosiphum nymphaeae (Linnaeus) and Rhopalosiphum padi (Linnaeus). Aphis gossypii, A. fabae, A. nasturtii, Hyalopterus pruni and Myzus (Nectarosiphon) persicae were found to be the most common and widely distributed species in the study.

Keywords: Aphidoidea, Aphid diversity, Aphid-host plants, Aphid, Konya

Cite this article as: Emir, Z., Bayindir Erol, A., Özdemir, I. (2025). Determination of aphid (Hemiptera: Aphidoidea) species on vegetable and fruit fields in central districts of Konya province. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 22-26. https://doi.org/10.31015/2025.1.3

INTRODUCTION

Türkiye has suitable climatic conditions and fertile soil, allowing the cultivation of different varieties of fruits and vegetables. When we look at the distribution of agricultural fields by province, Konya is the province with the most agricultural field (MEVKA, 2024). In 2023 year data, the vegetable growing field in Konya province was reported as 357.767 decares and the fruit growing field was 417.107 decares (TÜİK, 2024). Many problems are encountered in fruit and vegetable growing fields in Turkey. Among these, diseases and pests are important. Aphids are one of these pests that cause the most damage. Aphids are a significant insect group with regard to species diversity and density; there are 5.942 species of aphids worldwide, categorized into 538 genera (Favret, 2024). In Turkey, aphids have been observed to feed on both cultivated and wild plants and 675 aphid species have been recorded (Başer et al., 2024). Aphids belong to the superfamily Aphidoidea (Hemiptera); they feed on plant sap in the fruits, leaves, shoots, stems or roots of plants. As a result of their feeding, deformities result in the leaves, shoots, and fruits, and the plants show signs of stunting and curling of their leaves. During feeding, aphids release a lot of honeydew which provides a favorable environment for the growth of saprophytic fungus. Consequently, the leaves become covered with sooty mold which blocks photosynthesis and results in the yellowing of the leaves. More importantly, aphids can serve as vectors of many plant viruses. This virus transmission is the most significant damage done to plants and results in great plant losses (Toros et al., 2002).

Although there are many studies on aphids in Turkey, there are no studies on aphids in fruit and vegetable fields in the central districts of Konya province. This study, aimes to determine the aphid species and their hosts found in fruit and vegetable fields in the central districts of Konya province.

MATERIALS AND METHODS

Field Surveys and Collection of Aphid Samples

Depending on the climatic conditions, field surveys were carried out every week between May and October in 2022-2023. Samples were collected by cutting the leaves and stem parts of aphid-infested plants with pruning shears. The cut samples were first wrapped in paper and then placed in nylon bags labeled with respect to date, location and host plant type, then the samples were brought to the laboratory. Adult aphids were selected from the collected samples and transferred into Eppendorf tubes containing 96% ethyl alcohol using a brush.

Preparation and Classification

The Hille Ris Lambers (1950) technique was used in the preparation of the aphid samples. Measurements of morphological characteristics were made according to Hille Ris Lambers (1945, 1947a, 1947b, 1949, 1969, 1973), Börner (1952), Cottier (1953), Remaudiere (1954), Börner and Heinze (1957), Bodenheimer and Swirski (1957), Stroyan (1957, 1961, 1963, 1977, 1984), Shaposhnikov (1964), Tuatay and Remaudiere (1964), Eastop (1971, 1972), Bissel (1978), Blackman and Eastop (1984, 1994, 2000). The aphids were systematically classified from the catalog of Remaudiere and Remaudiere (1997) and Eastop and Hile Ris Lambers (1976).

RESULTS AND DISCUSSION

The aphid species and their host plants found on fruit and vegetable fields in central districts of Konya province (Karatay, Meram, and Selçuklu) are showed in Table 1. Twenty aphid species were identified. In the study, *A. gossypii* was recorded as the most common species followed by *A. fabae*, *A. nasturtii*, *Hyalopterus pruni* and *M. (Nectarosiphon) persicae*. The aphids were collected from a total of 22 host plants. The plants most preferred by aphids were determined as *Cucumis melo* L., *Cucumis sativus* L. and *Prunus amygdalus* Batsch.

Similarly, a survey conducted by Pirçek (2023) on aphid species found on fruit and vegetable fields in the Samsun Çarşamba plain in the north of Türkiye reported that *A. gossypii* and *M. persicae* being the most common species. The aphids species collected in that region were *Acyrthosiphon* (*Tlja*) lactucae (Passerini), *A. pisum* (Harris), *Aphis craccivora*, *A. fabae*, *A. gossypii*, *A. nasturtii*, *A. pomi* De Geer, *A. spiraecola* Pacth, *A. triglochinis* Theobald, *Brachycaudus* (*Thuleaphis*) amygdalinus, *B. helichrysi*, *Brevicoryne brassicae*, *Cavariella aegopodii* (Scopoli), *Dysaphis* (*Pomaphis*) pyri, *Hyalopterus pruni*, *Lipaphis erysimi* (Kaltenbach), *Myzus cerasi*, *M. persicae*, *Phorodon humuli* (Schrank), *Rhopalosiphum maidis* (Fitch), *R. padi* and *R. rufiabdominale* (Sasaki).

In another study surveying aphids on vegetables belonging to the Solanaceae family in Hatay province in the south of Türkiye, *Myzus persicae*, *Aulacorthum solani*, *Aphis fabae* and *A. nasturtii* were found to be dominant (Yalçınkaya, 2022). In the study carried out on fruit trees in the Isparta province and its districts in the south of Türkiye, *Myzocallis coryli* (Goetze), *Aphis pomi*, *Brachycaudus* (*Prunaphis*) *cardui*, *B. helichrsi*, *Dysaphis* sp., *D. devecta*, *D. plantaginea*, *D. pyri*, *Hyalopterus amygdali* (Blanchard), *H. pruni* (Geoffroy), *Corylobium avellanae* (Schrank), *M. cerasi*, *M. persicae* and *Eriosoma lanigerum* (Hausmann) species were recorded (Aslan and Karaca, 2005).

Fifteen aphids were detected in fruit plants studied in the Upper Çoruh Valley (Erzurum) in the east of Türkiye. *Aphis pomi, Hyalopterus pruni, Dysaphis devecta* (Walker), *D. (Pomaphis) pyri* and *Myzus cerasi* were recorded as the most common species (Narmanlıoğlu, 2013).

In the study carried out on peach orchards in Bursa province in the west of Türkiye, *Brachycaudus helichrysi*, *B. persicae*, *Hyalopterus pruni*, *Myzus persicae* and *Pterochloroides persicae* (Cholodkovsky) species were recorded (Sarıbal, 2019). In the study conducted on stone and pome fruit trees in Aydın province in the west of Türkiye, 8 genera and 18 aphids were recorded. *Hyalopterus pruni*, *Myzus persicae*, *M. cerasi*, *Aphis pomi* and *Dysaphis pyri* were the most common species (Karakaya, 2014).

CONCLUSION

The aphid fauna on fruits and vegetables in the central distiricts (Karatay, Meram, and Selçuklu) of Konya province was determined in this study. The findings further contribute to the aphid species and diversity of Türkiye and benefit future studies and aphid management in the Konya province. All identified aphid species are the first records for Konya province.

Table 1. List of aphid species and host plants found in central districts of Konya province

Brassica oleracea L.
•
Cucurbita pepo L.
Phaseolus vulgaris L. Rumex obtusifolius L.
Prunus amygdalus Batsch
Cucumis melo L.
Abelmoschus esculentus (L.)
Cucurbita moschata Duchesne
Cucurbita pepo L. Cucumis melo L.
Cucumis meto L. Cucumis sativus L.
Solanum melongena L.
Punica granatum L.
Citrullus lanatus (Thunb.) Matsum. &
Nakai
Capsicum annuum L.
Cucumis sativus L.
Cucumis melo L.
Cucumis sativus L.
Citrullus lanatus (Thunb.) Matsum. &
Nakai
Prunus amygdalus Batsch
Prunus amygdalusBatsch
Prunus persica (L.) Batsch
Prunus domestica L.
Prunus domestica L.
Brassica oleracea L.
Malus domestica L.
Pyrus communis L.
Prunus armeniaca L.
Prunus domestica L.
Prunus persica (L.) Batsch
Cucumis sativus L.
Prunus cerasus L.
Prunus avium L.
Prunus mahaleb L.
Prunus persica (L.) Batsch
Abelmoschus esculentus(L.)
Capsicum annuum L.
Lactuca sativa L.
Prunus amygdalus Batsch
Prunus armeniaca L.
Pyrus communis L.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

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

Acknowledgments

This study was carried out as part of Zadife EMİR's MSc thesis.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.4

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 27-33

Determination of suitable sowing date of safflower in Divarbakır conditions

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Article History Received:November 18, 2024 Revised: January 10, 2025

Accepted: January 16, 2025 Published Online: March 04, 2025

Article Info

Article Type: Research Article Article Subject: Agronomy, IndustrialCrops

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1587373

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Abstract

Safflower (Carthamus tinctorius L.) oil is widely used both as a cooking oil and in industrial applications and is tolerant to adverse weather conditions such as cold, drought and salinity. This study was conducted to determine the most suitable sowing date and safflower variety for the conditions in Diyarbakır, Türkiye, during the 2009-2010, 2010-2011 and 2011-2012 growing seasons. The experiment followed a randomized complete block design with split plots and four replications, using two safflower varieties: Remzibey-05 and Dinçer. Seeds were planted on the trial field of GAP UTAEM (GAP Internatiolan Agricultural Research and Training Center). The results showed that the highest seed yields were obtained from the Remzibey-05 variety sown on 15 December (2766 kg ha⁻¹) and 15 November (2755 kg ha⁻¹), and from the Dinçer variety sown on 1 December (2677 kg ha⁻¹). The lowest yield was recorded for the variety Remzibey-05 sown on 15 April (1005 kg ha⁻¹). Besides, the highest crude oil yield was obtained in Dincer cultivar with in 1st December 831 kg ha⁻¹ and the lowest was obtained from Dincer variety in 15th April sowing date with 332 kg ha⁻¹. Based on the results, sowing dates between 15 November and 15 December are recommended for optimal safflower production under Divarbakır conditions.

Keywords: Cultivar, Oil content, Protein ratio, Yield

Cite this article as: Kahraman, S., Karaaslan, D., Hatipoglu, A. (2025). Determination of suitable sowing date of safflower in Diyarbakır conditions. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 27-33. https://doi.org/10.31015/2025.1.4

INTRODUCTION

The safflower is oil plant. The flowers, leaves and seeds of the safflower have been used in different areas. Safflower which is more tolerant to drought, cold and salinity than other oil crops. (Kayacetin et al., 2012). Safflower (Carthamus tinctorius L.), recognized as a high-quality oil seed crop, is reported to have originated in southern Russia, Iran, Türkiye, Jordan, Iraq, and Israel (Knowles, 1989; Yaşar & Sezgin, 2023). Safflower is a very important plant in terms of contributing to the biodiesel, dye, high-quality oil and composition of fatty acids suitable for human consumption (Huth et all, 2015; Subaşı et all, 2023) and feed industry, being easily adapted to the application of rotation, being effective in reducing fallow areas, being able to grow in different types of soil, not needing special equipment in the cultivation process, and contributing to the sustainability of agricultural production and employment (Akgün and Söylemez 2022).

Türkiye's safflower production was 39 000 tons, on 32 129 hectares. The provinces with the highest safflower production in 2023 were Kayseri at 29.5%, Isparta at 13.1%, Konya at 12.4%, Kırşehir at 9.3%, Nevsehir at 8.8%, Ankara at 6.2% and Aksaray at 5.9%. In Türkiye, 85.2% of total safflower production occurs in these provinces. Diyarbakır province's production was 12 tons, on 9.9 hectares and average yield of 1 210 kg ha⁻¹ in year 2023 (Anon., 2023). Three cultivars had been developed until 2011 in Türkiye. Two of them, Dincer and Remzibey-05, are still in production. The cultivars in production have an oil rate of about 28-32% (Babaoglu and Guzel 2015).

Safflower oil quality is very high due to fatty acid composition. Its oil constitutes an major source of polyunsaturated fatty acid (Shivani et al., 2010). The fatty acid structure of herbal oil is a significant factor which affects its trading uses (Katar et al., 2014). Many clinical and laboratory studies support the use of safflower for cardiovascular disease, menstrual problems, and swelling and pain associated with trauma (Dajue and Mündel, 1996). Addition of safflower florets to foods is a widespread. Health concerns regarding synthetic food colourings may rise demand for safflower-derived foodcoloration (Weiss, 1983). Safflower is nutritionally like too live oil, with high levels of oleic or linoleic acid, besides lesser costly (Smith, 1996).

Safflower is can be used in biodiesel production. Besides, For the production of safflower, Türkiye has a suitable soil and climati econditions (Eryılmaz et al., 2014). Türkiye's biodiesel production, which is included in biofuels, which has special importance in energy submission security, is at risk. It is staminal to prioritize the cultivation of energy crops, especially in fallow fields (Karabaş, 2022). When safflower meal is compared with soybean meal which is nearly equal (Özek, 2016). Safflower cultuvars (Dinçer and Remzibey-05) are not suitable to second crop conditions (Sevilmiş et al., 2018). If the salt rate of the area where safflower cultivation will be carried out is closeto 300 millimoles, Linas and Olas varieties can be recommended, respectively. If the salt rate of the area where safflower cultivation will be carried out is 200 millimoles or below, it can be said that Dinçer andLinas varieties may be more suitable (Kurtuluş and Boydak, 2022).

Previous studies revealed that delayed sowing dates resulted in decreasing seed yields (Keles and Ozturk, 2012; İzgi, 2023). To achieve maximum grain yield in safflower cultivation for the conditions of Sistan region, sowing from early January to late January using, is recommended (Fanaei et al., 2024). For successful and economical safflower cultivation, suitable varieties and growing techniques should be determined. Suitable sowing date constitute a significant growing technique. Sowing date is among the most important factors designating the yield (El Bey et al., 2021). This study was conducted to determine the most variety and suitable sowing date for safflower in Diyarbakır conditions.

MATERIAL AND METHODS

This study was conducted in 2009-2010, 2010-2011 and 2011-2012 in Diyarbakır. Dinçer and Remzibey-05 varieties were used. Seeds were planted on the trial field of GAP UTAEM (GAP Internatiolan Agricultural Research and Training Center). The trial disigned randomized complete block in spling plot with four replication. The soil samples at 0-20 cm depth were analysed (Analysis was done in GAP UTAEM laboratory) and the soil structure the soil clay-loam, pH was 7.6, total salt concentration was 0.092%; the amount of phosphorus was 14.3 kg ha⁻¹ and potassium 1241.7 kg ha⁻¹, the ratio of organic matter was 0.78%. In the province of Diyarbakır the annual rainfall is mostly happened between October and June months. The climate situations of the trial area, are illustrated in Table 1.

Table 1.Long-term and 2009-2012 years climatic data of Diyarbakır province.

	Years	October	November	December	January	February	March	April	May	June
	2009-10	18.5	9.8	7.1	5.4	6.6	11.1	14.2	20.4	27.2
Average	2010-11	18.1	11.1	6.5	3.5	4.7	9.0	13.0	17.7	25.5
temperature (°C)	2011-12	16.4	6.4	2.3	2.4	1.9	5.1	15.2	19.6	27.7
	Long T.	17.0	8.9	3.8	1.7	3.5	8.2	13.7	19.1	26.3
	2009-10	42.0	70.6	83.5	80.9	79.9	66.6	60.4	49.3	29.1
Average humidity	2010-11	56.0	41.1	68.9	73.4	69.5	56.4	75.7	67.6	38.0
(%)	2011-12	41.6	58.8	73.9	84.4	68.2	59.2	58.5	58.0	27.8
	Long T.	48.0	67.1	76.7	77.1	72.8	65.6	63.2	56.3	35.9
	2009-10	62.4	55.6	87.2	113.4	40.2	68.7	22.4	31.6	11.2
Precipitation	2010-11	63.4	2.0	48.0	40.0	49.9	46.6	209.0	80.1	13.6
(mm)	2011-12	11.8	73.0	40.2	78.3	74.4	44.0	26.2	41.0	7.0
	Long T.	34.6	53.3	70.7	62.3	72.1	68.2	64.6	40.2	9.1

Prior to 15 - 20 cm deep soil was processed and for the levelling of the land plows and harrows were used. Plot lengths were 5 m, width were 1.8 m and each plot contained 6 rows. The seeds were sown in a way that there would be 3 kg seeds in each decare. In the parcels, 100 kg ha⁻¹ nitrogen and 50 kg ha⁻¹ phosphorus were used. 50 kg ha⁻¹ of the nitrogen and the whole of the phosphorous were given before planting. The remaining 50 kg ha⁻¹ nitrogen was applied when plants were 20-30 cm tall. The parcels, were harvested with a trial harvester. for the seed yields in July. After harvest, crude oil ratio (in 2010 and 2012) was determined by Nuclear Magnetic Resonance (NMR) device with protein ratio (in 2010) Kjehldal method. Analysis of variance across was calculated for each trait and LSD (Least significant difference) test was applied to match the variations (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

According to the findings of trial; the lowest was obtained from Remzibey-05 variety in the 15th April sowing date with 51.06 cm and the highest plant height obtained from Dinçer variety in the 15th December sowing dates with 122.99 cm. The average values of the varieties with regards to the sowing date were 54.51 cm in the 15th April sowing date with and 121.41 cm in the 15th December sowing dates. The plant height of Dinçer variety (99.82 cm) was higher than Remzibey variety (95.56 cm) (Table 2). Hatipoğlu et al. (2012) stated that plant height of safflower farming in Şanlıurfa between 45.3-127.9 cm. Coşkun (2014) stated that plant height of safflower farming in Bursa between 158.6 -100.5 cm. Atan et al. (2019) stated that plant height of safflower farming in Hatay between 143.83-163.67 cm. Öner and Şeker (2020) stated that plant height of safflower farming in Çorum between 82.37-107.6 cm. El Bey et al. (2021) stated that plant height of safflower farming in Samsun between 82.3-158.7 cm. Reported differences are likely to be, environmental conditions and variety.

The highest head number per plant was obtained with 15th November sowing date with 29.27 and the lowest was obtained in 15th April sowing date with 13.31. The head number per plant of Remzibey variety (23.58 cm) was higher than Dinçer variety (21.95) (Table 2). Hatipoğlu et al. (2012) stated that the number head of per plant in the conditions of Şanlıurfa changes between 10.5-31.8. Öz (2016) stated that head number per plant in the conditions of Bursa changes between 17.1-29.4. Atan et al. (2019) stated that head number per plant in the conditions of Hatay changes between 11.83-16.20. Öner and Şeker (2020) stated that head number per plant of safflower farming in Çorum between 7.28-10.43. Köse et al. (2021) stated that the number head of per plant in the conditions of Eskişehir changes between 9.2-11.7. Reported differences are likely to be, environmental conditions and variety.

Table 2. Mean values of safflower varieties at 2009-2010, 2010-2011 and 2011-2012 growing seasons.

	Plant h	eight (cm)	Head number per plant				
Sowing date	Va	rieties	Average	V	arieties	Average	
	Dinçer	Remzibey-05	Average	Dinçer	Remzibey-05	Average	
1 November	117.50 bc	114.94 c	116.22 b	25.85	27.65	26.75 b	
15 November	119.66 ab	120.56 ab	120.11 a	28.71	29.83	29.27 a	
1 December	120.21 ab	119.53 ab	119.87 a	26.46	30.64	28.55 ab	
15 December	122.99 a	119.83 ab	121.41 a	26.12	30.28	28.20 ab	
1 March	95.18 d	89.30 e	92.24 c	18.35	19.11	18.73 cd	
15 March	87.88 e	81.08 f	84.48 d	20.63	19.97	20.31 c	
1 April	77.17 g	68.39 h	72.78 e	16.06	17.96	17.01 d	
15 April	57.96 ı	51.06 j	54.51 f	13.44	13.18	13.31 e	
Average	99.82 a	95.56 b		21.95 b	23.58 a		
CV (%)	4.57			19.22			
LSD variety	1.19**			1.12**			
LSD sowing date	2.55**			2.50**			
LSD interaction	3.60**			ns			

ns: nonesignificant, **: significant at 0.01 level

The highest head diameter was obtained with 15th November sowing date with 2.41 cm and the lowest was obtained in 1st April sowing date with 2.08 cm. The head diameter of Dinçer variety (2.33 cm) was higher than Remzibey variety (2.08 cm). (Table 3). Hatipoğlu et al. (2012) stated that head diameter in the conditions of Şanlıurfa changes between 1.63-2.07 cm. İzgi (2023) stated that head diameter in the conditions of Mardin changes between 2.3-2.7 cm. Reported differences are likely to be, environmental conditions and variety.

The highest 1000 seed weight was obtained 15th November sowing date with 34.11 g and the lowest was obtained in 15th April sowing date with 31.19 g. The 1000 seed weight of Dinçer variety (34.08 g) was higher than Remzibey variety (30.85 g) (Table 3). Hatipoğlu et al. (2012) stated that 1000 seed weight in the conditions of Şanlıurfa changes between 37.3-42.5 g. Coşkun (2014) stated that 1000 seed weight in the conditions of Çanakkale changes between 39.00-33.78 g. Öz (2016) stated that 1000 seed weight in the conditions of Bursa changes between 34.1-36.9 g. Atan et al. (2019) stated that 1000 seed weight in the conditions of Hatay changes between 38.87-45.56 g. Köktaş and Güner (2023) stated that 1000 seed weight of safflower farming in Ankara between 39.00-44.42 g.

Table 3. Mean values of safflower varieties at 2009-2010, 2010-2011 and 2011-2012 growing seasons.

	Head dian	neter (cm)		veight (g)		
Sowingdate	Varie	eties	Average	Varieti	es	Average
	Dinçer	Remzibey-05	Average	Dinçer	Remzibey-05	Average
1 November	2.45	2.05	2.25 b	34.65	31.79	33.22 ab
15 November	2.54	2.28	2.41 a	36.10	32.12	34.11 a
1 December	2.44	2.16	2.30 b	34.25	31.25	32.75 ab
15 December	2.39	2.15	2.27 b	33.79	31.09	32.44 bc
1 March	2.25	2.02	2.14 c	33.64	30.04	31.84 bc
15 March	2.15	2.05	2.10 c	33.90	30.40	32.15 bc
1 April	2.19	1.97	2.08 c	33.23	30.81	32.02 bc
15 April	2.24	1.93	2.09 c	33.08	29.30	31.19 c
Average	2.33 a	2.08 b		34.08 a	30.85 b	
CV (%)	8.09			7.79		
LSD variety	0.06**			1.38**		
LSD sowing date	0.10**			1.44**		
LSD interaction	ns			ns		

ns: nonesignificant, **: significant at 0.01 level

According to the findings of trial; the highest crude oil ratio was obtained in Dinçer variety with in 1st November, Remzibey-05 cultivar with in 15th November with in sowing date with 33.7%, 33.6% and in Dinçer and Remzibey-05 variety with in 1st December sowing date with 33.4% respectively and the lowest was obtained from Dinçer variety in 1st April sowing date with 27.9%. The average values of the varieties with regards to the sowing date were 28.7% in the 1th April sowing date with and 33.4% in the 1th December sowing dates. The oil ratio of Remzibey variety (31.50%) was higher than Dinçer variety (30.90%) (Table4). Keleş and Öztürk (2012) stated that crude oil ratio in the conditions of Konya changes between 24.57-33.73%. Coşkun (2014) stated that crude oil ratio in the conditions of Çanakkale changes between 28.67-30.44%. Atan et al. (2019) stated that crude oil ratio in the conditions of Hatay changes between 34.38-38.49%. Baran and Andırman (2019) According to the results of the research, the highest oil content was obtained from the first sowing time (October 5) with 23.36% and the lowest sowing time (December 20) with 19.95%. Aslantaş and Akınerdem (2020) stated that crude oil ratio in the conditions of Konya changes between 21.1-29.0%.

According to the findings of trial; the highest protein ratio obtained from Remzibey-05 variety in the 15th December sowing dates with 22.06% and the lowest was obtained from Remzibey-05 variety in the 15th March sowing date with 16.32%. The average values of the varieties with regards to the sowing date were 17.33% in the 15th November sowing date with and 21.01% in the 15th April sowing dates. (Table4). Keleş and Öztürk (2012) stated that protein ratio in the conditions of Konya changes between 15.47-20.50%. Subaşı and Başalma (2021) stated that protein ratio in the conditions of Ankara and Bolu changes between 11.90-14.53%. Boydak et al (2024) stated that protein ratio in the conditions of Bingöl changes between 19.1-23.2%.

According to the findings of experiment, the highest seed yield was obtained in Remzibey-05 variety with in 15th December, 15th Novembers owing date with 2766 kg ha⁻¹, 2755 kg ha⁻¹ and in Dinçer variety with in 1st December sowing date with 2677 kg ha⁻¹ respectively and the lowest was obtained from Remzibey-05 cultivar in 15th April sowing date with 1005 kg ha⁻¹. The average values of the varieties with regards to the sowing date were 1012 kg ha⁻¹ in the 15th April sowing date with and 2634 kg ha⁻¹ in the 15th December sowing dates. The seed yield of Remzibey variety (2038 kg ha⁻¹) was higher than Dincer variety (1971 kg ha⁻¹) (Table 5). The seed yield depends on the level of fertility of the soil, environmental conditions, sowing dates and variety. Hatipoğlu et al. (2012) stated that in the conditions of Şanlıurfa the highest yield (4260 kg ha⁻¹) is taken on October 30 while the lowest yield (980 kg ha⁻¹) was obtained on April 5. Keleş and Öztürk (2012) stated that seed yield in the conditions of Konya changes between 437.0-1706.1 kg ha-1. Coşkun (2014) stated that seed yield in the conditions of Çanakkale changes between 2643.3-2374.4 kg ha-1. As a result safflower can sow as winter and summer, Remzibey 05 is more suitable than other two varieties for high seed. Ghorbanzadeh et al. (2014) in the autumn sowing times 2330 kg ha⁻¹ and in the spring sowing times 1405.4 kg ha⁻¹. Öz (2016) in the autumn sowing times 2380.0 kg ha⁻¹ and in the spring sowing times 1405.4 kg ha⁻¹. When considering the performances of varieties according to sowing times, the highest seed yield was given Remzibey-05 variety sown in the autumn with 3156.6 kg ha⁻¹. Yenice cultivar tha tsown in the spring has occured the lowest seed yield with value 1098.3 kg ha⁻¹. Atan et al. (2019) stated that seed yield in the conditions of Hatay changes between 1883.3-2627.8 kg ha⁻¹. Baran and Andırman (2019) According to the results of the research, the highest seed yield was

obtained at the first sowing time (October 5) with 2158.3 kg ha⁻¹ in Batman conditions. Koç (2019) stated that seed yield of safflower farming between 2310-3380 kg ha⁻¹ in 2018 year. Aslantaş and Akınerdem (2020) stated that seed yield of safflower farming between 423-839 kg ha⁻¹. Öner and Şeker (2020) stated that seed yield of safflower farming in Çorum between 1244-2928 kg ha⁻¹. İzgi (2023) stated that yield of safflower in the conditions of Mardin changes between 1907-3981 kg ha⁻¹.

According to the findings of trial; the highest crude oil yield was obtained in Dinçer cultivar with in 1st December 831 kg ha⁻¹ and the lowest was obtained from Dinçer variety in 15th April sowing date with 332 kg ha⁻¹ (Table5). Keleş andÖztürk (2012) stated that crudeoil yield in the conditions of Konya changes between 133.8-446.2 kg ha⁻¹. Delayed sowing dates resulted in decreasing seed and oil yields. Coşkun (2014) stated that crude oil yield in the conditions of Çanakkale changes between 761-725 kg ha⁻¹. Atan et al. (2019) stated that crude oil yield in the conditions of Hatay changes between 738.0-1011.7 kg ha⁻¹. Aslantaş and Akınerdem (2020) stated that crudeoil yield in the conditions of Konya changes between 87-213 kg ha⁻¹.

Table 4. Mean values of safflower varieties at 2009-2010, 2010-2011 and 2011-2012 growing seasons.

	Crude o	oil ratio (%)		Prote	in ratio (%)	
Sowing date	Varie	ties	Average	Va	rieties	Average
	Dinçer	Remzibey-05	Average	Dinçer	Remzibey-05	Average
1 November	33.7 a	31.8 c	32.8 b	19.49 1	20.49 c	19.99 d
15 November	32.2 b	33.6 a	32.9 b	17.05 o	17.61 1	17.33 h
1 December	33.4 a	33.4 a	33.4 a	18.79 j	20.39 e	19.59 e
15 December	30.2 de	31.8 c	31.0 c	19.85 g	22.06 a	20.95 b
1 March	30.6 d	30.4 d	30.5 d	19.59 h	17.76 k	18.68 f
15 March	29.2 g	32.0 bc	30.6 d	20.30 f	16.32 m	18.31 g
1 April	27.9 h	29.5 fg	28.7 f	19.77 h	21.58 b	20.67 c
15 April	29.6 fg	29.8 ef	29.7 e	21.58 b	20.44 d	21.01 a
Average	30.9 b	31.5 a		19.54	19.57	
CV (%)	1.23				0.23	
LSD variety	0.17**				0.04**	
LSD sowing date	0.27**				0.05**	
LSD interaction	0.38**				0.07**	

ns: nonesignificant, **: significant at 0.01 level

Table 5. Mean values of safflower varieties at 2009-2010, 2010-2011 and 2011-2012 growing seasons.

	Seed yield	(kg ha ⁻¹)		Crude oil y	yield (kg ha ⁻¹)	
Sowing date	Variet	ies	Average	Varie	eties	Average
	Dinçer	Remzibey-05	Average	Dinçer	Remzibey-05	Average
1 November	2418 cd	2276 cd	2347 b	739 bc	677 cd	708 b
15 November	2179 d	2755 a	2467 ab	575 e	783 ab	679 b
1 December	2677 ab	2401 cd	2539 a	831 a	713 cd	772 a
15 December	2502 bc	2766 a	2634 a	660 d	782 ab	721 b
1 March	1750 e	1784 e	1767 c	427 fg	469 fg	448 c
15 March	1735 e	1771 e	1753 с	468 fg	480 f	474 c
1 April	1487 f	1545 ef	1516 d	412 gh	460 fg	436 с
15 April	1019 g	1005 g	1012 e	332 1	356 hı	344 d
Average	1971 b	2038 a		555 b	590 a	
CV (%)	14.96			11.97		
LSD variety	44.4**			16.9**		
LSD sowing date	171.3**			48.2**		
LSD interaction	242.3**			68.2**		

ns: nonesignificant, **: significant at 0.01 level

CONCLUSION

This study evaluated the effect of different sowing dates and cultivars on the yield and quality of safflower under Diyarbakır conditions. The findings indicate that the most suitable sowing period for optimal seed yield is between 15th November and 15th December, with the highest yields obtained from the Remzibey-05 variety. The study also showed that later sowing dates, particularly in April, result in significantly lower yields. Additionally, the Remzibey-05 variety consistently out performed the Dinçer variety in terms of oil content and protein ratio, further reinforcing its suitability for the region. Overall, the results suggest that adjusting sowing times and choosing the right cultivar, such as Remzibey-05, can significantly enhance safflower productivity in Diyarbakır. These results provide valuable in sights forfarmers and agricultural planners, emphasizing the importance of optimal sowing times to maximize safflower yield and oilcontent in semi-arid region slike Diyarbakır.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they haven oconflict of interest.

Author contribution

The contribution of the authors to the presentstudy is equal.

Funding

This work was supported by a grant from Republic of Turkey Ministry of Agriculture and Forestry (Project No: TAGEM/TA/10/05/01/002).

Acknowledgement

Of the trial 2011-2012 years results "Determination of Suitable Sowing Date of Safflower in Diyarbakır Conditions. International Mesopotamia Agriculture Congress/22-25 September 2014 Diyarbakır-Turkey" were published.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.5

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 34-40

Evaluation of cotton production in Sanhurfa province in terms of harvesting methods and economic costs

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Article History

Received: December 23, 2024 Revised: February 1, 2025 Accepted: February 3, 2025 Published Online: March 11, 2025

Article Info

Article Type: Research Article Article Subject: Agricultural Machines

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1606098







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Abstract

The present study was conducted to analyze the economic revenues/costs for 2020/2021 period in Sanliurfa, where cotton production is intense. The main source of this study was the data obtained from 15 cotton farmers in the area. It was determined in the study that the cotton yield per decare varied between 450 kg and 660 kg, and the average yield was 562 kg/da, and the average costs were spraying, fertilizing, harvesting, irrigation, sowing and soil preparation, respectively. The cost of producing one kilogram of cotton was found to be 1.68 TL/kg. The average revenue per decare was found to be 3970 TL/da and the production cost was 945 TL/da. It has been determined that the mechanized harvesting system is more advantageous than manual harvesting according to harvesting cost, total cost and net profit values. For cotton production to be commercially sustainable, it is essential to enhance efficiency in production, determine support measures for cotton that do not adversely affect farmers in the current period, and prioritize mechanized harvesting methods.

under the terms and conditions of the Creative Keywords: Cotton, Harvest, Yield, Cost, Profitability

Cite this article as: Kup, F. (2025). Evaluation of cotton production in Şanlıurfa province in terms of harvesting methods and economic costs. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 34-40. https://doi.org/10.31015/2025.1.5

INTRODUCTION

Agricultural products have become the raw material of not only the food sector but also for many other sectors with the increasing population growth and the development of technology. Cotton has also become an important industrial plant providing raw material to many sectors with different usage areas. It is an industrial plant, which is very important in the economies of countries contributing to the production of textile with cotton fiber, oil production with seeds, and also for the animal husbandry with its pulp. According to the data of the International Cotton Advisory Committee (ICAC), the top 6 countries in cotton production in 2019/2020 in the world were China, India, the USA, Brazil, Pakistan, and Turkey (ICAC, 2020a). According to Turkish Statistical Institute 2019 data, fiber cotton production was 814000 tons in Turkey. Şanlıurfa, however, realized approximately 37% of the total production of fiber cotton with 300906 tons (TSI, 2021). Turkey imports cotton much more than it produces. According to TUIK data, it was estimated that 2.2 million tons of seed cotton were produced in our country in 2019/20 period, and the amount of fiber cotton corresponding to this amount was 814 thousand tons. The consumption coverage ratio of production increased to 63% in 2018/19, it decreased to 50% in 2019/20 (Republic of Turkiye Ministry of Trade, 2022). Cotton contributes to employment and is an important source of revenues for farmers as well as being important in national economies. The Turkish textile industry is an indispensable sector with its added value, the amount of foreign currency that textile exports bring to the economy of the country, and the employment volume created with the labor-intensive workforce. The strategic raw material of our textile industry, which is the locomotive sector of our country, is made up of cotton (Republic of Turkiye Ministry of Trade, 2022). Cotton has great economic importance for humanity with widespread and compulsory usage areas and for producer countries with the added value and employment opportunities it brings with it (Gencer et al., 2005). The textile and ready-made clothing sector made up approximately 25% of the total industrial employment as of 2011 (National Cotton Council, 2021a). It is important to increase the economic added value of cotton for countries and farmers. The agricultural policies of the countries, the use of proper techniques in cotton

production and harvesting, etc. affects the economic added value of cotton. Especially technical and economic inadequacies in harvesting and post-harvest storage and in ginning steps and the deficiency in practice cause great losses in the added value of all segments from agriculture to industry in cotton production (National Cotton Council, 2021b). Sawgin Machinery should be preferred as it has better effects on cotton quality characteristics (Sağlam et al., 2021). It was also reported that cotton was more exposed to natural conditions such as humidity, dew, rain, and sun with machine harvesting later than in manual harvesting, which causes the fiber color to become dull and the trash ratio high (Terzi & Kaynak, 2019). In another study, it was reported that farmers followed the substitute product price in cotton production, and the price increased in the substitute product affected the cotton supply negatively (Özüdoğru & Miran, 2015). It was also reported that despite the continuous increase in input costs in cotton production, the variability in output prices had negative effects on the profitability and sustainability of production (Belay et al., 2020).

In the present study, an economic analysis of cotton production was made in Şanlıurfa for the 2020/2021 period. The costs from sowing to harvesting of cotton in 15 enterprises were determined for economic analysis, and the revenues obtained directly or indirectly were compared by making statistical analyzes. Economic comparisons were also made between hand harvesting and machine harvesting.

MATERIALS AND METHODS

Cotton production fields

The main material of the study was the data obtained from 15 cotton enterprises, which were consulted in Haliliye and Eyyubiye Districts of the province of Şanlıurfa in 2020/2021 period. The net revenues were obtained by following the revenues-cost values in the whole process from the sowing of the cotton to the harvest and post-harvest sales to make the economic analysis of cotton. In general, statistical comparisons were also made between hand harvesting and machine harvesting besides the economic analysis.

Statistical analyses

Harvesting was performed by machine in 11 of the 15 identified enterprises, and by hand, in 4 of these. Statistical analyses were performed to examine the effects of harvesting methods on revenues-cost balance and the factors affecting net revenues. In the present study, descriptive statistics are given as mean, standard deviation, percentage, and frequency; and the Mann Whitney U Test was used to examine the difference in revenues-cost according to the harvest method. The All-Pairwise Method was used to determine the different groups; and the Spearman Correlation Analysis was used to examine the factors affecting net revenues. The critical decision value was taken to be 0.05 in the analyses, which were made with the SPSS 25.00 Package Program.

RESULTS AND DISCUSSION

The data from 15 businesses in 2020/2021 period

The revenues and costs tables were created as a result of the data received from 15 businesses in 2020/2021 period. The revenues-costs statements are given in Tables 1 and 2.

The data in the cotton revenues-expenditure table were obtained by following all the transactions from the sowing to the sales of cotton instantly. The sales price of the product from 1 decare (TL/da) was taken into account as direct revenues in the creation of the revenues statements. The supports for 2020/2021 period were taken into account in the indirect revenues. Cotton premium support (TL/da), diesel support (TL/da), and fertilizer support (TL/da)were used as indirect revenues. Many countries provide support for the survival and development of cotton production, which has high commercial importance. The fact that the demand for cotton is more than the production in Turkey requires the activation of the support policy tools for this product (Erdal & Erdal, 2008). Yılmaz & Gül (2015) reported that input costs must be reduced, product incentive premiums and supports must be increased for the development of cotton production. Ali et al. (2012) stated that input costs must be reduced, and support prices must be increased so that cotton producers do not face losses. According to ICAC data, the top 5 countries supporting the cotton industry in the 2019/2020 season were China, the USA, India, Turkey, and Greece, respectively. The support of Turkey to the cotton industry amounted to \$232 million. No payments were made for uncertified seeds since the 2012/13 season. The premium paid for seed cotton produced from certified seeds remained unchanged at 0.8 Turkish Liras (TL/kg) in 2018/19 and 2019/20 (ICAC, 2020b). The decision on the agricultural supports to be made in 2020 was published in the Official Gazette with the number of 3190 on 05.11.2020. In this respect, it was decided to provide 62 TL diesel and 4 TL fertilizer support per decare for cotton (Anonymous, 2021a). However, amendments were made by abolishing the 3190 decision on 05.11.2020 regarding the supports. This amendment was published in the Official Gazette with the decision number 3589 on 05.03.2021 (Anonymous, 2021b). According to the final decision, 62 TL diesel and 8 TL fertilizer support would be given per decare. The seed cotton premium was determined to be 1.1 TL/kg. The Republic of Turkiye Ministry of Agriculture and Forestry announced for 2020/2021 season that the seed cotton premium was increased by 37.5% to 1.1 Turkish Liras per kilogram (TOB, 2021). The cotton support premium (500×1.1) per decare was determined to be 550 TL/da because there was a 500 kg cotton restriction per decare.

 Table 1. Revenue Table of Cotton Production

	po	s	(da)			Revenues				
Lands	Harvest method	Cotton species	Sowing area (da)	Yield (kg /da)	Unit price (TL/ kg)	Premium support (TL/da)	Diesel support (TL/da)	fertilizer support (TL/da)	Selling price (TL/da)	Total revenue (TL/da)
1	Machine	Candia	100	650	6.2	550	62	8	4030	4650
2	Machine	Bomba	43	550	5.5	550	62	8	3025	3645
3	Machine	St 468	55	570	6.4	550	62	8	3648	4268
4	Machine	Fiona	95	600	6.7	550	62	8	4020	4640
5	Machine	Candia	75	600	6	550	62	8	3600	4220
6	Machine	Fiona	200	630	6.5	550	62	8	4095	4715
7	Machine	St 468	50	600	6.2	550	62	8	3720	4340
8	Machine	Set 499	40	500	6.5	550	62	8	3250	3870
9	Machine	Candia	100	660	6.3	550	62	8	4158	4778
10	Machine	Bomba	78	550	5.8	550	62	8	3190	3810
11	Machine	Candia	155	575	6.4	550	62	8	3680	4300
12	Hand	Candia	25	500	5	550	62	8	2500	3120
13	Hand	Candia	30	450	4.8	495	62	8	2160	2725
14	Hand	Candia	37	540	5.5	550	62	8	2970	3590
15	Hand	Candia	20	460	5	506	62	8	2300	2876
Aver	age		74	562	6	543	62	8	3356	3970

Table 2. Costs of Cotton Production

	Costs							(B)	
Lands	Soil preparation (Diesel) (TL/da)	Sowing (Diesel+Seed) (TL/da)	Spraying (Diesel+Pesticide) (TL/da)	Fertilizing (Diesel+ Fertilizer) (TL/da)	Irrigation (TL/da)	Harvest (TL/da)	Total costs (TL/da)	Net revenues (TL/da)	
1	28	55	317.5	268.5	70	150	889	3761	
2	21	55.5	238	260	70	120	764.5	2880.5	
3	19.25	60	272	250	70	140	811.25	3456.75	
4	30	67	271	270	70	150	858	3782	
5	21	55.5	341	260	70	160	907.5	3312.5	
6	17.5	55	320	250	70	150	862.5	3852.5	
7	30	65	280	270	70	140	855	3485	
8	37.8	52	270	235	70	120	784.8	3085.2	
9	28	60	350	275	70	150	933	3845	
10	21	55.5	290	260	70	120	816.5	2993.5	
11	20	60	272	250	70	140	812	3488	
12	36	66	270	245	70	700	1387	1733	
13	30	65	200	260	70	750	1375	1405	
14	15	52.75	294	264	70	405	1100.75	2489.25	
15	28	56	240	260	70	375	1029	1891	
Average	25.5	58.68	281.7	258.5	70	251.33	945.72	3030.68	

The expenses for soil preparation, sowing, spraying, fertilization, irrigation, and harvesting were taken into account for the costs table. The average expenses were found to be spraying, fertilizing, harvesting, irrigation, sowing, and soil preparation, respectively, from highest to lowest rate in the present study (Ali et al., 2012). It was found that cotton input costs were, land rental, pesticides, irrigation, fertilization, weed control, and seeds, respectively (Yılmaz & Gül, 2015). The first among the material costs of cotton production was the cost of pesticides that had a share of 7.2%, followed by the cost of fertilizer (6.7%), and water (4.0%), respectively (Yılmaz & Demircan, 2005). Reddy et al. (2018) reported that the most important factors affecting the cost increase in cotton production were manpower and fertilizers and also mechanization must be improved and fertilizer must be used wisely to reduce manpower and fertilizer costs. Parlakay et al. (2021) stated that there is an excessive input usage in cotton production with irrigation (36.79%), fertiliser-N (17.88%), and pesticide (8.22%).

According to the data obtained from 15 enterprises for 2020-2021 period, the cost of producing 1 kg cotton was found to be 1.68 TL/kg. Uğurlu (2020) reported that the cost of producing 1 kilogram seed cotton was 2.17 TL/kg. The seed cotton production costs (TL/kg) between 2010 and 2020 were 1.088, 1.254, 1.71, 1.71, 1.82, 1.96, 2.1, 2.34, 3.55 and 4.07, respectively (Republic of Turkiye Ministry of Trade, 2022).

According to Table 2, the average production cost of cotton was found 945 TL/da. Candemir et al. (2017) stated that the average cotton production cost was 856.64 TL/da with the data obtained from 42 cotton enterprises in Kahramanmaraş in 2013.

The data from 15 businesses in 2020/2021 period

The Spearman Correlation Analysis was made to analyze the factors affecting the net revenues in cotton production. The results are given in Table 3.

Table 3. Factors affecting the net revenues level

Measurements		Net Revenues (TL/da)
Saving Ana (da)	r	0.696*
Sowing Area (da)	p	0.01
\$7°.13 (L. /3.)	r	0.905*
Yield (kg/da)	p	0.01
II.: 4 Deine (TI /I.e.)	r	0.938^{*}
Unit Price (TL/kg)	p	0.01
Premium Support (TL/da)	r	0.706*
Fremum Support (1L/da)	p	0.01
Colling Drice (TI /de)	r	0.983*
Selling Price (TL/da)	p	0.01
Total Davanua (TI /da)	r	0.984^{*}
Total Revenue (TL/da)	p	0.01
Soil Departure (Discol) (TI/do)	r	-0.27
Soil Preparation (Diesel) (TL/da)	p	0.34
Sowing (Diesel+Seed) (TL/da)	r	-0.15
Sowing (Dieser+Seed) (TL/da)	p	0.59
Spraying (Diesel+Pesticide) (TL/da)	r	0.687*
Spraying (Dieser+Festicide) (TL/da)	p	0.01
Fertilizing	r	0.24
(Diesel+ Fertilizer) (TL/da)	p	0.39
Hanvest (TI /de)	r	-0.877*
Harvest (TL/da)	p	0.01
Total Costs (TI \da)	r	-0.799*
Total Costs (TL\da)	p	0.01

^{*}Significant relation at 0.05 level

It was found that the net revenues levels were correlated positively and strongly with cultivation area (p=0.01); and that the net gains of the products with higher cultivation area would be higher.

It was observed that net revenues levels were correlated positively and strongly with productivity (p=0.01); and that the net gains of the products with high efficiency would be higher.

It was determined that the net revenues levels were correlated positively and strongly with the Sales Price (TL/kg) (p=0.01); and that the net earnings of products with higher Sales Prices (TL/kg) would be higher.

It was found that net revenues levels were correlated positively and strongly with Premium Support (TL/da) (p=0.01); and that the net earnings of products with higher Premium Support (TL/da) would be higher.

It was determined that the net revenues levels were correlated positively and strongly with the Sales Price per Decare (TL/da) (p=0.01); and that the net earnings of products with higher Sales Price per Decare (TL/da) would be higher.

The net revenues levels were found to be correlated positively and strongly with Total Revenues (TL/da) (p=0.01); and that the net earnings of products with higher Total Revenues (TL da-1) would be higher.

Soil Preparation (Diesel) (TL/da) and Sowing (Diesel+Seed) (TL/da) costs were not significantly associated with the net revenues (p>0.05)

It was found that the net revenues levels were correlated positively and strongly with Spraying (Diesel+Pesticide) (TL/da) (p=0.01).

Fertilization (Diesel+Fertilizer) (TL/da) costs were not significantly related to net revenues (p>0.05).

It was found that the net revenues levels were correlated negatively and strongly with Harvest (TL/da) (p=0.01), and the net gains of the products with high harvest (TL/da) would be lower.

It was found that net revenues levels were correlated negatively and strongly with Total Expenses (TL/da) (p=0.01). It was observed that the net earnings of products with higher Total Expenses (TL/da) would be lower.

According to this result, it was also found that methods to reduce expenses must be applied to increase the net revenues. Reddy et al. (2018) stated that real-time soil analysis tests and the use of integrated pest control methods would be beneficial in reducing fertilizer prices.

Economic analysis of harvest methods

The Mann-Whitney U Test was used to analyze the harvesting methods in economic terms. The results are given in Table 4.

Table 4. Analysis of revenues and costs according to the harvest method

	Harvest method		
Measurements	Hand	Machine	p
	X±s.d.	X±s.d.	
Unit price (TL/kg)	5.08±0.30	6.23±0.35	0.24
Premium support (TL/da)	525.25±28.93	550±0.01	0.35
Selling price (TL/da)	2482.5±353.68	3674.18±387.49	0.01*
Total revenue (TL/da)	3077.75±378.29	4294.18±387.49	0.01*
Soil preparation (Diesel) (TL/da)	27.25±8.85	24.87±6.28	0.32
Sowing (Diesel+Seed)	59.94±6.57	58.23±4.63	0.48
(TL/da)	39.94±0.37	36.23±4.03	0.46
Spraying (Diesel+Pesticide) (TL/da)	251±40.55	292.86±34.61	0.06
Fertilizing	257.25+8.38	258.95+11.88	0.29
(Diesel+ Fertilizer) (TL/da)	237.23±8.38	238.93±11.88	0.29
Harvest (TL/da)	557.50±194.87	140.00±14.14	0.01*
Total costs (TL/da)	1222.94±184.92	844.91±52.26	0.01*
Net revenues (TL/da)	1879.56±454.07	3449.27±348.19	0.01*

^{*}Significant at 0.05 level

It was found that sales price measurements and premium support revenues were not at different levels according to the method of harvesting by hand and machine (p>0.05).

It was determined that the sales price per decare and the total revenues levels were at different levels according to the harvesting method. It was also found that the sales price per decare and total revenues levels were higher in machine harvesting methods (p=0.01).

It was found that the costs of Diesel, Sowing, Spraying, and Fertilization in Soil Preparation were not at different levels when compared to manual and machine harvesting methods (p>0.05).

It was determined that there are differences in the rates of harvesting expenses, total expenses, and net revenues according to the manual and machine harvesting methods. The reason for the difference was found to be the fact that the harvest costs and net gains were lower in the manual harvesting method and the total costs were higher (p=0.01). As a conclusion, it was found that the machine harvesting system is more efficient when compared to manual harvesting in terms of harvesting costs, total costs, and net revenues. Yılmaz & Gül (2015) reported that the total labor costs decreased with the increased size of the land, and the reason for this was that machine harvesting was preferred and the labor costs were more economical compared to manual harvesting. There has been a significant decrease in the number of cotton pickers coming to the GAP area in recent years because of the partial transition to irrigated agriculture. Labor problems are pushing producers towards machine harvesting. However, the lack of infrastructure and information makes it difficult to switch to machine harvesting in addition to the expensive picking machines to be used in the harvest. Contracting services related to the problem must be supported, machinery access must be included in the scope of incentives, and producers must be provided with low-interest and long-term loans to purchase harvesters (Gencer et al., 2005).

CONCLUSION

In the present study, the input-output analysis of cotton production was investigated by using statistical methods as a result of the data obtained from 15 farmers who produced cotton in Şanlıurfa in 2020/2021 period.

- It was found that the average yield was 562 kg/da in cotton production for product per decare.
- The average sales price of 1 kg cotton was found to be 6 TL/kg.
- A total of 11 out of 15 farmers preferred machine harvesting, and 4 preferred manual harvesting.
- The average revenue from cotton production was found to be 3970 TL/da per decare.
- The cost of growing 1 kg cotton was 1.68 TL/kg.
- The production cost in cotton was 945 TL/ha per decare.
- It was found in the study that the average values in the costs for cotton production were spraying, fertilizing, harvesting, irrigation, sowing, and soil preparation, respectively, from the highest to the lowest impact.

As a conclusion, cotton is a commercially important plant for countries. For this reason, it was concluded that we need to increase productivity, and cotton supports must be determined in sufficient amounts not to harm farmers for the current period and in order for cotton production to be commercially sustainable. There are especially two methods that must be applied to increase productivity. The first is to increase the amount of cotton production; and the other is to make effort to reduce production costs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

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

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.6

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 41-49

Total phenolics and total flavonoids in *Ginkgo biloba* leaves of the plant optimization of the extraction conditions

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Article History

Received: November 6, 2024 Revised: February 23, 2025 Accepted: February 28, 2025 Published Online: March 9, 2025

Article Info

Article Type: Research Article Article Subject: Chemical Engineering

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1580065







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Abstract

Due to the negative health effects of artificial antioxidants, consumer interest in natural products has increased in recent years. The importance of natural antioxidants derived from plant sources is gradually increasing in research on the use of antioxidants as preservatives to prevent oxidative deterioration of foods. Free radicals cause degradation reactions in foods. They also cause important problems such as cancer, progeria, and heart disease in living organisms. Eating foods high in antioxidants has an important impact on slowing and stopping health problems. Phenols and flavonoids, known for their antioxidant activity, are found in many medicinal plants and provide various biochemical benefits to living organisms. Many different methods are used to obtain natural antioxidants. Current research is moving in the direction of further developing these methods. In this study, the antioxidant content of Ginkgo biloba leaves was investigated. A highly efficient ultrasound-assisted extraction method with short extraction time and minimal solvent consumption was developed for the extraction of Ginkgo biloba leaves. Experimental conditions for extraction yield: ethanol concentration 25-100%, solid/solvent ratio 100 mg 30-70 ml-1 sample, extraction time 15-60 minutes, temperature 30-70 °C. The result of the experimental study: ethanol concentration: 75%, extraction time: 45 minutes, temperature: 50 °C found for the best extraction efficiency. Optimization results for the amount of phenolic substance: extraction time: 31.22 min, extraction temperature: 54.12 °C, ethanol concentration: 57.94%. Optimization results for the amount of flavonoid substance: extraction time: 47.88 min, extraction temperature: 36.34 °C, ethanol concentration: 69.51%.

Keywords: Ginkgo Biloba, Phenolic, Flavanoid, Extraction, Optimization

Cite this article as: Karahan, M.Y., Bulduk, I. (2025). Total phenolics and total flavonoids in Ginkgo biloba leaves of the plant optimization of the extraction conditions. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 41-49. https://doi.org/10.31015/2025.1.6

INTRODUCTION

Traditional medicine has long emphasized the importance of biologically active compounds obtained from plants in their medicinal use (Bhattacharjee T. et al. 2020, Hedayat K.M. et al. 2020, Raji R.N. et al. 2019). Plant extracts are widely used in therapeutic applications, especially due to their antioxidant and antimicrobial properties. Current research has proven these effects on various plant species and revealed that secondary metabolites of plants play important roles in health (Srivastava J.P. et al. 1996, Wallace R.J. et al. 2004, Reghu R. Et al. 2017, Fazal H. et al. 2011)

Ginkgo biloba L. is one of the oldest tree species that has existed for millions of years and is considered a "living fossil" (Singh, B. et al. 2008). This plant, which has managed to remain structurally unchanged for more than 200 million years, contains various biologically active compounds and provides protection against insects, bacteria, and fungi thanks to its natural defense mechanisms. Ginkgo biloba leaves are rich in powerful antioxidant compounds such as kaempferol, quercetin, and isorhamnetin. These flavonoids help prevent diseases associated with oxidative stress by eliminating free radicals (Záhradníková L. et al. 2007, Ronowicz J. et al. 2013, Atzori C. et al. 1993).

Ginkgo biloba extracts have been found to have antiparasitic, antifungal, antibacterial, antiviral, and DNA-protective effects (Silva A.M. et al. 2019, Li M. et al. 2019, Perry E.K. 1999). Ginkgo extracts have been used for thousands of years in the treatment of bronchitis and other respiratory tract infections as well as cardiovascular diseases (Kleijnen, J. et al. 1992). In Western countries, it has been used in the treatment of atherosclerosis and cerebrovascular insufficiency since the 1960s (Xie L. et al. 2003. Diamond B.J.; et al. 2000). It has also been reported to be effective in the treatment of circulatory system disorders such as depression, memory loss, headache, and vertigo (Diamond, B.J.; et al. 2000). In this context, it is seen that *Ginkgo biloba* offers a wide therapeutic potential thanks to the bioactive compounds it contains and is an important plant in the field of health, especially due to its antioxidant and antimicrobial effects.

Response surface methodology (RSM) is an approach that includes mathematical and statistical techniques based on the fit of experimental data to a polynomial equation. RSM allows for numerical analysis of the behavior of a dataset and can be effectively applied in cases where multiple variables affect a response. The main objective of the method is to optimize these variables simultaneously to maximize the organizational output (Bezerra M.A. et al. 2008). Orthogonal experimental design and uniform design can only determine the best combination of variable ratios but cannot consider the optimal quality in the entire domain. RSM can predict the output (response) of a multivariate quadratic equation and this method has been successfully applied in various fields such as food (Y.Y. Chen. et al. 2012, S.H. Wu. et al. 2013] and medicine (M. B. Lan. Et al. 2012, Y.K. Hong. et al. 2013). The two main approaches frequently used in RSM are Box-Behnken Design (BBD) (Bezerra M.A. et al. 2008, J. Prakash et al. 2013) and Central Composite Design (CCD) (Bezerra M.A. et al. 2008, T. Zhu. et al. 2012). CCD can provide advantages such as orthogonality, rotation, and flexibility by varying the number of center points.

This study aimed to optimize antioxidant extraction conditions from *Ginkgo biloba* leaves by RSM method. Using five-level, three-factor CCD, the effects of temperature, time, and ethanol concentration on the extraction process were analyzed. The obtained results can be evaluated as a theoretical guide for the industrial production of antioxidants from natural sources.

MATERIALS AND METHODS

Ginkgo biloba leaves used in the experimental studies were obtained from a herbalist in Afyonkarahisar province of Turkey. The leaves were dried in a dark room for 15 days and then ground in a mill to make a fine powder.

Chemicals such as Folin-Ciocalteu reagent, gallic acid and quercetin standards, and aluminum chloride hexahydrate, methanol, and sodium carbonate were obtained from Sigma-Aldrich Co. (Istanbul, Turkey). Ultrapure water used in the experiments was produced with the Milli-Q System to have a conductivity of less than $0.05~\mu S~cm^{-1}$. All other chemicals used were of analytical purity.

Quantification analyses were performed using a dual-beam UV-visible spectrophotometer (Shimadzu UV1800, Japan) operating with 1.0 cm quartz cells and UV-probe software.

The ultrasound-assisted extraction process was carried out in a Wisebath brand ultrasonic water bath with a power of 900 W and a frequency of 50 kHz, equipped with a digital timer and a temperature control unit that provides both temperature and time control. Ultrasound waves were generated at a fixed frequency of 50 kHz at the bottom of the bath and spread into the water.

Ultrasound-Assisted Extraction

Ultrasound-assisted extraction is a method that enables the efficient extraction of plant components. In this study, the extraction process was carried out in a Bandelin Sonorex ultrasonic bath operating at a frequency of 50 kHz. For the process, 500 mg of dried and ground *Ginkgo biloba* leaves were added into a 100 mL volumetric flask, followed by the addition of 30 mL ethanol. The flask was then covered with aluminum foil and placed in an ultrasonic bath. The liquid level in the flask was adjusted to equal the water level in the ultrasonic bath. The aluminum foil helped to maintain the solution concentration by preventing the evaporation of ethanol. The device was operated following the experimental conditions specified in Table 1. After the extraction process was completed, the extracts were filtered through a 0.45-micron membrane filter. The extracts were stored in the refrigerator until total phenolic and total flavonoid analyses were performed.

Identification of Total Phenolic and Flavonoid Content

Total phenolic compounds were determined in each extract using the Folin-Ciocalteu method according to the previously described procedure (Petrović M. et al. 2022). For analysis, 1 mL of extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 2 mL of ultrapure water, and 4 mL of sodium carbonate solution (75 g L $^{-1}$). The resulting mixture was kept at 20 °C in the dark for 40 min and then the absorbance value was measured with a spectrophotometer at a wavelength of 765 nm. Gallic acid solutions prepared in methanol (five different concentrations in the range of 50-250 μ g mL $^{-1}$) were used to create the calibration curve (Figure 1) and the results were expressed in gallic acid equivalents (GAE).

Total flavonoid content was determined using the Aluminum chloride colorimetric method (Lopes, J. D. et al. 2022). For analysis, 1.0 mL of extract, 4.0 mL of ultrapure water, and 0.3 mL of sodium nitrite solution (5%) were added to a 10 mL test tube. After waiting for five minutes, 0.3 mL of aluminum chloride solution (10%) was added. After six minutes, 4.0 mL of NaOH (4%) solution was added and then the total volume was completed to 10 mL

with ultrapure water. After the prepared solution was mixed well, the absorbance value against the blank solution was measured using a spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength of 510 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE) per 1 g of dried plant. The calibration curve was generated using quercetin solutions prepared in methanol (five different concentrations in the range of 50-250 µg mL⁻¹) (Figure 2) and the results were expressed as quercetin equivalents (QE).

Identification of Total Phenolic and Flavonoid Content

In order to determine the most suitable conditions for ultrasound-assisted extraction, the surface response methodology was applied. In this study, three independent variables were selected: extraction temperature, extraction time, and ethanol concentration. These factors were coded as X_1 , X_2 , and X_3 , respectively, and were examined at five different levels (Table 1). The evaluation was made by taking the averages of the data obtained from 20 different experiments performed in three replicates. Accordingly, total phenolic and total flavonoid contents were determined. Each factor was coded at five different levels according to the equation given below (Prakash Maran, J. et al. 2013).

Table 1. Specific Variables and Rates of Central Composite Design.

Variables	Units	Crombala	Code levels					
v ariables	Units	Symbols	-1.68	-1	0	+1	+1.68	
Ext. Temp.	°C	(X_1)	20	30	40	50	60	
Ext. Time	min.	(X_2)	30	40	50	60	70	
Ethanol Conc.	%	(X_3)	15	30	45	60	75	

$$Y = \beta_0 \pm \sum_{j=1}^{k} \beta_j X_j \pm \sum_{j=1}^{k} \beta_{jj} X_j^2 \pm \sum_{i < j = 2}^{k} \beta_{ij} X_i X_j$$
 Equation (1)

In this equation, Y represents the dependent variable, x_i and x_j are independent variables (i and j vary from 1 to k). β_0 is the constant term, β_j is the linear coefficient, β_{ij} is the interaction coefficient and β_{ij} is the quadratic coefficient. Here, k indicates the number of independent variables, and k = 3 is determined within the scope of this study (G. E. P. Box. et al. 1957, Samavati V. 2013, Prakash Maran J. et al. 2013). The data obtained from the experiments were evaluated using the generalized least squares method and multiple regression analysis. Pareto analysis of variance (ANOVA) was applied to determine the statistical parameters. Surface response analysis was performed using Minitab 16 software, and the results were reported as mean \pm standard error. P < 0.05 indicates statistically significant effects.

RESULTS AND DISCUSSION

Linearity of the Analytical Method

Absorbance values obtained against 5 different concentrations were plotted and calibration linearity was obtained. Five consecutive absorbance values were obtained for calibration of the device from 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm standard solutions.

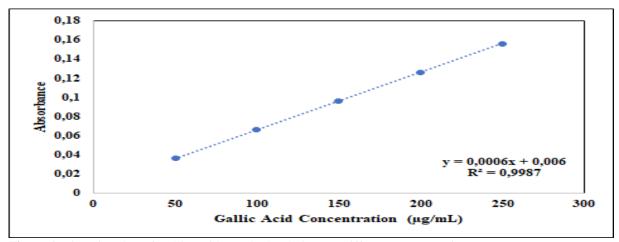


Figure 1. Linearity Plot Of Gallic Acid Standard Solutions at Different Concentrations.

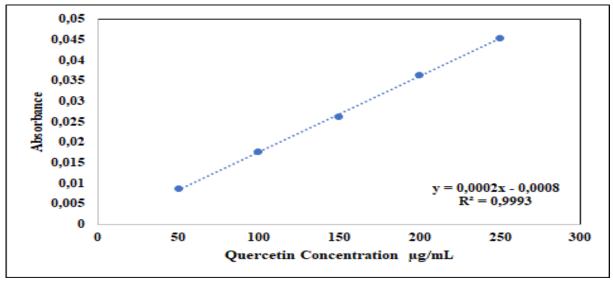


Figure 2. Linearity Plot of Quercetin Standard Solutions at Different Concentrations.

The central composite designs of the independent variables and the experimental results for total phenolic and total flavonoid contents are presented in Table 2.

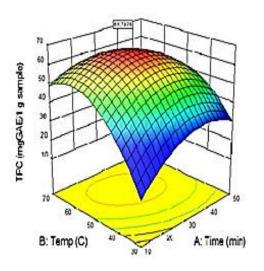
Table 2. Central Composite Designs of İndependent Variables and Experimental Results for Total Phenolic and Total Flavonoid Contents.

Experiment al study	Extraction temperature	Extraction time	Ethanol concentration	Total phenolic contents	Total flavonoid contents
	${}^{0}\mathbf{C}$	Minute	%	mg GAE g ⁻¹	mg QE g ⁻¹
1	30	30	45	35.00	6.16
2	40	40	30	41.67	7.49
3	30	50	45	60.94	8.50
4	50	50	45	52.78	8.29
5	40	60	30	51.87	10.51
6	30	50	45	57.49	9.30
7	30	50	45	61.70	8.58
8	10	50	45	47.66	6.97
9	20	40	30	33.69	6.68
10	40	40	60	50.33	8.06
11	30	50	15	41.23	10.16
12	40	60	60	58.79	11.34
13	30	70	45	53.52	10.93
14	30	50	75	60.96	7.61
15	20	60	60	61.62	8.39
16	30	50	45	62.26	10.73
17	20	40	60	45.16	9.38
18	30	50	45	63.47	11.79
19	30	50	45	64.59	12.01
20	20	60	30	50.55	9.38

Effect of Time on Extraction Efficiency

As shown in Table 1, it was observed that the extraction time at which the amount of phenolic and flavonoid substances was the highest increased continuously until the 45th minute, and after this time, the amount of total phenolic and total flavonoid substances decreased. The reason for this is that when the extraction time is raised, the cell walls of Ginkgo biloba leaves are completely separated and Ginkgo biloba leaves diffuse into the liquid

material. Overheating of Ginkgo biloba leaves during the long extraction time caused thermal degradation of the phenolic and flavonoid substance structure because phenolic and flavonoid molecules contain unstable chemical bonds such as unsaturated bonds, thus reducing the phenolic and flavonoid substance content. As a result, the preferable extraction time for phenolic and flavonoid extraction is 45 minutes. Response surface graphs showing the effect of extraction time and temperature on yield are shown in Figure 3.



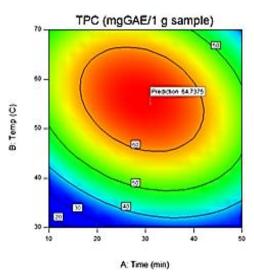
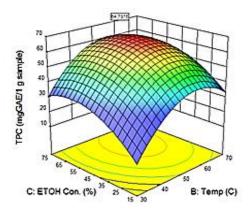


Figure 3. Surface Response Graphs Showing the Effect of Extraction Time and Temperature on Efficiency.

Effect of Temperature on Extraction Efficiency

Dried and ground Ginkgo biloba leaves were extracted at different temperatures. The amounts of total phenolic and flavonoid substances in the extracts were determined by UV-VIS spectrophotometer. The results showed that the amount of phenolic and flavonoid substances increased continuously until the extraction temperature of 50 °C and started to decrease after this point. High-temperature extractions improve extraction performance and mass transfer due to good desorption of solute from the active sites of the plant matrix. Initially, the increase in extraction efficiency with increasing temperature is because high temperature breaks phenolic and flavonoid substances from the plant cell and accelerates molecular movement. When the temperature increased above 50 °C, the extraction efficiency started to decrease. Temperatures greater than 50 °C cause the structure of phenolic and flavonoid substances to break down. Phenolic and flavonoid substances lose their activity and therefore the amount of phenolic and flavonoid substances decreases. As a result, the preferred temperature for phenolic and flavonoid extraction is 50 °C. Surface response graphs showing the effect of ethanol concentration and extraction temperature on yield are shown in Figure 4.



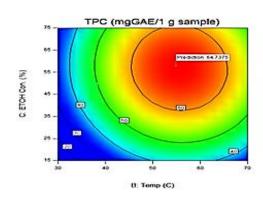
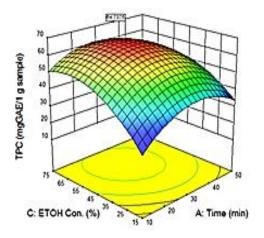


Figure 4. Surface Response Plots Showing The Effect of Ethanol Concentration and Extraction Temperature on Efficiency.

Effect of Ethanol Concentration on Extraction Efficiency

Ginkgo biloba leaf samples were extracted at different ethanol concentrations. Total phenolic and flavonoid content in the extracts were determined by Ultraviolet Visible Spectrophotometer. When the results were analyzed, the highest amount of phenolic and flavonoid substances was obtained at 75% ethanol concentration. As the

ethanol content increased up to 75%, an increase in total phenolic and flavonoid content was observed. As the ethanol concentration increased after this level, the amount of extracted substances decreased. Surface response graphs showing the effect of extraction time and ethanol concentration on the yield are shown in Figure 5.



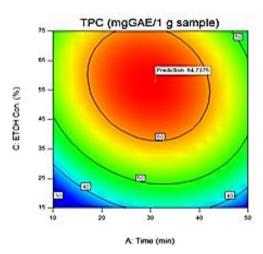


Figure 5. Response Surface Plots Show the Effect of Extraction Time and Ethanol Concentration on Efficiency.

Optimization of Ultrasonically Assisted Extraction by Surface Response Method

The single effects of the process parameters, known as the simultaneous single-factor approach, were implemented in the last section. This traditional model disregards the potential effects of interactions between the process parameters. The surface response methodology takes into account possible interactions. The table shows three parameters (Ethanol amount, time, and temperature) with minimum, medium, and maximum points.

20 different extraction methods were studied. The design was randomly selected by Expert software and the responses were recorded. Due to the software, a quadratic model using surface response methodology was obtained for the extraction yields, applying not only stepwise forward but also backward extinction regressions. A quadratic model using surface response methodology was derived from the software as given below. As a result of the analysis of the equation. 0.9569. The relationship between the predicted values and the experimental values is shown in Figures 6 and 7.

Model Fitting

The results of the ANOVA test for quadratic equations of the Design Expert 8.0.7.1 program are given in Equation 1 and Equation 2. The coefficients of the regression equation were calculated and the data were fitted in a second-order polynomial equation. The regression equation obtained from the ANOVA showed that the R2 (multiple correlation coefficient) was 0.9839. A value above 0.75 indicates that the model is appropriate. Regression analyses were performed at 95% confidence interval. The f value of the derived model is 41.46 and p < 0.0001 indicates that the derived model is appropriate.

This model calculated the overall variation in the data. Thus, this model was able to explain 98.39% of the variation in the response. R2 = 0.9584 and Predicted R2 = 0.9184. this shows that the model is good.

For a successful statistical model R2 value should be in the range 0-1.0. The adequate precision value of the current model is 28.388, which indicates that this model can be used for design. The adequate value is an indicator of the signal-to-noise ratio and values greater than 4 are essential preconditions for a model to be good. Simultaneously, relatively lower values of the coefficient of variation (CV = 3.13 %) indicate reliability and better precision of the values. The surface response method can be successfully applied to estimate the amount of flavonoids from the extraction of dried Ginkgo biloba leaves. Low values of the coefficient of variation indicate the reliability of the experimental results. In our study, the coefficient value (CV) is 3.13. The lower the coefficient of variation, the higher the reliability and sensitivity of the experimental results. In this framework, when the surface response graphs are analyzed; the possible conditions for the extraction of phenolic substances from Ginkgo biloba leaves; Time for extraction: 31.220, temperature: 54.119, Ethanol concentration: 57.944%. As a result, the optimum conditions of flavonoid extraction from Ginkgo biloba leaves: Time: 47.883 min., Temperature 36.336 °C, Ethanol concentration: 69.512%.

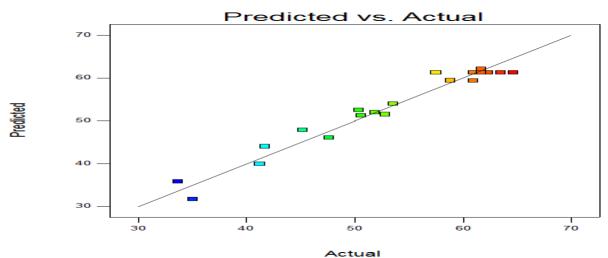


Figure 6. Linearity Plot of Experimental and Predicted Values of Total Phenolic Content in Ultrasonically Assisted Extraction.

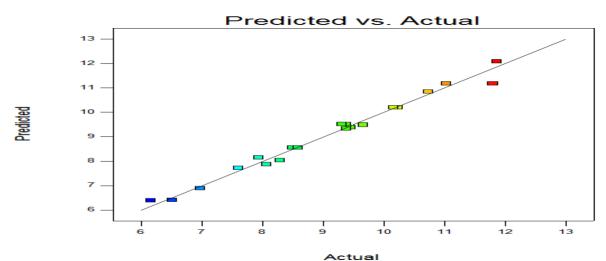


Figure 7. Linearity Plot of Experimental and Predicted Values of Total Flavanoid Content in Ultrasonically Assisted Extraction.

$$TPC = -193.90977 + 3.18511 * X_1 + 5.79490 * X_2 + 1.74633 * X_3 - 0.031185 * X_1^2 - 0.0460085 * X_2^2 - 0.012888 * X_3^2$$
 (Equation 2)

$$TFC = -8.61167 + 0.36503 * X_1 + 0.16116 * X_3 - 7.57172E - 004 * X_2 * -3.45813E - 003 * X_1^2 - 1.18677E - 003 * X_3^2$$
 (Equation 3)

 X_1 : Ethanol concentration. X_2 : Time. X_3 : Temperature

CONCLUSION

Response Surface Analysis Method was developed for the determination of total phenolic and total flavonoid content in the leaves of Ginkgo biloba, a member of the ginkgoinfa class, native to East Asia. Extraction temperature, extraction time, and solvent concentration were used as extraction parameters in the optimization. To find the optimal conditions where extraction temperature, ethanol concentration, and extraction time have a significant effect on the extraction results obtained by Ultrasonically Assisted Extraction, 20 different extractions were performed from Ginkgo biloba leaves at 3 different parameters (extraction time, extraction temperature, and ethanol concentration). Extraction parameters were optimized using Centrel Composite design in the Design Expert program. Extraction time (31.22 min), extraction temperature for total phenolics content: 54.11°C solvent concentration was 57.94% ethanol. The extraction time for total flavonoid content was 47.88 minutes, the extraction temperature was 39.33 °C, solvent concentration was 69.51% ethanol.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

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

Funding

No financial support was received for this study.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.7

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 50-56

The impact of abscisic acid application on grape quality attributes at harvest and post-harvest period

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Article History

Received: December 30, 2024 Revised: February 7, 2025 Accepted: February 10, 2025 Published Online: March 9, 2025

Article Info

Article Type: Research Article Article Subject: Agricultural Biotechnology Diagnostics

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Available at

tps://dergipark.org.tr/jaefs/issue/90253/1608549

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Abstract

Table grape varieties do not reach commercially acceptable colour levels in some growing seasons in viticultural areas. This situation has led to the consideration of applications that can enhance grape coloration. The study examined the effects of abscisic acid on 'Spil Karası' grape clusters and berries at harvest time and after harvest. A single application of 400 mg L⁻¹ ABA was made during the veraison period to evaluate its effects on berry coloration and post-harvest quality. Cluster weight losses were measured on days 7 and 15, while titratable acidity (TA), Brix, and fruit and rachis colour analyses were conducted on days 15 and 30 after harvest. The research results indicated that the application of ABA (abscisic acid) was not effective in terms of the parameters examined during harvest time analyses. The weight loss due to ABA application on the 15th day was 1.1% higher compared to the control. The ABA treatment group exhibited higher Lightness (L) values compared to the control group 30 days after harvest. The control group recorded the lowest Hue values at the same time point. However, this group displayed a high Chroma value. Conversely, the ABA treatment group showed a low Chroma (C) value during the same analysis period. Also the lowest This article is an open access article distributed L values were determined in ABA treatment and control groups 30 days after harvest in rachis.

Keywords: Grape coloration, Spil Karası, Lightness, Weight loss, Chroma

Cite this article as: Kandilli, G.G., Sen, A., Gulumser, H.B., Boz, Y. (2025). The impact of abscisic acid application on grape quality attributes at harvest and post-harvest period. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 50-56. https://doi.org/10.31015/2025.1.7

INTRODUCTION

The skin color of table grapes is one of the important fruit quality criteria for consumers (Palau et al., 2010), while the preservation of fruit quality after harvest is a critical factor for producers and in preventing post-harvest losses. The appearance of grapes, particularly their color, influences their commercial value, making colored varieties the most sought-after on the market due to their price and attractive visual appeal. Various regulators are applied to achieve coloration and ripening in grape berries. Abscisic acid is a critical regulator of the ripening process in non-climacteric fruits, with its functions involving epigenetic mechanisms (Li et al., 2024). ABA is a multifunctional phytohormone that begins to accumulate in grape berries as they ripen (Koyama et al., 2009). Abscisic acid (ABA) emerges as a key hormone in regulating alcohol dehydrogenase (ADH) gene expression and the synthesis of C6 volatiles during postharvest storage of grape (Chong et al, 2020). Due to its cost-effectiveness, ABA application has gained popularity, and its positive effects on berry color during veraison have been documented (Kataoka et al., 1982; Koyama et al., 2009), making it a potential alternative to treatments with ethephon (Cantin et al., 2007).

The color of grape berries is determined by the quantity and composition of anthocyanins in the skin (Ban et al., 2003). Anthocyanin accumulation in grapes begins at veraison, the onset of ripening, and it has been reported that this accumulation is at least partially regulated by the ABA (Hiratsuka et al., 2001; Ban et al., 2003). Several studies have shown that exogenous application of ABA increases the anthocyanin content in grape berries, thereby supporting the pigmentation of the skin (Ban et al., 2003; Jeong et al., 2004; Peppi et al., 2006; Yamane et al., 2006; Giribaldi et al., 2010).

Studies conducted on table grape varieties have demonstrated that ABA application is effective in achieving optimal coloration more rapidly compared to untreated grapes (Kataoka et al., 1982; Peppi et al., 2006, 2007; Cantin et al., 2007). In addition to abscisic acid, other chemicals such as ethylene, auxin, and brassinosteroids (BRs) have been studied to determine their effects on ripening and colouration. In the case of non-climacteric grapes, different results have been observed in its treatment with ethylene, with anthocyanin accumulation being observed in only some cases (Coombe and Hale, 1973; Delgado et al., 2004; Tira-Umphon et al., 2007). It has been observed that auxin delays ripening by slowing down anthocyanin accumulation (Coombe and Hale, 1973; Davies et al., 1997; Jeong et al., 2004; Wheeler et al., 2009). BRs may also play a role in grape berry ripening; however, the application of brassinazole, an inhibitor of BR synthesis, has been found to delay ripening (Wheeler et al., 2009). In addition to all these, abscisic acid (ABA) also plays a role in fruit ripening.

While numerous studies have examined the post-harvest effects of hormones like abscisic acid that enhance grape coloration, the efficacy and practical applicability of these findings remain unclear. Literature reviews, however, indicate that most research has primarily focused on coloration, with limited attention given to post-harvest quality assessments. The fact that the role of ABA in fruit ripening is still not fully understood has led us to focus our research on this aspect. This study seeks to address this gap. The Spil Karası grape variety was used in this research, and a single application of 400 mg L^{-1} ABA was made during the veraison period to evaluate its effects on berry coloration and post-harvest quality. Cluster and weight losses were measured on days 7 and 15, while acidity, Brix, and fruit and stem analyses were conducted on days 15 and 30.

MATERIALS AND METHODS

Locations and plant material

The experiment was conducted in a commercial vineyard situated in Fevziye village, Pamukova district, within Sakarya province (Figure 1A) in 2024. The geographic coordinates of the study site were 40°28'22.9"N, 30°06'12.5"E. The 'Spil Karası' grape variety was developed by the Manisa Viticulture Research Institute. The Spil Karası has a blue-black colored, seeded cultivar characterized by a conical cluster structure, with cluster weights ranging from 400 to 440 grams and individual berry weights of 4 to 5 grams.

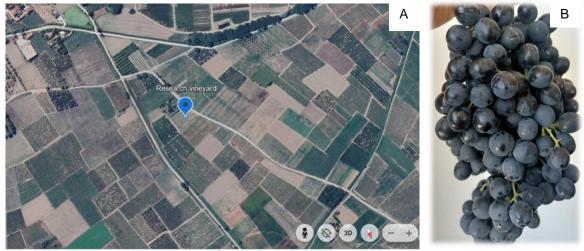


Figure 1. A) Research vineyard, B) Spil Karası grape (photo by G.G.Kandilli).

The experimental area

The vineyard was planted in 2021 with 'Spil Karası' grape cultivar (*Vitis vinifera* L.) grafted on 1103 Paulsen rootstock (Figure 1B). Vines were trained on a high wire cordon system at 120-150 cm above the ground.with two arms per vine. The vine rows were oriented south-southeast to north-northwest, with a spacing of 2 m between vines and 3 m between rows. The soil type is well-drained, clay-loam with pH 7.76. The vineyard was drip irrigated, and the irrigation amount was determined by monitoring soil, plant, and precipitation conditions every other day. This experiment was conducted using a completely randomized block design with three replicates, each replicate has three vines. The study utilized comparable vine capacity and crop load and standard cultural practices were followed (basal leaf removal, and shoot thinning).

Abscisic acid treatments

The ABA application was made one time during the veraison period, identified as the appropriate time in previous studies. (Li and Dami 2015, Karimi et al. 2016, Zhang and Dami 2012). Concentration of abscisic acid (ABA) was 400 mg. L-1. In each replicate group, a total of 9 vines were treated with three vines. No treatment was done to the control group.

Post harvest experiments and analysis of Brix, titratable acidity, berry and rachis colour

Only clusters meeting commercial standards were harvested. Clusters from each vine were harvested subsequent to the determination that the majority (75%) of the fruits had surpassed the commercial requirements of 17% total soluble solids (TSS). An initial analysis was conducted immediately following the harvest (T1), followed by two subsequent analyses (T2 and T3) at 15 days (DAH: days after harvest) intervals during the storage period. The grapes were stored under normal atmospheric conditions (NAC) at a temperature of $0\pm1^{\circ}$ C and a relative humidity of 85% - 90%.

A digital refractometer (Atago PAL-BX/ACID2) was used for Brix (%) and titratable acidity (%) analysis. The grape berry and grapes rachis colour was measured using chroma meter (Konica Minolta CR-400 Japan). The values obtained from colour analysis were converted to chroma and hue values by formulation.

Weight loss (WL): The percentage weight loss was calculated as the percentage of the harvest weight that had been lost, using the equation WL=((Wi-Wf)/Wi)*100. WL is the weight loss, Wi is the initial weight (g), Wf is the weight at each storage time (Trindate et al., 2023). The WL analyses carried out harvest, the followed two analyses 7 days interval in the postharvest periods.

Statistical analysis

The results of the study were subjected to one-way ANOVA at $p \le 0.05$ level and statistically significant means were compared by the LSD using the JMP statistical program The colour values (chroma, hue and L) values were illustrated by using scatter plot analysis in JMP (version. 7.0, SAS Institute Inc., Cary, NC) (SAS, 2003).

RESULTS AND DISCUSSION

The harvested grapes cluster and berry some morphological responses to ABA treatment were investigated. The treated grapes and the control group grapes did not differ statistically, as Table 1 illustrates. In other studies reported in parallel with our findings, that the quality attributes berry weight (Cantin et al, 2007, Yamamoto et al., 2015), berry width and length (Shahab et al., 2020, Yamamoto et al., 2015) and cluster weight (Gu et al., 2011) remained unaffected in ABA treatments. However Wheeler et al. (2009) found that ABA application increased berry weight. Weight loss, a straightforward and quantifiable parameter, is commonly used to effectively evaluate the impacts of treatments on the postharvest quality of fruits (Crisosto et al. 2001). There was no significant difference between ABA-treated and control plants in yield.

Table 1. Cluster and berry size at harvest time

Treatment	Cluster weight (g)	Berry weight (g)	Berry width (mm)	Berry length (mm)
ABA	410.3	4.29	1.82	2.12
Control	483	4.68	1.84	2.19
p	NS	NS	NS	NS

NS: Non significant (p≤0.05)

The impact of ABA treatment on weight loss on the 7th and 15th day after harvest was examined, and it was found that the weight loss due to ABA application on the 15th day was 1.1% greater than that of the control (Table 2). After harvest, table grapes are highly vulnerable as they experience significant water losses due to rachis and pedicel desiccation, leading to browning, weight reduction, and berry softening (Romero et al.,2020). The study found that weight loss, a critical factor limiting the shelf life of the products, increased over the storage period. Although the extent of weight loss varied with the treatment, it consistently grew with time. These weight losses during storage adversely impacted other quality attributes, leading to concurrent declines in fruit appearance and discoloration of the rachis and pedicels as maturity progressed. Weight loss, a straightforward and quantifiable parameter, is commonly used to effectively evaluate the impacts of treatments on the postharvest quality of fruits (Crisosto et al. 2001). Undesirable outcomes for fresh grapes during post-harvest storage include loss of mass, shattering, and decay, as these factors diminish the overall quality of grape clusters (Neto et al., 2017).

Table 2. Cluster weight loss during postharvest

Treatment	1	7	15	WL (%)
ABA	472	358	245	4.8
Control	466	383	292	3.7

Cluster weight: g, 1: Harvest, 7: 7 DAH, 15: 15 DAH.

The analysis of ABA application's effect on Brix and acidity at harvest and post-harvest revealed that the treatment group berries had higher Brix content only in the analyses conducted 15 days after harvest. The acid content did not change significantly with ABA treatment (Table 3). Similar results were reported in other studies investigating the effect of ABA on grape titratable acidity and brix (Ferrara et al. 2015, Gu et al., 2011, Shahab et al., 2020). Conversely, certain studies have found that the application of ABA can enhance the total soluble sugar (Wheeler et al., 2009, Kok, 2022) and reducing sugar levels in grape berries (Sun et al., 2019). The application of

abscisic acid to grape clusters during post-harvest storage was found to decrease the titratable acidity and increase the total-soluble solids content (Chong et al., 2020).

Table 3. Influence of ABA application on Brix and acidity levels over postharvest periods

Treatment	Brix (%)	Titratable acidity (%)
T1	18,23	0,48
C1	19,70	0,69
T2	19,20 a	0,66
C2	18,50 b	0,54
T3	20,57	0,69
C3	20,10	0,70

Different letters denote statistically significant differences between treatments and postharvest periods based on LSD test ((p< 0.05) . T: ABA Treatment, C: Control, * The numbers next to the treatment abbreviations represent the number of days after harvest when the measurements were take (1: Harvest, 2: 15 DAH, 3: 30 DAH).

To comprehend the relationship between chroma and hue values and L, the response variable in this investigation, a three-dimensional scatter plot was employed. In both the berry colour and rachis colour plots, the highest L value was obtained in the analysis at harvest time. Shahab et al., (2020) and Yamamoto et al., (2015) reported that the application of abscisic acid resulted in a lower L value in harvested grapes. A lower L value is often associated with a more intense, darker berry color according to existing research (Roberto et al., 2012; Tecchio et al., 2017). The L values of the ABA treatment 30 days after harvest are higher than those of the control group in berry colour (Figure 2A). The lowest hue values were recorded in the control group 30 days after harvest. However, this group showed a high chroma value. On the other hand, a low chroma value was observed in the ABA treatment at the same analysis period. Kok 2022 found that abscisic acid application during the veraison period decreased the hue values of grapes at harvest time. However, the current investigation was unable to determine the effect of ABA application on harvest-time hue values.

The lowest L values were determined in ABA treatment and control groups 30 days after harvest in rachis (Figure 2B). High chroma and hue values were measured in both treatment and control groups at harvest, and average chroma values were measured in both groups 30 days after harvest. There was no significant distribution of the colour values measured in both the berry colour and the rachis colour in the analyses carried out 15 days after the harvest. Rachis browning is a prevalent issue that compromises the quality and marketability of table grape clusters. To consumers, a green rachis signifies freshness, whereas a brown rachis can lead to consumer rejection and eventually, fruit waste (Lichter, 2016). While water loss is acknowledged as the primary contributor to this problem, measurements have typically focused on the weight loss of the entire grape cluster (Balic et al., 2012), with limited investigations specifically examining the rachis (Hamie et al., 2022). Increased anthocyanin levels and antioxidant capacity have been associated with improved resistance of the grape rachis against browning (Qin et al., 2022).

During the early stages of fruit development, endogenous abscisic acid content is closely associated with fruit water loss. In the initial phase of fruit development, ABA content declined; nevertheless, it increased continuously after the color change period (Chong et al., 2020).

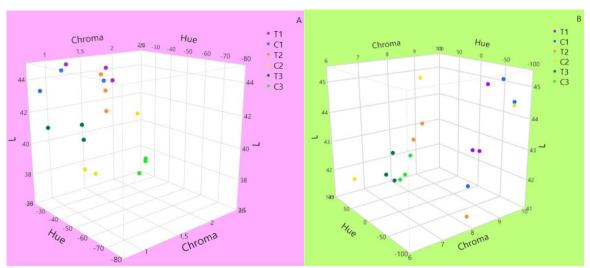


Figure 2. Scatter plots for Chroma, Hue and L values. A) Grapes berry color (n=3), B) Grapes rachis color (n=3), T: ABA treatment, C: Control; 1: Harvest time, 2: 15 days after harvest, 3: 30 days after harvest.

Grapes exhibit an accumulation of anthocyanins, the pigments responsible for their color, during the veraison stage of development. This process appears to be regulated, at least in part, by the plant hormone abscisic acid (Owen et al., 2009). Exogenous abscisic acid application enhanced the accumulation of anthocyanins, particularly the petunidin and malvidin types and quantitative real-time PCR analysis demonstrated that ABA treatment around the véraison stage led to the upregulation of genes encoding enzymes involved in the biosynthesis of both general flavonoids and anthocyanins (Katayama-Ikegami et al. 2016). Recent studies have shown that ABA treatment can alter global DNA methylation patterns in grapes. These findings indicate that ABA induces changes in the DNA of genes related to ripening and stress response during grape maturation (Li et al., 2024). In addition, ABA affects the transcription of genes and the activity of proteins involved in sugar accumulation and metabolism during grape ripening (Wheeler et al., 2009).

CONCLUSION

In conclusion this study offers a new perspective on the effects of ABA application during the both at harvest time and in the post-harvest period. While previous ABA studies have primarily focused on harvest measurements, the examination of quality parameters during the post-harvest period is limited. This study contributes to this understudied area. The results indicate that ABA application did not impact cluster weight, berry size and weight, Brix, or titratable acidity, which is consistent with previous findings. However, a key finding is that ABA application increased cluster weight loss and accelerated a darker coloration, characterized by higher L (lightness) and lower chroma (saturation) values, in the post-harvest period. The measurement of grape rachis color further enhanced the originality of this study.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

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

Acknowledgements

We would like to thank grape grower Metin Cömert for the collaboration. Also many thanks to Yusuf Demir, Development & Regulatory Manager at Sumitomo Chemical Türkiye, for supplying absisic acid.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.8

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 57-67

Export similarity index as a barometer of Turkey's agricultural machinery and equipment export competitiveness

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Article History

Received: January 7, 2025 Revised: February 21, 2025 Accepted: February 27, 2025 Published Online: March 9, 2025

Article Info

Article Type: Research Article Article Subject: Agricultural Economics

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Available at

/dergipark.org.tr/jaefs/issue/90253/1614997

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Abstract

This is study aims to evaluate Turkey's agricultural machinery and equipment export performance with the aid of the Export Similarity Index (ESI) from a relative and holistic perspective with the leading countries in the global export market (China, Germany, United States of America [USA], Japan, Italy). For this purpose, export data for 2002-2021 were extracted from the Trade Map database. According to the Harmonized System (HS) as a 6-digit code system, the ESI value was calculated for 39 agricultural machinery and equipment product groups (subproduct groups 69, 82, 84, 87). The results of the study showed that, the ESI values regarding Turkey's agricultural machinery and equipment exports with China, Germany, the USA, and Italy increased in this mentioned period. These increase in ESI values indicated that, the countries export similar products and therefore the competition between them has increased. However, the decreases in Turkey's ESI values compared to Japan mean that, the similarity in the products they export and the competition between the two countries have decreased. Turkey's ESI value of "67.19" with Italy in 2021 shows that ,Turkey is in high competition with Italy in agricultural machinery and equipment exports. In addition, Turkey's ESI value of "26.46" with China in 2002 increased to "46.23" in 2021. This increase in the ESI value shows that, Turkey and China are increasingly exporting similar products and that competition between the two countries is increasing. China's performance is a serious threat to Turkey. To strengthen Turkey's agricultural machinery and equipment export performance, comprehensive export strategies that include both companies in the sector and state-led efforts are needed.

Keywords: Export, Competitiveness, Export Similarity Index, Agricultural Machinery and Equipment Sector

Cite this article as: Aktas Cimen, Z., Ertekin, C. (2025). Export similarity index as a barometer of Turkey's agricultural machinery and equipment export competitiveness. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 57-67. https://doi.org/10.31015/2025.1.8

INTRODUCTION

Agriculture, one of the most important sectors for humanity, is the practice of cultivating natural resources to sustain human life and provide economic gain. It is one of the most powerful sectors to end extreme poverty, increase shared prosperity and nourish people. Agricultural lands are the basic building blocks of agricultural production. According to the World Bank (World Bank [WB], 2024), agricultural lands constitute 34.04% of the world's surface area. In this area, China ranks first with a share of 10.89%, the United States (US) ranks second with a share of 8.49%, Australia ranks third with a share of 7.60% and Turkey ranks thirty-fourth with a share of 0.80%. The ratio of Turkey's agricultural land area to Turkey's surface area is 0.48%. With this ratio, Turkey ranks 34th in the world among 210 countries (ANNEX 1). The increasing global population puts great pressure on the agricultural sector. The rapid growth of the world's population also causes an increase in food demand. This means that agriculture should produce more. In addition, it is important to make agriculture more efficient, productive and environmentally friendly for a sustainable future. Therefore, in agriculture, in addition to other approaches, limited areas should be used more effectively and efficiently. This can be achieved with precision agriculture practices that carry out agricultural production more intelligently and efficiently. In addition, agricultural lands decreased by 1.62% worldwide from 2002 to 2021, while the world population increased by 25.19% in the same period. In Turkey, agricultural lands decreased by 7.54% in the period 2002-2021, while the population increased by 25.50% (WB, 2024). The increase in food demand caused by the increasing population and the globalization of food markets increase international competition in agriculture. This increase in competition makes it important to reduce production costs and use resources effectively and efficiently to be strong in the global market (Hartanto et al., 2019). Agricultural machinery and equipment that shape agriculture (ANNEX 2) play an important role in increasing the efficiency of food production against increasing food demands. Especially developing biotechnology, robotics, and automation systems increases the capacity of agricultural machinery and equipment and encourages sustainable agricultural practices. In this context, the agricultural machinery and equipment sector stands out not only as an industry branch, but also as an important factor in shaping the global agricultural economy and food security. In addition, agricultural machinery and equipment help achieve environmental sustainability goals as well as productivity increase (Patel et al., 2024a). Sustainability is an important driving force in shaping the future of agricultural machinery. Developments such as smart agricultural technologies and digitalization, water-saving irrigation systems, and biological solutions for pest control offer solutions to minimize environmental impacts. Information technology and data-driven processes have changed the processes in the plantation and cattle industry. Machines are no longer alone, but are connected to the cloud and other machines and equipped with special sensors for precision agriculture (Patel et al., 2024a). There is increasing emphasis on developing ecofriendly equipment that reduces carbon footprints. Electric and hybrid tractors that run on renewable energy sources are becoming increasingly popular in line with global efforts to address climate change. Moreover, the agricultural machinery and equipment market is trending towards integrated farming systems. This involves combining various agricultural activities such as crop cultivation and livestock management to create synergies that increase agricultural productivity, which is driving the growth of the agricultural machinery and equipment market (IndustryARC, 2024a). Moreover, agricultural machinery and equipment are an important input in the agricultural process and play a major role in increasing yields as well as reducing input costs such as labor, seeds, and fertilizers, thus increasing agricultural incomes (Banerjee and Punekar, 2020) (Table 1).

Table 1. Farming Machines and Tools: An Overview Benefits Analysis (Patel et al., 2024b).

Equipment	Function	Types	Benefits
Tractors	Power source for various farming tasks	Utility, row-crop, orchard, garden, inustrial	Versatility, efficiency
Plows	Turning and loosening soil	Moulboard, chisel, disc	Improved soil structure, weed control
Harrows	Breaking up soil clods, levelling soil	Disc, tine, chain	Seedbed preperation, moisture retention
Cultivators	Stirring soil around crops	Spring-tooth, field	Weed control, aeration
Seeders	Sowing seeds uniformly	Broadcast, air	Efficient planting, precision
Planters	Precise planting of sees	Row crop, precision	High germination rates, reduced seed waste
Transplanters	Planting seedlings	Vegetable	Efficient transplanting, labor savings
Sprinkler systems	Distribution water	Centre pivot, lateral move	Uniform water application, reduced labor
Drip irrigation	Watering plants at root level	Drip lines, emitters	Water conservation, increased efficiency
Flood irrigation	Flooding fields with water	Furrows, basins	Simple, low-cost
Sprayers	Applying pesticides and herbicides	Boom, air-blast	Pest and disease control, efficient application
Dusting equipment	Applying powdered chemicals	Usters, blowers	Targeted application, reduced drift
Combine harvester	Harvesting grains	Self-propelled, tractor mounted	Efficient harvestng, high capacity
Forage harvesters	Harvesting forage crops	Self-propelle, trailed	Efficient harvestng, high-quality forage
Balers	Compressing crops into bales	Square, round	Hay and straw storage, transportation
Mowers	Cutting grass or crops	Rotary, sickle, flail	Efficient mowing, pasture management
Grain handling equipment	Moving an storing grains	Augers, conveyors, grain dryers	Efficient handling, preservation
Grain storage bins	Storing harveste grains	Steel, concrete	Secure storage, pest control
Feeding equipment	Distributing feed	Feeders, mixers	Efficient feeeing, Reduced labor
Watering equipment	Providing water to animals	Waterers, troughs	Clean water Access, animal welfare
Manure spreaders	Distributing manure as fertilizer	Broadcast, injection	Nutrient recycling, soil improvement
Agricultural Drones	Monitoring crops, spraying	Multi-rotor, fixed-wing	Precision agriculture, data collection
GPS Systems	Precision farming, mapping fields	Receiver, software	Accurate field operations, data analysis

The size of the global agricultural machinery and equipment market was assessed at USD 192.10 billion in 2023. This market is expected to reach USD 206.80 billion in 2024 and reach USD 324.88 billion by 2030 with a CAGR of 7.79% (GII Global Information, 2024). The agricultural machinery and equipment market, largely defined by the need to increase agricultural productivity and efficiency, is of vital importance to meet global food demand fueled by population growth. According to Trade Map 2021 data, Turkey, which ranks thirtieth in world exports with a share of 1.02%, is a country where agricultural mechanization is important due to its ability to produce in all seasons in terms of climatic conditions. Turkey's machinery exports are ranked fifth in agricultural machinery and equipment exports among 22 sectors (TARMAKBIR, 2022). Aktas Cimen and Ertekin (2023), in their studies on the 2001-2021 period using Trade Map data, showed that the share of agricultural machinery and equipment exports in world exports has remained quite stable, but the share of agricultural machinery and equipment exports in Turkey's total exports has generally increased steadily. As the global economy grows, the need for a deeper understanding of the various benefits of trade also increases. This article aims to reveal Turkey's competitiveness by comparing its export similarity to the world market in the agricultural machinery and equipment sector with the leading countries in the world in the sector. This study comparatively reveals Turkey's export similarity to the global market against ten countries in the ranking of export revenues in the world agricultural machinery and equipment sector for the period 2002-2021. No existing study has been found in the literature that determines the competitiveness of the agricultural machinery and equipment sector on a global scale. As a result, this study is expected to fill this gap in the literature.

Overview of the Agricultural Machinery and Equipment Market

The agricultural machinery and equipment sector continues to be equipped with modern technologies in line with the goals of increasing productivity, optimizing the workforce, and environmental sustainability. The global agricultural machinery market volume is 171.4 billion USD, of which 8.3 billion USD is France, 8.2 billion USD is Germany and 3.3 billion USD is Turkey (Centre for Sustainable Agricultural Mechanization, 2023). When the export data for the agricultural machinery and equipment sector in Turkey and the world is examined, an increase is observed (Table 2).

Tablo 2. Course of Agricultural Machinery and Equipment Exports in Turkey and the World (2002-2021).

Years	Turkey Agricultural Tools and Equipment Export (x1000 US \$)	Turkev	Turkey Agricultural Tools and Equipment Export (x1000 US \$)	World Change (%)
2002	103085	-	25693391	-
2003	228288	121.46	29587322	15.16
2004	262052	14.79	36383071	22.97
2005	283701	8.26	40514027	11.35
2006	317204	11.81	44807336	10.60
2007	414278	30.60	54540516	21.72
2008	564629	36.29	70206795	28.72
2009	485673	-13.98	50780145	-27.67
2010	532938	9.73	54769620	7.86
2011	626603	17.58	70406035	28.55
2012	806325	28.68	72907729	3.55
2013	853309	5.83	74700191	2.46
2014	1022732	19.85	73441172	-1.69
2015	945804	-7.52	63892286	-13.00
2016	860669	-9.00	62107793	-2.79
2017	951580	10.56	70440948	13.42
2018	1127056	18.44	75898338	7.75
2019	1243226	10.31	73187422	-3.57
2020	1264995	1.75	73160595	-0.04
2021	1646014	30.12	90904116	24.25

Reference: Prepared by the authors based on Trade Map (2023) data.

As can be seen from the table, Turkey's agricultural machinery and equipment sector exports in the 2002-2021 period (excluding crisis years) show a better outlook than the sector's world exports. In the 2009 financial crisis, Turkey's agricultural machinery and equipment sector exports decreased by 13.98%, while the sector's world exports decreased by almost twice as much as Turkey's exports (27.67%). A similar situation was experienced in

the sector in 2015, 2019 and 2020. While world agricultural machinery and equipment exports decreased by 2.79% in 2016 alone, Turkey's agricultural machinery and equipment exports experienced a decrease of more than 3 times the sector's world exports (9.00%) due to the coup attempt, losses in the tourism sector, and global and geopolitical developments. However, in the period 2002-2021, Turkey's agricultural machinery and equipment sector exports have increased much more than the sector's world exports and contribute more to the national income.

Among the top ten countries contributing to the world economy with agricultural machinery and equipment exports, China's export performance is remarkable (Table 3).

Table 3. Top Ten Countries and Their Shares in World Agricultural Machinery and Equipment Export Revenues Ranking According to HS Codes (2021, x1000 US \$).

HS 69	Export Value	Country Share (%)	HS 82	Export Value	Country Share (%)
China	30695409	42.41	China	23196436	30.29
Italy	6429404	8.88	Germany	10387146	13.56
Spain	5150524	7.12	USA	4740156	6.19
Germany	4392013	6.07	Japan	3964131	5,18
India	2314625	3.20	Taipei, China	3590191	4.69
USA	2297719	3.17	Switzerland	2216323	2.89
Japan	1831989	2.53	Korea	2113170	2.76
Turkey	1616311	2.23	Italy	2054260	2.68
Mexico	1493034	2.06	Poland	1864043	2.43
Poland	1316015	1.82	Belgium	1761357	2.30
			Turkey (28th)	326131	0.43
WORLD	72380725	79,49	•	76590397	73.40
HS 84	Export Value	Country Share (%)	HS 87	Export Value	Country Share (%)
China	547585389	21.99	Germany	245903308	16.31
		• • • • • • • • • • • • • • • • • • • •	Germany Japan	245903308 137909344	16.31 9.14
Germany	547585389	21.99			
Germany USA	547585389 268562339	21.99 10.79	Japan	137909344	9.14
Germany USA Japan	547585389 268562339 209284552	21.99 10.79 8.41	Japan USA	137909344 122202361	9.14 8.10
Germany USA Japan Italy	547585389 268562339 209284552 147382033	21.99 10.79 8.41 5.92	Japan USA China	137909344 122202361 120021475	9.14 8.10 7.96
Germany USA Japan Italy HK*, China	547585389 268562339 209284552 147382033 108057165	21.99 10.79 8.41 5.92 4.34	Japan USA China Mexico	137909344 122202361 120021475 115018307	9.14 8.10 7.96 7.63
Germany USA Japan Italy HK*, China	547585389 268562339 209284552 147382033 108057165 95090192	21.99 10.79 8.41 5.92 4.34 3.82	Japan USA China Mexico Korea	137909344 122202361 120021475 115018307 67015410	9.14 8.10 7.96 7.63 4.44
Italy HK*, China Holland	547585389 268562339 209284552 147382033 108057165 95090192 90145570	21.99 10.79 8.41 5.92 4.34 3.82 3.62	Japan USA China Mexico Korea Spain	137909344 122202361 120021475 115018307 67015410 54144088	9.14 8.10 7.96 7.63 4.44 3.59
Germany USA Japan Italy HK*, China Holland Mexico	547585389 268562339 209284552 147382033 108057165 95090192 90145570 85268563	21.99 10.79 8.41 5.92 4.34 3.82 3.62 3.42	Japan USA China Mexico Korea Spain Belgium	137909344 122202361 120021475 115018307 67015410 54144088 51734829	9.14 8.10 7.96 7.63 4.44 3.59 3.43
Germany USA Japan Italy HK*, China Holland Mexico Korea	547585389 268562339 209284552 147382033 108057165 95090192 90145570 85268563 76018245	21.99 10.79 8.41 5.92 4.34 3.82 3.62 3.42 3.05	Japan USA China Mexico Korea Spain Belgium France	137909344 122202361 120021475 115018307 67015410 54144088 51734829 50825662	9.14 8.10 7.96 7.63 4.44 3.59 3.43 3.37

Reference: Prepared by the authors based on Trade Map (2023) data.

China ranks first in the agricultural machinery and equipment product groups coded HS 69 (42.41%), HS 82 (30.29%) and HS 84 (21.99%), while it ranks fourth in the world in the HS 87 (7.96%) coded product group, which is understood to be advantageous in its competitiveness in agricultural machinery and equipment exports. The USA ranks third in the world in the export of the three agricultural machinery and equipment product groups coded HS 82, 84 and 87, and sixth in the world in the export of the HS 69 coded product group. Japan, the world's third largest economy after the USA and China, ranks fourth in the world in the export of agricultural machinery and equipment coded HS 82, 84, and second in the export of agricultural machinery and equipment coded HS 87 with a share of 9.14%, while it ranks seventh in the export of the HS 69 coded product group. Germany, which ranks fourth after the USA, China and Japan with its strong economy, ranks second in the world in HS 82 and 84 coded agricultural machinery and equipment exports, ranks first with a share of 16.31% in the HS 87 coded product group, and ranks fourth in the world in the HS 69 coded product group. Italy, the 3rd largest national economy in the Eurozone, ranks second in the HS 69 coded agricultural machinery and equipment product group, eighth in the HS 82 coded product group, fifth in the HS 84 coded product group and tenth in the HS 87 coded product group. Turkey, which ranks thirtieth in world exports, has been determined to be eighth in the HS 69 coded product group, seventeenth in the world exports in the HS 87 coded product group, twenty-sixth in the world exports in the HS 84 coded product group and twenty-eighth in the world in the HS 82 coded product group. In the global market for agricultural machinery and equipment exports, Turkey seems to have a greater advantage in the export of the HS 69 coded product group compared to the export of the HS 82, 84 and 87 coded product groups when compared to the developed economies of the world.

Literature Review: Competition Between Exporting Countries

World production has grown beyond expectations since the 1960s (Bayoumi, 1995). With increasing growth, countries have wondered about the effects of structural changes in international trade on trade performance and how countries can adapt to these changes (Nguyen et al., 2017). In addition, production increases have given rise

to the concept of "competitiveness" in the 1970s (Siudek and Zawojska, 2014). However, defining and measuring the concept is an interesting and controversial issue (Falciola et al., 2020). Competitiveness, a multidimensional concept, is classified in the literature at the firm, industry and country levels. Competitiveness at the firm level is defined as the firm's ability to design, produce and/or market products that are superior to those offered by its competitors, considering price and non-price attributes (Ajitabh and Momaya, 2004). Industry-level competitiveness refers to the ability of large firms within the national industry to compete in the global market (Aktan and Vural, 2004). Competitiveness at the country level is defined as the ability of a country to create, produce, distribute and/or provide services while gaining increasing returns on its resources in international trade (Buckley et al., 1988). This ability is shaped particularly through the firms and industries of that country. The global competitiveness of countries may be concentrated in certain sectors, and no country has the same advantages in terms of competitiveness in all sectors (Aktan and Vural, 2004). National competitiveness also reflects the power of a country against its competitors in the global economy. The interest of countries in global competition is increasing day by day, and this interest seems to be relatively higher in developed and developing countries (World Economic Forum, 2024). At the same time, competition is a type of relationship between countries, and these relationships can also "play a binding role" between different countries or regions (Liu et al., 2020). Due to the lack of a universally accepted definition of "competitiveness", countries' export data is used as a tool to evaluate a nation's "sales ability". Because foreign trade data allow comparisons to be made between countries (Saxena and Salze-Lozac'h, 2010).Bir ülkenin uluslararası pazardaki rakiplerini belirlemek için kullanılan İhracat Benzerlik Endeksi (Export Similarity Index-ESI), ilk olarak 1960'larda tanıtılmıştır. Tarım alet ve ekipmanları sektöründe ESI ile yapılan çalışmalara rastlanmamıştır. Bu nedenle yerli ve yabancı literatürde ESI'nin kullanıldığı çalışmalara yer verilmiştir.

Aktas Cimen and Kosekahyaoglu (2023), analyzed the concentration of Turkey and BRICS (Brazil, Russia, India, China, South Africa) exports on a country basis for the period 2001-2021 using the Gini-Hirschman Index (GHI) according to Trade Map 2021 data, while the Export Similarity Index (ESI) was used for export similarity to the European Union (EU) common market. The findings of the study show that, according to GHI values, product diversity in Turkey's exports to the United Kingdom, the United States, and Germany has increased significantly over time, while product diversity in exports to Italy and Spain has followed a fluctuating course. The ESI results determined that Turkey and BRICS are in high competition in the relevant product groups in the EU market (ESI value 60.75) and pose a threat to each other.

Wang and Liu (2015), examined the world, American and Indian markets on the export similarity of agricultural machinery between China and the European Union (EU) between 2007-2013 using the export similarity index. For this purpose, they used the export similarity index, showing that China and the EU have a higher export similarity index in the developed countries market, which leads to fierce competition in export products. Accordingly, it is emphasized that effective measures should be taken to strengthen inter-regional trade cooperation between China and the EU and reduce bilateral trade friction.

Özoğul (2018) examined the distribution of trade by country, and stated that developed countries are in a leading position in the trade of agricultural machinery and equipment as well as agricultural products. This situation shows that intra-industry trade is widespread in the agricultural machinery sector. The main reason for this is that developed countries can meet the demand in the sector within themselves thanks to their state-of-the-art machinery production. In 2015, China became the largest exporter of agricultural machinery worldwide. This country is followed by the USA, Germany, Japan and the Netherlands. China is the most important exception among exporting countries. Thanks to its cheap labor, China has started to come to the fore in the agricultural machinery sector as in every field. For this reason, it is emphasized that it is in a more advantageous position especially in the production of cost-effective but low-quality machinery.

Celik et al. (2020), announced that, Turkey's main countries for agricultural machinery export in 2017 USA, Azerbaijan, Italy, Iraq, and Sudan. Agricultural machinery sector is the 7th in the machinery sector.

This article aims to fill the gap in the existing literature by providing an empirical analysis of the course of Turkey's export similarity to the global market and the change in its competition over time with the leading countries (China, Germany, USA, Italy, Japan) in the global agricultural equipment export market.

DATA SET AND METHOD

This study evaluates the export competitiveness of Turkey's agricultural tools and equipment sector in the global market through ESI analysis.

Data Set

The research data in the study were obtained by selecting the "machinery" classification within the "goods" classification via the Trade Map database. The classification according to the Customs Tariff Statistics Position Numbers (GTIP) for agricultural machinery and equipment is given in ANNEX 2. The data covers 200 countries worldwide at different development levels that export agricultural tools and equipment for the period 2002-2021. In addition, 10-year trade data is considered sufficient to examine the trade competitiveness and trade potential of any commodity (Jagadeesh et al., 2024).

Method

Competitiveness in trade is generally defined as the capacity of a sector to increase its share in international markets against its competitors. The competitiveness index is an indirect measure of a country's international market power assessed through its share of world markets in selected export categories (Mikic and Gilbert, 2009).

Export Similarity Index (ESI)

ESI is an index that measures the similarity of export sections of any two countries (or groups of countries) to a third market (Finger and Kreinin, 1979). The ESI value can measure the comparative threat that a country poses to another in the global market and reveal the course of the relative development of the country's exports (Schoot, 2004). ESI, which measures the degree of overlap in the export structures of countries, provides an idea about the competitive pressures that countries face in different periods (Riad et al., 2012). The limitation of the index is that it only takes into account the structure of exports, not their level, and therefore the index values can be misleading when the size of the economies included in the analysis is very different (Mikic and Gilbert, 2009). Unlike other indices, ESI emphasizes structural rather than quantitative aspects of exports by using standardized international trade data (Schoot, 2004). ESI values also help measure competitive threats between countries and assess the complexity of exports (Pomfret, 1981). The index expresses the share of total exports of a specific product from the examined region in total world exports of the same product. The ESI proposed for analysis is formulated as follows (Finger and Kreinin, 1979; Mikic and Gilbert, 2009; Vlasenko et al., 2020):

$$ESI = \sum \min[X_k (jw), X_k (mw)] \times 100$$

Here;

Xk (jw) is the share of the export value of product k (product group) of country "j" to the world in its total exports to the world,

Xk (mw) is the share of the export value of product k of country "m" to the world in its total exports to the world. In other words, it takes the smaller of the sectoral export shares (in percentage) in each product category and adds them up (Mikic and Gilbert, 2009).

The index takes a value between "0" and "100". The value of "0" indicates that the export outlooks of the countries are not similar (there is no competition between countries) in terms of sector or product (product group), while the value of "100" indicates that the countries have perfect similarity (competition between countries) in terms of sector or product (product group) (Schoot, 2004; Saricoban and Kosekahyaoglu, 2017; Li et al., 2022; Wani et al., 2024). Increases in the ESI value may trigger increased competition and trade wars in the future. High ESI values indicate the existence of intense global market competition and limited prospects for inter-sectoral trade through regional arrangements (Schoot, 2004). ESI can evaluate the competitiveness of Turkey with the top five countries in agricultural machinery and equipment exports.

RESULTS

Turkey, on the other hand, ranks eighth in the HS 69 coded product group, seventeenth in the HS 87 coded product group, twenty-sixth in the HS 84 coded product group, and twenty-eighth in the HS 82 coded product group in the global agricultural machinery and equipment export market. Based on the data set and method explained in the previous section, Turkey's export similarities with the top five leading countries in the global agricultural machinery and equipment export market (China, USA, Germany, Italy, and Japan) were analyzed. In the study, where the export data of the HS 69, 82, 84, and 87 coded product groups for the period 2002-2021 were taken into account, the course of Turkey's agricultural machinery and equipment export similarities with China, USA, Germany, Italy, Japan in the global market over 20 years is observed in Figure 1.

Export Similarity of Turkey and China

China ranks first in the HS 69, 82, and 84 coded product groups and fourth in the HS 87 coded product group in the global agricultural machinery and equipment export market. Figure 1 shows that the global agricultural machinery and equipment export similarity between Turkey and China increased over the 20 years between 2002 and 2021. Turkey and China, which had the lowest ESI value in 2003 (21.67) in the analyzed period, reached the highest ESI value (46.23) in 2021. The agricultural machinery and equipment ESI value of Turkey and China increased from 26.46 in 2002 to 46.23 in 2021. The increase in the ESI value indicates that Turkey exports similar products to China in the global agricultural machinery and equipment export market, and therefore the competition between Turkey and China in agricultural machinery and equipment exports has increased.

Export Similarity of Turkey and Germany

Germany ranks first in the HS 87 coded product group, second in the HS 82 and 84 coded product groups, and fourth in the HS 69 coded product group in the global agricultural machinery and equipment export market. In the twenty-year period in which the agricultural machinery and equipment export similarity of Turkey and Germany was analyzed for the period 2002-2021, it was observed that the export similarity of both countries generally followed a horizontal course until 2019, with small increases and decreases; and a decrease in the ESI value was

observed as of 2020. In the analyzed period 2002-2021, Turkey and Germany, which had the lowest ESI value in 2003 (48.96), reached the highest ESI value (69.80) in 2019. The agricultural machinery and equipment ESI value of Turkey and Germany increased from 54.03 in 2002 to 65.66 in 2021. This increase in the ESI value shows that Turkey exports similar products to Germany in the global agricultural machinery and equipment export market and that competition between them has increased.

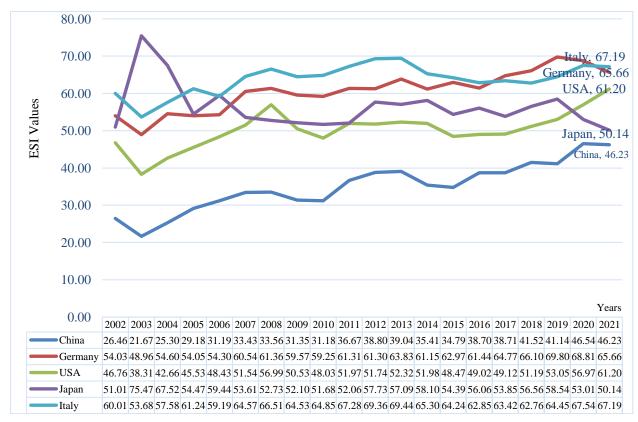


Figure 1. Course of ESI Values (2001-2022).

Export Similarity of Turkey and the USA

The USA ranks third in the HS 82, 84, and 87 coded product groups and sixth in the HS 69 coded product group in the global agricultural machinery and equipment export market. In the 2002-2021 period, when the global agricultural machinery and equipment export similarity of Turkey and the USA was analyzed, it was seen that there was a general upward trend, although there were significant increases and decreases in the twenty years (Figure 1). The ESI values of Turkey and the USA showed an increasing trend between 2004-2008, while they decreased in 2009 and 2010. The ESI values of Turkey and the USA, which showed small increases and decreases in the 2011-2015 period, have been showing an increasing trend, especially since 2016. In the analyzed period of 2002-2021, Turkey and the USA, which had the lowest ESI value in 2003 (38.31), reached the highest ESI value (61.20) in 2021. The agricultural machinery and equipment ESI value of Turkey and the USA means that they export similar products in the global agricultural machinery and equipment export market and that competition between them has increased.

Export Similarity of Turkey and Japan

Japan ranks second in the HS 87 coded product group, fourth in the HS 82 and 84 coded product groups and seventh in the HS 69 coded product group in the global agricultural machinery and equipment export market. In the study analyzing the similarity of Turkey and Japan's global agricultural machinery and equipment exports for the period 2002-2021, significant increases and decreases are observed in the twenty years and it is observed that the general trend is decreasing (Figure 1). It is understood from Figure 1 that the ESI values of Turkey and Japan have shown a steady decrease, especially since 2019. In the study, it is seen that Turkey and Japan had the lowest ESI value (50.14) in the period 2002-2021 in 2021. This decrease in the ESI values of Turkey and Japan indicates that the exports of similar products and the competition between them have decreased in the global agricultural machinery and equipment export market.

Export Similarity of Turkey and Italy

Italy ranks second in the HS 69 coded product group, fifth in the HS 84 coded product group, eighth in the HS 82 coded product group, and eleventh in the HS 87 coded product group in the global agricultural machinery and

equipment export market. Figure 1 shows that the global agricultural machinery and equipment export similarity between Turkey and Italy increased over the 20 years between 2002 and 2021. It is seen that Turkey and Italy, which had the lowest ESI value in 2003 (53.68) in the analyzed period, reached the highest ESI value (69.44) in 2013. The agricultural machinery and equipment ESI value of Turkey and Italy increased from 60.01 in 2002 to 67.19 in 2021. This increase in the ESI value shows that Turkey exports similar products to Italy in the global agricultural machinery and equipment export market, and therefore the competition between Turkey and Italy in agricultural machinery and equipment exports has increased. In the study analyzing Turkey's export similarities to China, the USA, Germany, Italy, and Japan in the agricultural machinery and equipment sector, the countries were ranked as Italy (60.01), Germany (54.03), USA (46.76), Japan (51.01), China (26.46) according to the ESI values in 2002, while the ranking changes to Italy (67.19), Germany (65.66), USA (61.20), Japan (50.14), China (46.23) in 2021. The biggest difference in ESI values in the twenty years in China (19.77), USA (14.44), Germany (11.63), Italy (7.18), and Japan (-0.87). Turkey's positive ESI difference values with China, the USA, Germany, and Italy indicate that they exported increasingly similar products in the agricultural machinery and equipment sector in the 2002-2021 period and that competition between them increased, while the negative difference in the ESI value between Turkey and Japan indicates that the similarity between the agricultural machinery and equipment export products of the two countries has decreased, and therefore the competition between them has weakened. In addition, the largest difference in ESI values is seen in Turkey's export similarity with China. According to Trade Map 2021 data, it is understood that China, which is the leader in world exports with a share of 15.20%, exported increasingly similar products to Turkey in agricultural machinery and equipment exports to the global market in the 2002-2021 period. This result means that China is increasingly posing a threat to Turkey in the global agricultural machinery and equipment export market.

CONCLUSION

Global population growth and environmental pressures caused by the climate crisis, combined with limited agricultural land, create pressure to increase agricultural production and increase productivity. The increasing world population also increases the demand for food, but limited agricultural land and environmental factors necessitate the development of more efficient and sustainable methods to meet this demand. At this point, modern agricultural machinery and equipment both increase production volumes and reduce environmental impacts. The production of agricultural machinery and equipment has necessitated international trade with its roles in providing agricultural production and productivity increases. While developed countries export high-tech agricultural machinery and equipment, developing countries are trying to increase their agricultural production efficiency by importing these products. Exports of agricultural machinery and equipment facilitate access to these machines and equipment.

The similarity of Turkey to the global agricultural machinery and equipment export market with the top five countries (China, USA, Germany, Italy, Japan) in the global market for the period 2002-2021 was analyzed using ESI. In the index calculation, agricultural machinery and equipment export similarities between Turkey and leading countries were calculated in the HS 69, 82, 84, and 87 coded product groups included in the agricultural machinery and equipment classification (based on 39 product groups subject to export). According to ESI values, China ranks first in the HS 69, 82, and 84 coded product groups, and fourth in the HS 87 coded product group in the global agricultural machinery and equipment export market. Germany ranks first in the HS 87 coded product group, second in the HS 82, and 84 coded product groups and fourth in the HS 69 coded product group. The USA ranks third in the HS 82, 84, and 87 coded product groups, and sixth in the HS 69 coded product group, and seventh in the HS 69 coded product group. Italy ranks second in the HS 82 and 84 coded product group, fifth in the HS 84 coded product group, eighth in the HS 82 coded product group, and eleventh in the HS 87 coded product group. Turkey ranks eighth in the HS 69 coded product group, seventeenth in the HS 87 coded product group, twenty-sixth in the HS 84 coded product group, and twenty-eighth in the HS 82 coded product group.

In the 2002-2021 period, it is seen that Turkey has increasingly exported similar products to China, the USA, Germany, and Italy in the agricultural machinery and equipment sector, and that competition between them has increased. The decrease in Turkey's ESI values with Japan means that the similarity of agricultural machinery and equipment export products of the two countries has decreased, and therefore the competition between them has weakened. In 2021, Turkey's agricultural machinery and equipment ESI value rankings for China, the USA, Germany, Italy, and Japan in the global market are Italy (67.19), Germany (65.66), the USA (61.20), Japan (50.14), and China (46.23). These ESI values show that Turkey exports very similar products to Italy in the agricultural machinery and equipment market and that there is high competition between them, while Turkey exports less similar products to China in the agricultural machinery and equipment market and that the competition between them is weaker. However, the biggest difference in ESI values in the 20 years from 2002 to 2021 is seen in Turkey's export similarity with China. This result shows that Turkey and China are increasingly exporting similar products in the global agricultural machinery and equipment export market and that competition between the two countries

is increasing. According to Trade Map 2021 data, it can be said that world export leader China (with a share of 15.20%) poses more of a threat to Turkey.

In this case, to cope with the environmental threats created by the global climate crisis as well as the increase in food demand caused by the increasing Turkish population, it is extremely important for Turkey to quickly implement policies to improve its competitiveness in agricultural machinery and equipment product groups, where it does not have much of a competitive advantage so that the agricultural machinery and equipment sector can remain strong in global competition. In addition, it is of great importance to support and develop Turkey's agricultural machinery and equipment export performance with appropriate export and trade policies. For this, there is a need to develop comprehensive export strategies that include both companies in the sector and state-led efforts. In addition, incentives, tax reductions, etc. Supporting the agricultural machinery and equipment sector with policies will be very important applications in both maintaining a competitive advantage and gaining a competitive advantage in product codes where the competitive advantage is weak.

It is thought that agricultural machinery and equipment play important roles in meeting the food demand of the increasing world population and coping with the dangers created by the climate crisis. Therefore, findings regarding competition in the agricultural machinery and equipment export market can deepen the knowledge of stakeholders such as researchers, academics, institutions, and decision makers. It can be effective in making strategic decisions for both companies and countries in the global economy. The limitations of this study are that the ESI analysis was conducted with the top 5 countries in agricultural machinery and equipment exports. Subsequent scientific studies can deepen the ESI analysis regarding agricultural machinery and equipment exports by including more countries or to a large common market such as the European Union.

Automation and new technologies should meet the needs of farmers, and be easy to use and efficient. Therefore, farmers should be encouraged to invest in these high technologies. Especially the increase in farm sizes and the use of high technology are very important for manufacturers in the sector.

The number of machines sold may decrease in parallel with the growth of agricultural land and the increase in the capacity and size of the machines used. However, this does not negatively affect the turnover because more expensive machines will be sold. Farmers want innovative machines to be used for special use and production, multi-purpose machines are important and they want features that can be used in niche production areas to be included.

Machines that consume minimum energy, safety, efficiency, comfort, and versatility are also among the factors that should be taken into consideration. The future of the tractor and agricultural machinery sector will develop in parallel with the future of the agricultural sector. The main goal of development should be to increase the financial power of farmers.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

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

Data availability

Data will be made availabale on request.

Consent to participate

The authors consent to participate.

Funding

No funding was received to conduct this research.

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International Journal of

Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.9

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 68-81

Ampelographic characterization of some grape genetic resources in the Aegean region of Türkiye

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Article History

Received: January 12, 2025 Revised: February 25, 2025 Accepted: March 1, 2025 Published Online: March 9, 2025

Article Info

Article Type: Research Article Article Subject: Pomology and Treatment, Oenology and Viticulture

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Available at

tps://dergipark.org.tr/jaefs/issue/90253/1618260

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Abstract

Viticulture has an ancient history worldwide, and thousands of grape cultivars are grown in different countries. Some of these grape cultivars are the same cultivar, but they are grown with different names, and similarly, other varieties are grown with the same name. To prevent this confusion, grape varieties or genotypes must be defined differently. The most widely used definition in the world is ampelographic, and different grapes are preserved by being identified in this way. In this study, 29 of the local grape cultivars or genotypes collected from different vineyard areas of our country, especially in the Aegean Region, and taken under protection were defined regarding 53 different ampelographic characters. As a result of the definitions, it was identified that all of the cultivars/genotypes were seeded and belonged to the Vitis vinifera L. species. According to the similarity dendrogram data from the definitions, the similarity rate between the defined cultivars/genotypes changed between 0.53 and 0.89. The highest similarity rate (0.89) was obtained from the Ak Üzüm and Nuri Bey genotypes with lightcoloured berries. It is seen that all cultivars and genotypes are different from each other according to the 53 criteria evaluated. According to the results of the 53 different characters evaluated, it was determined that the varieties/genotypes were the same in terms of the 50th (seed formation) and 48th (intensity of the flesh colouration with anthocyanin) characters. But, there were differences in terms of other characters. According to the results obtained from the study, it was revealed that cultivars/genotypes differed at varying rates, and cultivars /genotypes whose definitions were made were protected for future studies regarding their identified characteristics.

Keywords: Vitis vinifera, Dendrogram, Similarity, Cultivars, Genotypes, Identification

Cite this article as: Kesgin, M., Kakci, H., Yildiz, N., Atak, A. (2025). Ampelographic characterization of some grape genetic resources in the aegean region of Türkiye. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 68-81. https://doi.org/10.31015/2025.1.9

INTRODUCTION

Viticulture has an ancient history worldwide, and thousands of grape cultivars are grown in different countries. Among the grapevine species cultivated thousands of years ago and spread over a wide area worldwide, the species with the most significant number of cultivars is Vitis vinifera L. (De Lorenzis, 2024; Baltazar et al., 2025). However, in recent years, some interspecific hybrid cultivars developed in breeding studies have increased production areas (Atak, 2024). These cultivars are grown intensively, especially in the European continent and Türkiye (İşçi and Altındişli, 2024). In Anatolia, which is among the genetic resources of the grapevine, many grape cultivars have been produced for different purposes since ancient times (Winkler, et al., 1974; Taskesenlioglu et al., 2022; Kaya et al., 2023).

As in many countries, there is a very rich variety of grapes in Türkiye and different widely accepted identification techniques are used to identify these cultivars. Researchers have been using ampelographic methods for many years to identify grape cultivars or genotypes, and in recent years, molecular methods have also begun to be used for identification purposes (Vivien and Pretorius, 2000; Atak et al., 2012). In addition, chromatographic

and spectrophotometric methods are also used for identification purposes, where grape berries are identified in terms of their different contents (Rapp, 1988; Temerdashev et al., 2024).

Grapevine cultivar identification is essential for ensuring product authenticity, managing quality control, and maintaining regulatory compliance. In some cases, grape leaves used for consumption can be more valuable than the fruit itself (Moncayo et al., 2016; Koklu et al., 2022; Carneiro et al., 2024).

Some researchers compared and identified grape cultivars and genotypes by examining and scoring different parts of the grapevine plant, such as fresh shoots, lignified shoots, leaves, flowers, berries and seeds (Sargolzaei et al., 2021; Bodor-Pesti et al., 2023; Hbyaj et al., 2024).

In Türkiye, grape cultivars are registered according to approximately 50 ampelographic identification criteria for registration of grape cultivars for two years. They are registered if a difference is detected in at least one criterion. Therefore, the selected cultivars and genotypes must be identified based on different characteristics during registration in breeding and clonal selection studies (Atak et al., 2013; Kara et al., 2023).

In addition, cultivars or genotypes that are the same despite being grown under different names and cultivars and genotypes that are grown under the same name in different places but have mutated due to climate, soil and other factors and have now become different need to be defined (Dettweiller et al., 2000; Labra et al., 2004; Yılmaz et al., 2020). According to the findings obtained from the definitions, it will be determined whether these cultivars or genotypes are the same or different, and cultivar confusion will be prevented.

In this study, some grape cultivars/genotypes collected from different parts of Türkiye and preserved as grapevine genetic resources were identified by determining their important ampelographic characteristics to be used with their defined characteristics in future breeding studies.

MATERIALS AND METHODS

Plant Material

Grape varieties and genotypes grown in the Aegean Region but whose numbers have been decreasing over time were collected to prevent their extinction and their important characteristics were identified within the scope of this study. The material for this study consisted of 29 cultivars/genotypes from the Aegean Region Genetic Resources Parcel within the Manisa Viticulture Research Institute, located within the central borders of Manisa province. The cultivars/genotypes were grafted onto 1103 P and planted at a 3 m x 1.5 m distance. They were planted in 6-8 vines each. They are 12-14 years old and short-pruned in the double-arm cordon training system. A training system was created with concrete poles and a low-trunk 6-wire V system. The soil structure of the experimental area is clayey-loamy, the organic matter content is approximately 1% and the soil pH is 7.9. Photographs of the cultivars and genotypes used in the study (except for two genotypes) are given in Figure 1. Temperature data (lowest, highest, average) for the experimental area in 2024 are given in Figure 2.

Method

Ampelographic characterization

In this study, 53 characters selected from the OIV descriptive list (2nd edition) for grape varieties and *Vitis* species, published by the International Organization for Grape and Wine (OIV, 2009), were used for identification. The criteria in this list were used in the ampelographic identifications of 29 varieties/genotypes. According to the recommendation of the descriptive list published by OIV for grape varieties and genotypes, criteria with high discrimination properties were selected for identification. The names and explanations of the OIV characters used in the study are given in Table 1. Shoot tips were examined when they reached approximately 25 cm in length, and the first four young leaves were evaluated within the scope of this study. The definitions of mature leaves were made in the period between the fruit set and the verasion and in the leaves in the clusters located in the middle part of the shoots. The clusters were measured when they reached harvest maturity. For berry characteristics, examinations were made when the maturity index of samples from the middle of the cluster reached at least 25.

Ampelographic clustering

According to international descriptors, the mean values of the definition data obtained in different years (2022-2024) were transformed into numerical scales. In cases where two-year differences were observed, definitions were made by looking at the values in the third year. These data obtained within the scope of the study were analysed with the help of a distance matrix with the NTSYSpc 2.0 program (Rohlf, 2000). The data in the clustering dendrogram were calculated based on the Unweighted Pair Group of the Arithmetic Mean (UPGMA). Genetic similarity status was determined according to the degree to which each of the cultivars and genotypes had a common scale with each other.



Figure 1. Photos of the genotypes

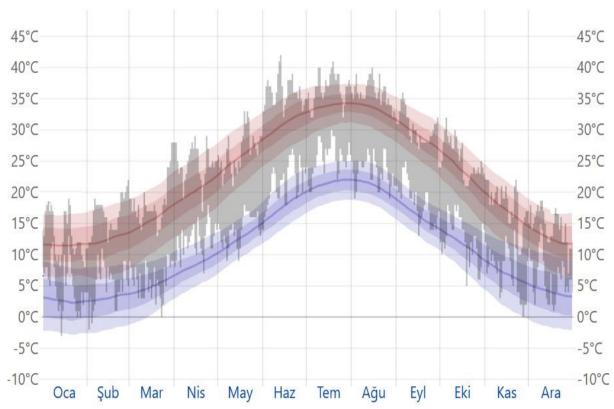


Figure 2. Temperature data (lowest, highest, average) for the experimental area in 2024

Table 1. OIV codes and descriptions are used to identify grape genotypes.

Vegetation Stage	Code Order	OIV Code	Characteristics	Notes and Explanations					
	1	301	Time of bud burst	1=very early 3=early 5=medium 7=late 9=very late					
Phenology	2	Time of bud burst 301 Time of bud burst 302 Time of full bloom 303 Time of beginning or ripening (veraison) 304 Time of physiologic of full maturity of the anthocyanin colorat prostrate hairs of tip 4 Young Shoot: den prostrate hairs on tip 6 Shoot: attitude (before of internodes) 8 Shoot: colour of dor of internodes 8 Shoot: color of vent of internodes 16 Shoot: number consecutive tendrils 51 Young leaf: color upper side of blade (4) Young leaf: dens prostrate hairs between veins on lower side of (4th leaf)	Time of full bloom	1=very early 3=early 5=medium 7=late 9=very late					
Phen	3	303	Time of beginning of berry ripening (veraison)	1=very early 3=early 5=medium 7=late 9=very late					
	4	304	Time of physiological stage of full maturity of the berry	1=very early 3=early 5=medium 7=late 9=very late					
	5	Young Shoot: intensis anthocyanin coloration prostrate hairs of tip Young Shoot: densi	Young Shoot: intensity of anthocyanin coloration on prostrate hairs of tip	1=absent or very weak 3=weak					
J	5 3 anthocyanir prostrate ha 6 4 Young Sh prostrate ha	Young Shoot: density of prostrate hairs on tip	1=none or very sparse 3=sparse 5=medium 7=dense 9=very dense						
nd Young Lee		6	Shoot: attitude (before tying)	1=erect 3=semi erect 5=horizontal 7=semi droping 9=droping					
, Shoot, a	8	7	Shoot: colour of dorsal side of internodes	1=gren 2=green with red stripes 3=red					
Young Shoot,	9	8		1=gren 2=green with red stripes 3=red					
You	10	16		1=discontinuous(2 or less) 2=subcontinuous or continues (3 or more)					
	11	51	Young leaf: color of the upper side of blade (4 th leaf)	1=green 2=yellow 3=bronze 4=copper reddish					
	11 Upper side of blade (4 th le Young leaf: density prostrate hairs between may veins on lower side of blade (4 th le	prostrate hairs between main veins on lower side of blade	1=none or very sparse 3=weak 5=medium 7=strong 9=very dense						
Flower	13	151	Flower: sexual organs	1=male 2=male to hermaphrodite 3=hermaphrodite 4=female with upright stamina 5=female					

				I			
				1=entire			
				2=three			
	14	68	Mature leaf: number of lobes	3=five			
				4=seven			
				5=more than seven			
				1=absent			
			Mature leaf: area of	2=petiol point red			
	15	70	anthocyanin coloration of	3=red until the first bifurcation			
	13	70	main veins on upper side of	4=red until the 2nd bifurcation			
			blade				
				5=red beyond the 2nd bifurcation			
				1=both sides concave			
				2=both sides rectilinear			
	16	76	Mature leaf: shape of teeth	3=mixture between notes 2 and 4			
				4=both sides convex			
				5=one side concave one side convex			
				1=very wide open			
				2=open			
			Mature leaf: degree of	3=slightly open			
	17	79	opening / overlapping of	4=slightly overlapping			
			petiole sinus	5=overlapping			
				6=strongly overlapping			
				U 11 U			
	4.0		Mature leaf: shape of base of	1=U shaped			
	18	80	petiole sinus	2={ shaped			
			-	3=V shaped			
	19	081-1	Mature leaf: teeth in the	1=none			
	19	001-1	petiole sinus	2=occurrence of 1 or 2 teeth in the petiole sinus			
			35. 1.0	1=none			
Y	20	081-2	Mature leaf: petiole sinus	2=occurrence on one side of petiole sinus			
ld ap			base limited by veins	3=occurrence on both sides of petiole sinus			
gr				1=U shaped			
elo	21	083-1	Mature leaf: shape of base of	2={ shaped			
ď	21	003-1	upper lateral sinuses	3=V shaped			
Mature Leaf (Ampelography).			Mature leaf: teeth in the	1=none			
a (22	083-2					
[eg			upper lateral sinuses	2=frequently occurring			
			Mature leaf: density of	1=none or very weak			
1 1			prostrate hairs between the	3-weak			
ďα	23	84	main veins on lower side of	5=medium			
				7=dense			
			blade				
			blade	9=very dense			
			blade				
			Mature leaf: density of erect	9=very dense 1=none or very low			
	24	85		9=very dense 1=none or very low 3=low			
	24	85	Mature leaf: density of erect	9=very dense 1=none or very low 3=low 5=medium			
	24	85	Mature leaf: density of erect hairs between the main veins	9=very dense 1=none or very low 3=low 5=medium 7=high			
	24	85	Mature leaf: density of erect hairs between the main veins	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high			
	24	85	Mature leaf: density of erect hairs between the main veins on lower side of blade	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low			
			Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low			
	24	85	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium			
			Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high			
			Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high			
			Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low			
			Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak			
			Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm)			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm)			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 5=medium (about 105 mm) 5=medium (about 135 mm)			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 135 mm) 7=long (about 165 mm)			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 135 mm) 7=long (about 165 mm) 9=very long (about 195 mm and more)			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 135 mm) 7=long (about 165 mm) 9=very long (about 195 mm and more) 1=very short (up to about 65 mm)			
	25 26 27	86 87 601	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein N1	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 135 mm) 7=long (about 165 mm) 9=very long (about 195 mm and more) 1=very short (up to about 65 mm) 3=short (about 85 mm)			
	25	86	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein N1 Mature leaf: length of vein	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 135 mm) 7=long (about 165 mm) 9=very long (about 195 mm and more) 1=very short (up to about 65 mm) 3=short (about 85 mm) 5=medium (about 105 mm)			
	25 26 27	86 87 601	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein N1	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 165 mm) 9=very long (about 195 mm and more) 1=very short (up to about 65 mm) 3=short (about 85 mm) 5=medium (about 105 mm) 5=medium (about 105 mm) 5=medium (about 105 mm) 5=medium (about 105 mm)			
	25 26 27	86 87 601	Mature leaf: density of erect hairs between the main veins on lower side of blade Mature leaf: density of prostrate hairs on main veins on lower side of blade Mature leaf: density of erect hairs on main veins on lower side of blade Mature leaf: length of vein N1 Mature leaf: length of vein	9=very dense 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very low 3=low 5=medium 7=high 9=very high 1=none or very weak 3=weak 5=medium 7=dense 9=very dense 1=very short (up to about 75 mm) 3=short (about 105 mm) 5=medium (about 135 mm) 7=long (about 165 mm) 9=very long (about 195 mm and more) 1=very short (up to about 65 mm) 3=short (about 85 mm) 5=medium (about 105 mm)			

		I	T	1 1 (1 127)			
				1=very short (up to about 35 mm)			
			Mature leaf: length of vein	3=short (about 55 mm)			
	29	603	N3	5=medium (about 75 mm)			
				7=long (about 95 mm)			
				9=very long (about 95 mm)			
				1=very short (up to about 15 mm)			
				3=short (about 25 mm)			
	30	604	Mature leaf: length of vein	5=medium (about 35 mm)			
			N4	7=long (about 45 mm)			
				9=very long (about 55 mm and more)			
				1=very short (up to about 30 mm)			
			Mature leaf: length petiole	3=short (about 50 mm)			
	31	605	sinus to upper lateral leaf	5=medium (about 70 mm)			
	31	003	sinus to upper lateral lear				
			Silius	7=long (about 90 mm)			
				9=very long (about 110 mm and more)			
				1=very short (up to about 30 mm)			
			Mature leaf: length petiole	3=short (about 45 mm)			
	32	606	sinus to lower lateral leaf				
		sinus	7=long (about 75 mm)				
				9=very long (about 90mm and more)			
				1=very short (up to about 15 mm)			
			Moture lo-f: 11 C	3=short (about 25 mm)			
	33	611	Mature leaf: length of vein	5=medium (about 35 mm)			
			N5	7=long (about 45 mm)			
				9=very long (about 55 mm and more)			
				1=very short (up to about 6 mm)			
				3=short (about 10 mm)			
	34	612	Mature leaf: length of tooth	5=medium (about 14 mm)			
	34 612	N2	7=long (about 18 mm)				
				9=very long (about 22 mm and more)			
				1=very narrow (up to about 6 mm)			
			Mature leaf: width of tooth	3=narrow (about 10 mm)			
	25	(12					
	35	613	N2	5=medium (about 14 mm)			
				7=wide (about 18 mm)			
				9=very wide (about 22 mm and more)			
				1=very short (up to about 6 mm)			
	2.5	c1.4	Mature leaf: length of tooth	3=short (about 10 mm)			
	36	614	N4	5=medium (about 14 mm)			
				7=long (about 18 mm)			
				9=very long (about 22 mm and more)			
				1=very narrow (up to about 6 mm)			
			Mature leaf: width of tooth	3=narrow (about 10 mm)			
	37	615	N4	5=medium (about 14 mm)			
				7=wide (about 18 mm)			
				9=very wide (about 22 mm and more)			
				1=wide open (up to about -35 mm)			
			Mature leaf:	3=open (about -15 mm)			
	38	618	opening/overlapping of	5=closed (about -5 mm)			
			petiole sinus	7=overlapping (about 25 mm)			
				9=very overlapping (about 45 mm and more)			
				1=very short (up to about 80 mm)			
				3=short (about 120 mm)			
	39	202	Bunch: length (peduncle	5=medium (about 160 mm)			
	5)	202	excluded)	7=long (about 200 mm)			
				9=very long (about 240 mm and more)			
				1=very narrow (up to about 40 mm)			
ch				3=narrow (about 80 mm)			
Bunch	40	203	Bunch: width	5=medium (about 120 mm)			
_ B	70	203	Zanen. widui	7=wide (about 160 mm)			
				9=very wide (about 200 mm and more)			
				1=very loose, 3=loose, 5=medium, 7=dense			
	41	204	Bunch: density	9=very dense			
				1=long cylindrical bread cylindrical			
	42	208	Bunch: shape	2=narrow conical broad conical			
		l	<u> </u>	2-narrow comear broad comear			

			T	
				3=funnel shaped
				1=very low (about 200 mm and more)
			Bunch: weight of a single	3=low (about 300 g)
	43	502	bunch	5=medium (about 500 g)
				7=high (about 700 g)
				9=very high (about 900 g and more)
				1=very short (up to about 8 mm)
				3=short (about 13 mm)
	44	220	Berry: length	5=medium (about 18 mm)
				7=long (about 23 mm)
				9=very long (about 28 mm and more)
				1=very small (up to about 8 mm)
				3=small (about 13 mm)
	45	221	Berry: width	5=medium (about 18 mm)
				7=large (about 23 mm)
				9=very large (about 28 mm and more)
				1=flat
				2=roundish
			3=elliptic	
				4=ovate
	46	223	Berry: shape	5=obtuse ovate
				6=obovate
				7=cylindric
				8=arched
				1=green yellow
				2=rose
	47	225	Berry: color of skin	3=red
Веггу				4=grey
Веі				5=dark red violet
				6=blue black
	40	•••	Berry: intensity of the	1=none or very weak, 3=weak, 5=medium
	48	231	anthocyanin coloration of	7=strong, 9=very strong
			flesh	• • •
	49	236		1=none, 2=muscat, 3=foxy,
	50	241	flavour	4=herbaceous, 5=others
	50	241	Berry: formation of seeds	1=none, 2=rudimentary, 3=complete
				1=very short ($\leq 3.8 \text{ mm}$)
				3=short (5 mm)
	51	242	Berry: length of seeds	5=medium (6,2 mm)
				7=long (7,4 mm)
				9=very long (≥ 8,6 mm)
				1=very low (up to about 10 mg)
				3=low (about 25 mg)
	52	243	Berry: weight of seeds	5=medium (about 40 mg)
				7=high (about 55 mg)
				9=very high (about 65 mg and more)
				1=very low (up to about 1 g)
				3=low (about 3 g)
	53	503	Berry: single berry weight	5=medium (about 5 g)
				7=high (about 7 g)
				9=very high (about 9 g and more)

RESULTS AND DISCUSSION

While the ampelographic identification results obtained with this study are given in Table 2, the genetic similarity dendrogram formed according to these results is given in Figure 3. According to the data obtained from the definitions and scores, the cultivars and genotypes show similarities with each other at rates varying between 0.53 and 0.89. It is seen that all cultivars and genotypes are different from each other according to the 53 criteria evaluated.

The results obtained from 53 different characters evaluated showed that all cultivars and genotypes had a seeded structure in terms of the 50^{th} criterion, which is the seed condition. Similarly, it was determined that all were colourless regarding the 48th criterion, the anthocyanin colouration intensity in the flesh of the berry. In addition, it was defined as a result of the definitions that the number of consecutive tendrils was discontinuous (2+0+2) since all cultivars and genotypes were V. vinifera cultivars. It was determined that only two of the cultivars

and genotypes had a muscat aroma in terms of the 49th criterion, which is the particularity of flavour. In contrast, all the others did not have a unique taste. It was determined that only two genotypes were different from the others in terms of the "petiole sinus base limited by veins" examined in the mature leaves in terms of the 20th criterion. It was understood as a result of the definition studies that the cultivars and genotypes showed quite different characteristics from each other in terms of all the other criteria.

According to the dendrogram, the cultivars and genotypes are divided into two main branches. While it is seen that the Siyah Yuvarlak genotype differs considerably from the other genotypes in the first main branch, in the second main branch, the Bağdat Siyahı and Bülbül genotypes differ greatly from the other cultivars/genotypes and are located in a separate branch.

The highest similarity rate (0.89) was obtained from the Ak Üzüm and Nuri Bey genotypes with light-coloured berries. Despite having different berry colours, the Ak Dimrit and Ufak Dimrit genotypes showed a high similarity rate of 0.85. Similarly, Balçova Karası and Beyaz Kokulu genotypes, despite their different berry colour, showed similar characteristics in many other respects and had a high similarity rate in the dendrogram. A similarity of over 0.80 was also found between the Yuvarlak Kara and Ufak Kara genotypes and Sivri Kara and Al İdris genotypes.

Although ampelographic (morphological) identification studies with different numbers of characters are used to distinguish or identify many grape varieties, genotypes or hybrids from each other, they sometimes may not give the desired results. Ampelographic characters are related to many conditions, but they are especially closely related to ecological factors and different growth stages of the grapevine. Therefore, they can sometimes be insufficient in distinguishing genotypes. Nevertheless, ampelographic characters are often needed in determining close agronomic mutations (Ortiz et al., 2004).

Some values related to the berry characteristics of the cultivars or genotypes used in the particular study can greatly affect the similarity ratio. Sabir et al. (2009) obtained a match among seedless hybrids and hybrids with seeds in the UPGMA dendrogram based on ampelographic data. They characterized 41 ampelographic descriptors. It was also concluded that the relationship between genotypes was highly related to the origin of the places where they were grown. In this study, high similarities were obtained between some cultivars and genotypes collected from close geographical regions. Researchers have also attempted to identify differences using molecular markers for identification. The dendrogram constructed by the two approaches was the varieties are highly similar, especially in terms of where they are clustered and the differentiation of the groups to which they belong. Another similar study was conducted by Atak et al. (2012) with hybrid grape genotypes. The researchers compared the hybrid genotypes by making both ampelographic and molecular definitions. They emphasized that, especially in ampelographic definitions, seedless ones showed more similarities to each other and could differ significantly from seeded ones.

Davies and Savolainen (2006) also reported that biodiversity is phenotypic and genetic variation, and the numbers of morphological changes along the branches of the phylogenetic tree were significantly correlated with the number of reconstructed changes in genetic characters.

Chadha and Randhawa (1974) reported that leaf morphological investigations are essential. They emphasized that grapevine leaf characteristics without the observation of other organs would be sufficient for the classification of grapevine cultivars. During the past decades, several refinements and specifications related to sampling, methodology, and data evaluation have been reported, which makes measurements faster and more accurate with higher discriminative power. (Preiner et al., 2014; Bodor-Pesti et al., 2023).

Recently, morphometric variability between and within species, cultivars, clones, and clone candidates was explored, and traits with discriminative power were highlighted. These traits are not necessarily the same in all investigations. The reasons for this are the different sample sets and those external factors that influence the morphometric traits. Related studies show that biotic and abiotic factors and vineyard management practices modify the ampelometric characteristics (Silvestroni et al., 1990; Bodor et al., 2013). Also, the climatic condition is significant, as year-to-year studies can show big differences (Chitwood et al., 2021). Observation of similar differences in our study shows that conducting identification studies in different years will yield more realistic results.

The differences between varieties and genotypes can be clearly revealed with identification studies, and synonyms or homonyms can be determined. After identification studies conducted by Maletic et al. (2015) with Croatian genetic resources, many synonyms and homonyms were detected, and unique genotypes were selected. Stavrakaki and Binari (2017) conducted a similar study with varieties from Greece. The researchers determined the synonyms, homonyms and variations of the varieties they identified as a result of their studies. Similarly, in our study, the differences between all varieties/genotypes were revealed after identification.

Ateş et al. (2011) also observed great differences among the varieties examined regarding ampelographic characters in their study of ten grape varieties regarding 52 ampelographic characters. They especially reported that certain characteristics played a particular role in the constitution of the ampelographic dendrogram. In our study, it was determined that while few ampelographic data (especially 48th and 50th definition criteria) showed common characteristics among varieties and genotypes, most of them showed great differences.

As a result, according to the findings obtained from our study, 29 varieties or genotypes differed from each other in terms of selected ampelographic criteria at varying rates. Thus, important ampelographic descriptions of these grapes collected from the Aegean Region, many of which are in danger of extinction, have been made and safely preserved in the genetic resource parcel for use in subsequent scientific studies.

Table 2. OIV notes of genotypes are defined within the scope of the study.

Order No	OIV Code	Siyah Yuvarlak	Ak Dimrit	Yuvarlak Kara	Hacı Balbal	Bostancı	Ufak Dimrit	Erkenci Dimrit (Demirhan)	Kayırcık	Kürt üzümü	Pembe Gemre Type
1 2	301 302	7 5 5	1 3	1 3	1 5	3	1 3	1 3	3	7 7	1 5
3	303	5	3 5	3 3 5 3	5 5	5 5	3	3 5	5 5	5	5 3
4	304	7	3	5	7	7	3	5	7	7	7
5 6	3	1			3	1	3	1_	1	1	1
6 7	4 6	7 1	9 3	1 1	9 1	3	9	7 1	3	1 1	1 1
8	7	2	2		3	1	1	2	2	2	3
9	8	1	2 2	2 2	3	2	2	2	2 2	1	3
10	16	1	1	1	1	1	1	1	1	1	1
11	51	3	3	3	3	3	4	3	2	1	3
12	53	7	9	1	9	1	9	9	1	1	3
13 14	151 68	3	3 4	3 4	3 3 3 2	3	3	3	3	3	3
15	70	2		3	3	2				1	
16	76	2 3	3 2	3 3 3	2	2 2	2 2	3 5	2 3	3	3 2
17	79	2	3	3	3	5	4	2	2	3	2
18	80	1	1	3 2	1	1	1	3	1	3	1
19 20	081-1 081-2	1 1	1 1	1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
20 21	083-1	1	3	2	2	3	2	1	2	2	1
22	083-2	1	1	1	1	1	1	2	1	1	1
23	84	5	7	1	7	1	7	7	1	1	1
24	85	1 -	1	1	3	1	3	3	1	1	1
25 26	86 87 601 602 603	5 1	5 1	1 1	3 1	1 1	5 1	3 1	1 1	1 1	3
20 27	601	5	5	7	3	5	5	5	3	5	1 7
28	602	5	5	7	5 5	7	7	7	7	5	7
29	603	7	7	7	5	7	7	5	5	5	7
30	604	9	9	9 5	7	9	9	7	7	9	9
31 32	605 606	5 7	3 5	5 7	3	5 5	5 5	5 5	5 5	5 5	3
33	611	5	5		3 5 3 3	5	5	3	3	3	3
34	612	3	3	5	3	7	3	5 5	5	3	5
35	613	3	3	5 5 5 3	3	7	3	5	5	3	5 5 3
36 37	614 615	3	3			5 7	3	5	3	5	
38	618	5 3	5 3	5 5	5 7	7	5 7	5 3	5 3	3 1	5 3
39	202	7	7	7	5	7	5	7	7	7	3
40	203	3	3	7	3	3	5	5	5	3	5
41	204	5 2 5 5	5 2	5	5 2	7	7	7	7	5	5
42 43	208 502	2	3	1 5	2 5	1 3	2 3	2 5	2 7	2 5	1 3
43 44	220	5	5	3 7	3 7	3	3	3	9	5	3
45	221	5	5	5	5	5	5	5	5	5	5
46	223	5 4	3	1	3	2	2	2	3	2	5 4
47	225	2	1	5	2	1	5	5	1	1	1
48	231	1	1	1	1	1	1	1	1	1	1
49 50	236 241	1 3	1 3	1 3	1 3	1 3	2 3	2 3	1 3	1 3	1 3
51	242	5	5	7	7	7	5	5	7	7	5
52	243	5	5 5	9 5	5	5	5	5	5	7	5
53	503	3	5	5	7	5	3	3	7	5	3

Table 2. Continue

Order No Ord	Sivri Kara Siyah Asma Ak Üzüm Balçova Karası Beyaz Gut	
Ord OIV Jelin Č	Siyah Siyah Ak İ Ak İ Beya	D
1 301 3 3 7 1	1 3 7 3 1 7	
2 302 7 3 7 3	5 5 3 3 5	
3 303 5 3 5 5	5 5 5 5 5	
4 304 7 5 3 7	5 5 9 5 7	
5 3 1 1 1 3 6 4 3 7 1 9	1 1 1 3 1	
6 4 3 7 1 9 7 6 1 1 1	1 9 1 3 7 3 3 3 3 3 3 3	
8 7 3 1 1 2	1 1 1 1 1 1	
9 8 2 2 2 1	2 2 2 2 2 2	
10 16 1 1 1 1	1 1 1 1 1 1	
11 51 3 3 3	1 1 1 3 4 3	
12 53 1 5 1 7	1 3 1 1 7 1	
13 151 3 3 3 3 14 68 3 2 3 3	3 3 3 3 3	
14 68 3 2 3 3 15 70 2 1 2 1	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
15 70 2 1 2 1 16 76 4 2 2 2	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
16 76 4 2 2 2 17 79 2 2 1 2 18 80 2 2 2 1	3 4 5 2 4 3	
18 80 2 2 1 1	2 1 1 2 2 1	
19 081-1 2 1 2 1		
20 081-2 1 1 2 1	$1 \qquad 1 \qquad 1 \qquad 1 \qquad 1 \qquad 1$	
21 083-1 1 3 3 1	3 1 3 2 1 3	
22 083-2 1 1 1 1	1 1 1 1 1	
23 84 1 5 1 5	3 5 3 3 5 1	
24 85 1 1 1 5 25 86 3 5 3 3	1 1 1 1 3 3 3 5 1 5 7 1	
26 87 1 1 1 3	1 3 1 1 3 3	
27 601 5 5 5 5	5 5 7 5 5 3	
28 602 5 7 7 9	5 5 7 7 5 5	
29 603 7 7 7 7	5 5 7 5 5	
30 604 9 7 9 9 31 605 5 5 5 3	9 7 9 9 7 7	
	3 3 7 5 3 3	
32 606 7 5 5 3 33 611 5 3 3 3	5 3 7 7 1 3	
33 611 5 3 3 3 34 612 3 5 5 5	3 3 5 3 3 3 3 5 3 5 3	
	3 3 5 3 5 3	
35 613 3 5 5 5 36 614 3 3 5 3	1 3 3 3 3 1	
37 615 5 3 3	5 5 7 3 5 3	
38 618 3 3 9 5	5 7 7 3 7 3	
39 202 5 5 9 5 40 203 5 7 5 3 41 204 5 7 5 5	5 7 7 7 7 7	
40 203 5 7 5 3	3 5 5 5 3 3	
41 204 5 7 5 5	5 7 5 5 3 5	
42 208 2 2 2 2 43 502 5 5 5 3	2 2 2 2 2 2 3 5 3 3 3 3	
43 502 5 5 5 3 44 220 7 5 7 7	3 7 7 5 5 7	
45 221 5 5 5 7	5 7 5 5 5 7	
46 223 4 2 2 1	1 2 2 2 2 1	
47 225 5 2 2 1	5 6 1 5 1 6	
48 231 1 1 1 1	1 1 1 1 1	
49 236 1 1 1 1	1 1 1 1 1	
50 241 3 3 3 3	3 3 3 3 3	
51 242 7 7 7 5 52 243 0 5 5 5	7 7 7 7 5 5 5 5 9 5 7 5	
52 243 9 5 5 5 53 503 7 5 5 7	5 5 9 5 7 5	

Table 2. Continue

Order No	OIV Code	Alİdris	Kara Parmak	Beyaz Kokulu	Nuri Bey	Hurma	Kara Dimrit	Siyah Pekmezlik (Demirhan)	Gelin Üzümü-3	Bülbül
1	301	3	3 3 5 5	3 5	7	7	3	3	1	7
2 3	302 303	5 5	<i>5</i>	5 5	5 5	7 5	3 5	3 5	3 5 3	3 5 5
4	304	5	5	9	7	7	5	7	3	5
5	3	1	1	1	1	1	1	1	1	1
6	4	1	1	1	1	1	7	7	3	3
7	6	3	3	3	3	3	3	3		
8	7	1	1	1	1	1	1	1	1	1
9 10	8 16	2 1	2 1	2 1	2 1	2 1	2 1	2 1	2	2 1
11	51	2	2	3	3	3	3	3	3	1
12	53	1	1	1	1	1	3	7	3	
13	151	3	3	3	3	3	3	3	3 3 2 3 2 2	3 3 2 2 2 2 3
14	68	2 2	3	1	2	3 5	4	4	3	3
15	70	2	4	1	1		3	1	2	2
16 17	76 79	5 4	4 2	2 2	4 4	4	3 2	5 2	3	2
18	80	1	1	1	1	2 2	1	1	2	3
19	081-1	1	1	1	1	1	1	2	1	1
20	081-2	1	3	1	1	1	1	1	1	1
21	083-1	1	3	3	3	1	3	1	3	3
22	083-2	1	1	1	1	1	1	2	1	1
23	84	1	1	3	5	5	7	7	3	1
24 25	85 86	3	1 5	1 5	1 5	1 5	3 7	5 7	1 3	1 1
26	87	1	1	3	3	1	3	5	1	1
27	601	5		5	5		5	3		3
28	602	5	5 5	5	7	5 5	7	5	3 5	3
29	603	5	5 7	5	5	7	7	5	5 7	5
30	604	9		9	9	9	9	7		3 3 5 5 3 3 3 5 5
31	605 606	5 7	5	7 7	5 5	3	3	3 1	5 5	3
32 33	611	5	5 3	3	3	3	3 5	3	1	3
34	612	3	3		5	5	3	5		5
34 35	612 613	3	3 3	3	5	5 5	3	5	3	
36	614	3	1	1	3	5	3	3	3	3
37	615	5	5	3	5	5	3	3	3	3
38 39	618 202	9 5	3 7	3 7	7 7	7 7	3 7	3 5	3 7	3 7
40	202	3	5	7	7	5	7	5	7	7
41	204	3 5	7	5	5	7	7	9	5	5
42	208	2	2	2	2	2	1	2	2	5 2 3 7
43	502	3	3	3	3	5	7	5	7	3
44	220	5	7	7	7	9	5	7	5	7
45 46	221 223	5 2	5 3	7 2	7 2	5 5	5	5 2	5	7 2
46 47	225 225	3	3	1	$\frac{2}{2}$	2	1 5	3	6 1	1
48	231	1	1	1	1	1	1	1	1	1
49	236	1	1	1	1	1	1	1	1	1
50	241	3	3	3	3	3	3	3	3	3
51	242	5	7	7	7	5	5	7	7	7
52 53	243 503	5	5	5	7	5	5	5	5	9
53	503	3	5	3	5	7	3	5	5	7

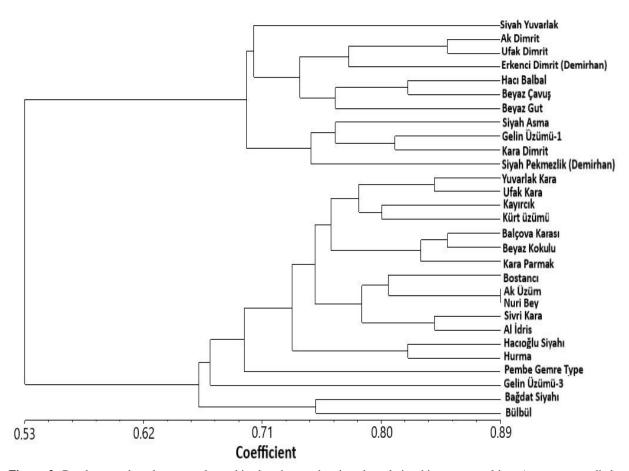


Figure 3. Dendrogram based on ampelographic descriptors showing the relationship among cultivars/genotypes studied (Dissimilarity Coefficient Euclidean Distances Squared, UPGMA)

CONCLUSION

This study revealed the ampelographic identification and differences of 29 grape varieties or genotypes collected from the Aegean region. Our study has revealed those that are highly similar to each other and those that are highly different from each other. With the adverse effects of climate change, changing consumer demands and increasing production costs, grape genetic resources are under serious threat in many countries. Unfortunately, many grape varieties grown locally have begun to disappear. These genetic resources must be identified and preserved in the coming years due to their resistance to different biotic and abiotic stress conditions and potential to be suitable for changing consumer demands. In an environment where even wild vines are gaining excellent value today, it is inevitable that our genetic resources, local grapes, will be needed in the coming years. It is essential to identify all genetic resources in different parts of our country in other ways, such as in this study, to determine the different ones and to protect them for the next generations.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

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

Data availability

Data will be made available on request.

Consent to participate

The authors consent to participate.

Funding

This study was supported by TAGEM with project number TAGEM/TBAD/16/A01/P01/012.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.10

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 82-89

An ecological study of Matricaria chamomilla L. var. chamomilla

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Article History

Received: December 7, 2024 Revised: February 26, 2025 Accepted: March 1, 2025 Published Online: March 9. 2025

Article Info Article Type: Research Article Article Subject: Ecology

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https://dergipark.org.tr/jaefs/issue/90253/1596655

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Abstract

In this study, the phytoremediation potential and essential element utilization potential of Matricaria chamomilla var. chamomilla species were investigated and the ecological characteristics of the species were determined. The plant and soil samples were collected from the Karaoren road in Aksaray province in April (2023). The research focused on the consantration of the following minerals: Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn in plant and soil samples. ICP-MS was used for plant samples and XRF device was used for soil samples. The obtained data were analyzed statistically by SPSS (version 25). According to the analysis results, while the concentrations of Al, Co, Cu, Mn, Ni, and S in the soil were above optimal values, the concentrations of Al, Co, Cu, Mn, Ni, and Pb in the plants were within the optimal range. In the stem part of the plant, the concentration of Cu, Mn, Pb and Zn elements was found being below the reference values. But, Cr and Fe concentrations in the plant were determined above reference values. However, the Bioconcentration Factor (BCF) value was low for all elements in the plant and was less than 1. This means that the potential use of this species in phytoremediation is quite limited.

Keywords: Matricaria, Chamomilla, Trace Element, Phytoremediation, **Ecological Features**

Cite this article as: Demir, A., Eskin, B., Rashidi, A. (2025). An ecological study of Matricaria chamomilla L. var. chamomilla. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 82-89. https://doi.org/10.31015/2025.1.10

INTRODUCTION

Chamomile, belongs to the Asteraceae (Compositae) family, has been known as a world-famous plant since ancient times (Mahdavi 2020). Matricaria chamomilla is a native plant of Southern and Eastern Europe, which is now widely grown in Germany, Hungary, France, Russia, the former Yugoslavia, Brazil, North Africa, India Asia, Northand South America, Australia, and New Zealand (Singh, 2011). In Turkey the natural distribution areas of this species are Thrace, Western and Southern Anatolia (Turkish Plants Data Service 2024).

In M. chamomilla (Figure 1) the glabrous stem can grow up to 45 centimeters. Lower leaves are 5-7 cm long, glabrous and oblong. Capitula is usually solitary but sometimes it is subcozymbose. Phyllaries may be oblanceolate, obtuse, or acute. The ligules are patent at first, after that they become reflexed. The ray achenes of coronas with 5 whitish ribs on their back surfaces are brown in color (Davis et al. 1975).

M. chamomilla L. var. chamomilla is an annual plant which is known as May daisy. Also it is known as chamomile, medicinal chamomile, common chamomile, babunc, akbubac, papatya and papaçya in Turkey, this plant has a wide ecological abundance area and is distributed up to 900 meters above sea level. Geologically, this plant is widely distributed worldwide, growing in various habitats such as roadsides, waste dumps, and cultivated areas (Cemek et al. 2008; Salamon, 2009; Ozdemir et. al. 2021)

Chamomile which has been used in traditional medicine for thousands of years, has exhibited various biological activities such as anti-inflammatory, antioxidant, antiseptic, antispasmodic, antiallergic, antidiabetic, anticancer and anti-microbial in numerous pharmacological studies. These properties scientifically support the plant's wide use in traditional medicine (Mihyaoui et al. 2022). Chamomile is also used extensively in the pharmaceutical, food, hygiene and cosmetic industries in nowadays. Chamomile has gained increasing commercial value worldwide due to high demand from the pharmaceutical and cosmetic industries (Catani et al. 2021). It is consumed

in the form of tea, oil, and extracts, this plant has not only maintained its place in traditional medicine but has also become an indispensable part of modern industry (Solouki et al. 2007).

Current scientific studies on *M. chamomilla* focus on the plant's anatomical, genetic, taxonomic, medicinal, chemical, pharmacostatic, cosmetic, nutritional and floristic properties (Solouki et al. 2007; Inceer and Ozcan, 2011; Ayran at al., 2018; Inceer and Bal, 2019; Yaz et al. 2023). However, it has been observed that ecological studies about investigating this plant's capacity for heavy metal accumulation and trace element utilization are relatively limited. Therefore, in this study, the focus was on the trace element utilization potential of naturally growing *M. chamomilla var. chamomilla* in Aksaray based on plant-soil interactions. Additionally, it was investigated whether this plant has phytoremediation potential or not in this study.

When the literature is examined, it is known that studies on heavy metals are mostly concentrated in regions where there is environmental pollution and the number of studies on heavy metal accumulation in the natural environment is quite low (Osma et al. 2023). For this reason, it was thought that elements such as Al (aluminum), Ba (barium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Ni (nickel), Pb (lead), S (sulfur) and Zn (zinc) would play an important ecological processes during plant growth and development. Therefore, this research is highly important in terms of determining these ecological processes.

MATERIALS AND METHODS

Description of the sampling sites

Aksaray province in the Central Kizilirmak section of the Central Anatolia Region is located between the 38-39 north parallels and the 33-35 east meridians (Eskin and Doganay, 2019). It is surrounded by Nevsehir in the east, Nigde in the southeast, Konya in the west, Ankara in the north and Kirsehir in the northeast. Aksaray's surface area is 7798 km², and its altitude above sea level varies between 900 and 3300 meters (Coskun, 2016). 433,055 people have been living in this area (Turkish Statistical Institute, 2024; Aksaray Governorship, 2024).



Figure 1. General View of the M. chamomilla L. var. chamomilla

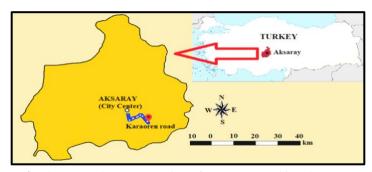


Figure 2. The research area: Location of Karaoren road in Aksaray, Türkiye

The Collection, Preparation and Analysis of Samples

Samples of *M. chamomilla L. var. chamomilla* were collected from Karaoren road in Aksaray, Turkey (Figure 2) during the flower period in April. Five plants and soil samples were taken from five different locations within the study area where the species population was dense. Soil samples were taken from under each plant were mixed and turned into a single sample. The soil samples (approximately 1000 g each) were taken from 0-15 cm depth using a shovel. The collected plants were dried in an oven at 80°C for 48 hours. The dried plants were ground with a hammer and sieved through a 1.5 mm sieve. Then, 0.2 g of plant samples were put in Teflon containers, and 4 ml of 65% HNO₃ was added to them. Next, all the plant samples were mineralized in the microwave oven (CEM MARS 5) at 145°C for 5 min 165°C for 5 min and 175°C for 20 min. The samples were left to the mineralization and cooling process were filtered through Whatman filters. Then, it was completed to 50 ml with ultrapure water

and placed in falcon tubes. A multi-element standard solution of -1000 ppm (Merck) was prepared from the stock solution. The numerical values of heavy metals and mineral elements were identified by an Inductively Coupled Plasma Mass Spectrometer device (ICP-MS; Thermo, Xseries 2 Serial number: SN02132C).

Numerical data for the analysis of the same minerals in soil samples were obtained via XRF device (Pan Analytical, Axios Max Serial number: DY5970). In this study, the soil samples were ground to $20~\mu m$ size in bearing tungsten carbide vane. 5 grams of the powder sample was mixed homogeneously with 1 gram of Micropulver Wachs C. Then the wax and sample mixture were pelletized under pressure by a Die Attacher. The obtained sample was placed on XRF device, and the element values were identified (Demir et al., 2021).

The Scheibler calcimeter method was used to determine CaCO₃ values in the soil (Caglar 1949). pH measurement was done by an electronic pH meter. Also, the organic matter measurements were made according to Walkley-Black (Black, 1965).

Additionally, total phosphorus values in the soil were determined by the Olsen and Sommers method (1982). and available potassium values in the soil were measured by photometric method.

Statistical Analyses

Pearson correlated average and standard deviation values were determined by IBM SPSS Statistics 25 software. The obtained values were demonstrated a statistical significance as **P<0.01 and *P<0.05 level (2-tailed).

Bioconcentration factor

The Bioconcentration Factor (BCF) is an index that shows a plant's sensitivity to metal pollution. BCF is a numerical value that indicates how much of a metal a plant accumulates in its tissues compared to the concentration of that metal in the surrounding soil. Plants with high BCF values (>1) can accumulate more metal from the soil through phytoremediation (Ghosh and Singh, 2005; Osma et al. 2023; Rashidi et al., 2024).

BCF = metal concentration in the plant tissue/metal concentration in the soil

RESULTS AND DISCUSSION

In this research, the concentrations of Al, Co, and Ni in the roots, stems, leaves, and flowers of M. chamomilla were found to be within optimal ranges. The concentrations of Cu and Mn were determined to be at the limit values in both the root and leaf tissues, while the stem and flower tissues exhibited concentrations below the limit values. Also Pb element is below the limit values only in the stem, it was at the limit values in all other plant parts. Zn element remained below limit values in all parts of the plant. The consentration of Cr, Fe and Zn elements in the soil are at limit values, but Al, Co, Cu, Mn, Ni, and S elements are above the limit values. When the concentrations of elements in this plant parts were evaluated, it was observed that the values for Al, Co, Cu, Fe, Mn, and Pb were ranked as root>leaf>stem>flower, while the values for Cr and Zn were ranked as leaf>root>flower>stem. The amount of elements is lined up leaf>root>stem>flower for Ba, stem>root>leaf>flower for Ni, and root>stem>leaf>flower for S (Table 1 and Table 2). Therefore, it can be said that this plant species take all the essential elements necessary for its growth and development from the soil in which it grows. At the same time, the Fe element is found in concentrations above the optimum values in all plant parts other than the stem. Fe is an essential micronutrient for plants. It plays an important role in many physiological processes such as photosynthesis, chlorophyll formation and enzyme activities (Kacar and Katkat, 2010). In addition, in M. chamomilla var. chamomilla Cr concentrations exceeded optimal levels in all plant parts. The high level of total Cr in the plant may be due to the conversion of absorbed chromium in the form of chromate (CrO₄-²) to the nontoxic Cr (III) form in the roots by Fe (III) reductase enzymes. (Zayed et al., 1998; Yildiz et al., 2011) This transformation can be considered as a defense mechanism of the plant against chromium toxicity. Also, it can be said that M. chamomilla var. chamomilla generally prefers clay-loamy and slightly alkaline soils as its natural habitat. Because these soils provide the optimal conditions for its growth and development. However, the alkaline soils reduce the solubility of Cr in the soil. This may affect the uptake of Cr by plants (Shahid et al 2017). Moreover, the more detailed research is needed to better understand the distribution, transformation, and M. chamomilla var. chamomilla availability of different forms of (Cr(III) and Cr(VI)) in the soil. As a result, biomonitoring M. chamomilla var. chamomilla throughout its entire vegetation period and evaluating the plantsoil interaction is of great importance for both scientific research and applied fields. Such studies will allow us to better understand the growth and development dynamics of M. chamomilla var. chamomilla, reveal its relationship with the soil in detail, and make the best use of this plant.

When the Bioconcentration Factor (BCF) ratios for the *M. chamomilla var. chamomilla* plant are evaluated, it is seen that the BCF value for all elements is less than 1 (<1) (Table 1). It means that the plant is not accumulating the elements from the soil very effectively. The concentration of the element in the plant tissue is lower than the concentration in the soil. This shows that the species does not have the potential to be used in phytoremediation. The accumulation of elements in the plants can be significantly affected by various factors such as soil texture, soil pH, organic matter content, and environmental conditions. Therefore, further research is crucial to fully understand the bioaccumulation potential of this plant species and to identify the key factors influencing BCF values under varying environmental conditions.

Table 1. Chemical Analysis of the Plant Parts (root, stem, leaf and flower) and Soil Samples of P. M. chamomilla var. chamomilla and Soil Samples.

Elements	Root	Stem	Leaf	Flower	Soil	Bioconcentration
			mg/kg			Factor (BCF)
Al	626.90	46.43	622.88	258.28	66300	0.023
Ba	14.03	9.20	14.23	2.85	411.2	0.098
Cr	1,69	1.06	1.81	1,11	100	0.0567
Co	0.41	0.10	0.41	0.19	33,3	0.033
Cu	7.13	2.90	7.08	4.07	38,05	0.55
Fe	483.95	38.72	483.89	271.18	41350	0.030
Mn	69.03	8.95	68.69	23.56	800	0.212
Ni	2.71	4.05	2.52	1.47	78,8	0.136
Pb	1.65	0.04	1.64	0.30	25,9	0.140
\mathbf{S}	2.50	2.34	2.08	1.65	313,9	0.027
Zn	12.78	9.27	13.91	11.51	69,05	0.687

Tablo 2. Optimum Values (Min.-Max.) of Elements for Plant and Soil Samples

Elements	Values in Plant	Values in Soil	
	mg/kg		
Al	7-3400	10000-40000	
Ba	-	-	
Cr	0.1-0.5	5-120	
Co	0.02-0.5	1-10	
Cu	5-30	5-30	
Fe	5-250	5000-50000	
Mn	30-300	270-525	
Ni	0.1-5	10-50	
Pb	0.05-3	10-30	
S	-	10-157	
Zn	20-150	10-300	

Refrences for limit values in soil and plant (Kabata-Pendias and Pendias, 2001; Ghosh and Singh, 2005; Jones and Jacobsen, 2005; Barker and Pilbeam 2007; Kabata-Pendias and Mukherjee, 2007; Kacar and Katkat, 2010; Blum, et al. 2014)

Table 3 shows that the physical analysis results of soil samples were taken from the distribution area of *M. chamomilla* var. *chamomilla*. When these results are evaluated, it can be observed that the natural habitat of M. *chamomilla* var. *chamomilla* consists of clay-loamy and slightly alkaline soils with a pH of 7.62. The level of CaCO₃ is low (5.3%), and the Saturation value is 0.026 % (non-saline). In addition, the organic matter concentration of the soil was found to be of low (1.38 %) value. These findings indicate that *M. chamomilla* var. *chamomilla*. has a certain tolerance to soil type and chemical properties. In the study at the *M. chamomilla* plant was conducted by Rezaeih et al., pH 8.2, CaCo₃ 5.9 %, organic matter 1.2 %, P₂O₅ 12.2 mg/kg, K₂O 430 mg/kg and texture clay-loam were determined in the soil (Rezaeih et al., 2015) In this case, in both studies, it is seen that the plant prefers soils with similar properties in terms of ecological properties such as texture, CaCo₃, organic matter. Therefore, the structure, pH, organic matter content of the soil affect the ability of plants to absorb nutrients.

 Table 3. The Physical Analysis Results of the Soil Samples of M. chamomilla var. chamomilla Habitats

Soil		
Analysis Type	Numerical value	Status
Texture (%)	57.09	Clayey-loamy
CaCO ₃ (%)	5.3	Low Chalky
pН	7.62	Slightly Alkaline
Saturation (%)	0.026	Without salt
Organic Matter (%)	1.38	Low
Phosphorus (P ₂ O ₅) kg/da	3.66	Low
Potassium (K ₂ O) kg/da	189.36	Adequate

In the study, the correlation calculations among root with stem, leaf and flower and between soil with root, stem, leaf and flower were investigated. No significant positive or negative correlation was found between Ba element in the root and Co in the flower and S in the leaf, between Cr in the root and Fe in the leaf, and between Fe in the root and Ba in the stem in this study. In addition, high correlation results were observed for the nutrients Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S and Zn between root and stem, leaf and flower (Table 4). This shows that the nutrients are transported and stored between different organs of the plant.

According to the correlation calculations between soil with root, stem, leaf and flower (Table 5), Ba soil-Mn stem, Cu Soil-Ba rooot, stem, Fe soil-Mn Leaf, Ni Root, Mn soil-Ba leaf, Pb root-Cr stem, Co flower, S leaf, S root-Zn flower have high value (>1). Correlation matrix (Table 5) also showed high positive correlations (>0.999, >0.720) between Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S and Zn and Zn in soil and Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S and Zn in root, stem, leaf and flower. Except for Ba in the leaf, there are low correlations (>0.699, >0.001) between Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S in the soil and Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S in the root, stem, leaf and flower. These results are very important for understanding the plant-soil interactions and determining the nutrient needs of plants.

Table 4. The Correlation Relationship of Mineral Nutrients Between Root and Other Plant Parts

Correlation N	Matrix (R)										
Root											
	Al	Ba	Cr	Co	Cu	Fe	Mn	Ni	Pb	S	Zn
Al Stem	0.996	0.984	0.950	0.774	0.994	0.989	0.153	0.987	0.931	0.971	0.866
Al Leaf	0.916	0.111	0.999**	0.527	0.975	0.983	0.175	0.985	0.999*	0.995	0.657
Al Flower	0.908	0.984	0.999*	0.510	0.970	0.979	0.194	0.982	0.999*	0.993	0.642
Ba Stem	0.975	0.999*	0.984	0.679	0.999*	0**	0.016	0.999*	0.972	0.994	0.766
Ba Leaf	0.965	1**	0.991	0.646	0.997*	0.999*	0.028	0.999*	0.982	0.998*	0.761
Ba Flower	0.957	0.999*	0.991	0.625	0.994	0.998*	0.982	0.998*	0.987	0.999*	0.742
Cr Stem	0.251	0.003	0.142	0.755	0.064	0.025	0.999*	0.011	0.198	0.067	0.640
Cr Leaf	0.937	0.995	0.999*	0.575	0.986	0.992	0.117	0.993	0.995	0.999*	0.700
Cr Flower	0.082	0.173	0.309	0.755	0.106	0.145	0.988	0.159	0.363	0.237	0.500
Co Stem	0.963	0.882	0.785	0.944	0.896	0.878	0.484	0.871	0.749	0.830	0.985
Co Leaf	0.955	0.999*	0.995	0.620	0.994	0.997*	0.999	0.998*	0.987	0.999*	0.738
Co Flower	0.251	0	0.142	0.755	0.064	0.025	0.999*	0.011	0.198	0.067	0.640
Cu Stem	0.997*	0.983	0.949	0.777	0.993	0.988	0.158	0.986	0.929	0.970	0.868
Cu Leaf	0.869	0.966	0.992	0.435	0.946	0.958	0.277	0.962	0.998*	0.981	0.574
Cu Flower	0.743	0.889	0.944	0.233	0.856	0.875	0.475	0.882	0.961	0.917	0.387
Fe Stem	0.851	0.956	0.988	0.403	0.934	0.947	0.311	0.952	0.995	0.973	0.545
Fe Leaf	0.925	0.991	0**	0.546	0.231	0.987	0.152	0.989	0.997*	0.997*	0.674
Fe Flower	0.915	0.987	0.999**	0.526	0.975	0.983	0.175	0.985	0.999*	0.995	0.657
Mn Stem	0.774	0.909	0.958	0.278	0.879	0.897	0.434	0.903	0.973	0.934	0.429
Mn Leaf	0.970	0.999**	0.988	0.662	0.998*	0.999	0.006	1**	0.977	0.996	0.774
Mn Flower	0.936	0.994	0.999*	0.572	0.985	0.991	0.121	0.993	0.995	0.999*	0.697
Ni Stem	0.999*	0.956*	0.906	0.845	0.973	0.964	0.272	0.960	0.880	0.935	0.920
Ni Leaf	0.978	0.998*	0.982	0.688	0.999*	0.999*	0.027	0.999*	0.970	0.993	0.796
Ni Flower	0.996	0.940	0.883	0.871	0.961	0.949	0.320	0945	0.855	0.916	0.939
Pb Stem	0.949	0.998*	0.996	0.604	0.991	0.996	0.081	0.997	0.990	1**	0.725
Pb Leaf	0.783	0.916	0.999*	0.293	0.886	0.904	0.420	0.910	0.976	0.940	0.443
Pb Flower	0.990	0.993	0.966	0.526	0.975	0.983	0.175	0.994	0.999*	0.995	0.657
S Stem	0.930	0.993	0.999*	0.558	0.982	0.989	0.138	0.991	0.996	0.998*	0.685
S Leaf	0.251	0	0.142	0.755	0.064	0.025	0.999*	0.011	0.198	0.067	0.640
S Flower	0.896	0.979	0.998*	0.486	0.963	0.973	0.221	0.976	1**	0.990	0.620
Zn Stem	0.999**	0.963	0.916	0.831	0.979	0.964	0.272	0.967	0.892	0.944	0.685
Zn Leaf	0.162	0.093	0.232	0.693	0.026	0.065	0.997	0.079	0.287	0.158	0.568
Zn Flower	0.998*	0.980	0.943	0.788	0.991	0.985	0.176	0.983	0.922	0.965	0.877

^{**} Correlation is significant at level of 0.01 (2-tailed), * Correlation is significant at level of 0.05 (2-tailed)

Table 5. Correlation Relationship of Mineral Nutrients Between Soil and Plant Parts

 $Correlation\ Matrix\ (R)$

Soil											
	Al	Ba	Cr	Co	Cu	Fe	Mn	Ni	Pb	S	Zn
Al Root	0.861	0.779	0.970	0.958	0.967	0.970	0.964	0.997	0.251	0.998	0.277
Al Stem	0.900	0.829	0.947	0.979	0.985	0.987	0.982	0.987	0.170	0.999*	0.197
Al Leaf	0.992	0.965	0.792	0.992	0.987	0.985	0.989	0.883	0.158	0.937	0.131
Al Flower	0.994	0.970	0.780	0.989	0.984	0.982	0.986	0.874	0.177	0.930	0.150
Ba Root	0.962	0.913	0.877	0.999	1**	0.999**	0.999**	0.945	0.003	0.980	0.024
Ba Stem	0.951	0.897	0.894	0.997*	0.999*	0.999*	0.998*	0.957	0.033	0.986	0.060
Ba Leaf	0.964	0.916	0.873	0.999*	1**	0.999*	1**	0.943	0.964	0.916	0.873
Ba Flower	0.971	0.927	0.873	0.999*	0.999*	0.998*	0,999*	0.933	0.038	0.972	0.011
Cr Root	0.990	0.961	0.801	0.994	0.990	0.988	0.991	0.890	0.142	0.942	0.116
Cr Stem	0.275	0.409	0.477	0.033	0.001	0.011	0.014	0.322	1**	0.196	0.999*
Cr Leaf	0.435	0.559	0.320	0.204	0.172	0.159	0.185	0.155	0.985	0.026	0.073
Cr Flower	0.435	0.559	0.320	0.204	0.172	0.159	0.185	0.155	0.985	0.026	0.980
Co Root	0.420	0.287	0.936	0.628	0.653	0.663	0.643	0.863	0.755	0.790	0.773
Co Stem	0.694	0.585	0.999*	0.848	0.653	0.871	0.858	0.980	0.500	0.947	0.523
Co Leaf	0.972	0.929	0.856	0.999**	0.999*	0.998*	0.999*	0.931	0.044	0.970	0.017
Co Flower	0.275	0.409	0.477	0.033	0.001	0.011	0.014	0.322	1**	0.196	0.999*
Cu Root	0.941	0.883	0.907	0.995	0.997*	0.998*	0.996	0.965	0.064	0.991	0.091
Cu Stem	0.897	0.825	0.949	0.977	0,984	0.986	0.981	0.988	0.176	0.999*	0.202
Cu Leaf	0.999*	0.987	0.724	0.973	0.965	0.962	0.969	0.931	0.260	0.895	0.234
Cu Flower	0.980	0.998*	0.560	0.903	0.888	0.882	0.894	0.692	0.460	0.780	0.436
Fe Root	0.953	0.901	0.890	0.998*	0.999*	0.999**	0.999*	0.954	0.025	0.985	0.052
Fe Stem	0.999*	0.992	0.699	0.965	0.956	0.952	0.959	0.810	0.294	0.879	0.268
Fe Leaf	0.989	0.959	0.806	0.994	0.991	0.989	0.992	0.894	0.134	0.945	0.107
Fe Flower	0.992	0.965	0.792	0.992	0.987	0.985	0.989	0.883	0.158	0.937	0.131
Mn Root	0.292	0.425	0.461	0.051	0.019	0.005	0.032	0.305	0.999*	0.179	0.999*
Mn Stem	0.988	1**	0.598	0.922	0.909	0.903	0.914	0.725	0.418	0.808	0.393
Mn Leaf	0.958	0.907	0.883	0.999*	0.999**	1**	0.999*	0.950	0.010	0.982	0.037
Mn Flower	0.984	0.949	0.824	0.997*	0.994	0.993	0.996	0.908	0.103	0.954	0.077
Ni Root	0.958	0.907	0.884	0.999*	0.999**	1**	0.999*	0.950	0.011	0.982	0.038
Ni Stem	0.840	0.754	0.979	0.946	0.956	0.960	0.952	0.999*	0.289	0.995	0.315
Ni Leaf	0.947	0.892	0.899	0.996	0.998	0.999	0.998*	0.960	0.045	0.988	0.072
Ni Flower	0.811	0.720	0.988	0.929	0.940	0.945	0.936	0.999*	0.103	0.954	0.077
Pb Root	0.996	0.975	0.766	0.986	0.980	0.977	0.982	0.863	0.198	0.921	0.172
Pb Stem	0.976	0.936	0.846	0.999*	0.998*	0.997*	0.998*	0.924	0.064	0.966	0.037
Pb Leaf	0.990	1**	0.611	0.928	0.915	0.910	0.920	0.735	0.403	0.817	0.379
Pb Flower	0.923	0.859	0.927	0.988	0.993	0.994	0.991	0.977	0.114	0.996	0.141
S Root	0.977	0.937	0.844	0.999*	0.997*	0.996	0.998*	0.922	0.067	0.965	0.040
S Stem	0.987	0.955	0.814	0.996	0.992	0.991	0.994	0.900	0.120	0.949	0.093
S Leaf	0.275	0.409	0.477	0.033	0.001	0.011	0.014	0.322	1**	0.196	0.999*
S Flower	0.997*	0.976	0.762	0.985	0.979	0.976	0.981	0.860	0.204	0.919	0.178
Zn Root	0.561	0.438	0.980	0.745	0.767	0.775	0.758	0.933	0.640	0.878	0.660
Zn Stem	0.853	0.771	0.973	0.954	0.963	0.967	0.960	0.998*	0.264	0.997*	0.290
Zn Leaf	0.361	0.490	0.395	0.124	0.092	0.079	0.105	0.234	0.995	0.106	0.993
Zn Flower	0.889	0.815	0.954	0.973	0.980	0.983	0.978	0.991	0.194	1**	0.220

^{**} Correlation is significant at level of 0.01 (2-tailed), * Correlation is significant at level of 0.05 (2-tailed)

CONCLUSION

The consentration of Al, Co, Cu, Mn, Ni, and S in the soil is above the optimum values, and it can be said that the soil where *M. chamomilla* var. *chamomilla* grows is contaminated by heavy metals, specifically Al, Co, Cu, Mn, Ni, and S. The fact that *M. chamomilla* var. *chamomilla* grows in these soils with high heavy metal pollution is an indicator of the high biomonitoring value of this species.

Cr and Fe rates in the plant are above optimum values, however, the BCF value of *M. chamomilla var. chamomilla* is low. Therefore, its potential for phytoremediation applications can be considered limited. *M. chamomilla var. chamomilla* generally prefers clay-loamy and slightly alkaline soils as its natural habitat. The soil is not only a growing medium for plants, but also a source of nutrients. The structure, pH, organic matter content of the soil affect the ability of plants to absorb nutrients. Further research should be conducted to monitor this plant during the vegetation period in terms of plant physiology and growth parameters, soil properties and nutrient levels. These detailed monitoring studies will enable a deeper understanding of the factors causes to the plant's low BCF value, allowing for a more accurate assessment of its phytoremediation potential.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare no conflict of interest.

Author contribution

Authors' individual contributions to the article are equal.

Acknowledgments

The authors would like to thank Aksaray University Scientific and Technological Application and Research Center for its contribution to the experimental analyses in the present study. In addition they would like to thank botanist experts Dr. Seher Karaman for identifying *M. chamomilla* var. *chamomilla* plant.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.11

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 90-97

Effect of different pyrolysis temperatures on biofertilizer properties of microalgal biochar and energy analysis

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Article History

Received: December 8, 2024 Revised: February 25, 2025 Accepted: March 5, 2025 Published Online: March 10, 2025

Article Info

Article Type: Research Article Article Subject: Agricultural Energy

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Available at

dergipark.org.tr/jaefs/issue/90253/1598105

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Abstract

In this study, Chlorella sp. (Cs), Chlorella vulgaris (Cv), Neochloris conjuncta (Nc), Botryoococcus braunii (Bb), and Scenedesmus obliquus (So) microalgae strains were cultivated in channel type ponds. The microalgal biomasses obtained were divided into two groups (350 and 600 °C). The microalgal biomasses in the first group were biocharized at two different pyrolysis temperatures, while those in the second group were untreated crude microalgal biomasses. As a result of the energy input-output analysis of both groups of microalgal biomasses, the highest net energy gain was calculated in the un-treated Cv strain with 52.41, while the lowest value was calculated in the biocharification process of So and Bb strains at 600°C with 13.03. In all groups, the energy efficiency, energy ratio, and net energy gain of the Cv strain were found to be higher than other microalgae strains. When the bio-fertilizer, biostimulant data, and energy data are evaluated together, it's concluded that it's most appropriate to prefer the Cv microalgae strain.

Keywords: Microalgal biomass, Microalgal biochar, Pyrolysis, Biofertilizer, This article is an open access article distributed Biostimulant, Energy efficiency

Cite this article as: Uysal, O. (2025). Effect of different pyrolysis temperatures on biofertilizer properties of microalgal biochar and energy analysis. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 90-97. https://doi.org/10.31015/2025.1.11

INTRODUCTION

The increasing trend in the global population is also similar to industrialization. It is known that the increase in industrialization is proportional to the use of fossil fuels. It is stated that the use of fossil fuels and the increase in atmospheric CO₂ levels accordingly exceeds the critical threshold value today. This situation reveals the climate crisis, drought, and the need for efficient use of water resources. Among the many energy alternatives, biofuels are likely to emerge as strategically important sustainable fuel sources in the foreseeable future (Uysal, 2022). Since the amount of C sequestered in the soil (1100 Gt; 1Gt = 1000000 tons) is much higher than that in the atmosphere (750 Gt) (Sundquist, 1993), an average of 60 Gt CO₂ is released annually from the soil to the atmosphere. This CO₂ released into the atmosphere is mostly formed during the respiration of microorganisms during the decomposition of organic matter in the soil. One of the most useful practices to reduce the increasing CO₂ concentration in the atmosphere is the process of converting CO₂ stored in biomass by photosynthesis into a more stable form of C called biochar by pyrolysis (Spokas and Reicosky, 2009). Since biochar contains more and more stable aromatic C than soil organic matter, its degradation is very slow. Biochar has been reported to persist in soil for 100 to 1000 years and has 10 to 1000 times higher organic matter than most soil organic matter. Therefore, unlike organic matter traditionally used in agriculture, biochar is expressed as an additive that will enrich C and positively affect soil quality (Lehmann and Joseph, 2015; Verheijen et al., 2010).

In recent years, studies on the reduction of atmospheric CO₂ have encountered a significant amount of biochar application. Since biochar application causes significant changes in the chemical, physical, and biological properties of the soil, it also affects the C and N dynamics in the soil (Van Zweiten et al., 2014). There are applications such as green manure, barn manure, compost, and biochar in studies to increase organic matter in soils (Akkeçeci and Özkan, 2022). While some of these applications provide nutrients directly to the soil, some of them have a healing effect on soil properties. The properties, application amounts, and decomposition degrees of organic materials added to the soil to increase the organic matter are significant for the expected benefits. The balance of decomposition and addition in the preservation of organic matter in soils is an important indicator of sustainable soil management. The good development of plants is closely related to the physical, chemical, and biological properties of the soil's environment. One of the applications made to optimize these properties is the application of organic material to the soil. Biochar applications are considered as a remarkable approach for soils that are tired after the practices during agricultural production. In almost all countries in the world, biochar applications to soils attract a great deal of attention. Biochar is a product resulting from the conversion of organic materials (of plant and animal origin) into materials containing high carbon and mineral substances by gasification at high temperatures, in an oxygen-free or very little oxygen environment (Akgül, 2017). It has been shown that the biochar obtained by the pyrolysis of biomass can make a positive contribution to issues such as reducing the loss of nutrients by washing, reducing the bioavailability of environmental pollutants, enriching carbon in the soil, reducing greenhouse gas emissions and improving soil fertility (Ippolito et al., 2012). In addition to the effect of biochar on C storage in the soil, many studies have been published investigating the effect of N₂O release in fertilized agricultural lands. It is known that N₂O, whose contribution to global warming is 298 times higher than carbon dioxide, is 100 years longer in the atmosphere (IPPC, 2013). In the last century, it has been reported that a significant amount of N₂O has been released into the atmosphere due to the use of nitrogen fertilizers in agricultural lands (Park et al., 2012). N₂O is a thermodynamically strong oxidant, but kinetically refractory towards decomposition and reduction. This kinetic barrier can be overcome through binding and activation with metal ions such as Fe or Cu (Tolman, 2010). The presence of Cu and Fe in the micronutrients required for microalgae cultivation, as well as the presence of microelements in microalgal biomass, is an indication that this kinetic barrier will be overcome. Microalgae is a material with a high potential for biochar as it is a sustainable and renewable resource (Yu et al., 2017). Microalgae's use in the production of biochar is being explored due to various advantages and potential application areas. Carbon Sequestration: Microalgae absorb carbon dioxide from the atmosphere through photosynthesis. This characteristic allows microalgae to potentially serve as a carbon sink during growth, contributing to the formation of biochar. Bioenergy Production: The incorporation of microalgae in biochar production can generate energy during the bioenergy production process. Biochar serves as a biomass fuel in energy production. Soil Improvement: Microalgae biochar can be used to enhance soil quality. The addition of biochar to soil may increase water retention capacity, improve soil structure, and enhance nutrient retention for plants. Carbon Cycling: Microalgae biochar can contribute to controlling the carbon cycle. By providing longterm carbon storage in the soil, it may help reduce carbon emissions into the atmosphere (Cheah et al., 2015; Suganya et al., 2016). Agricultural Applications: When applied to agricultural soils, microalgae biochar may stimulate plant growth and increase yield. It can also support environmental sustainability by reducing pesticide and fertilizer usage. Industrial Applications: Microalgae biochar can find applications in the production of carbonbased materials in industrial processes. Adding microalgal biochar to the soil can have several positive effects. Here are some potential impacts of adding microalgal biochar to the soil (Ayaz et al., 2021; Zhang et al., 2021). Improvement of Soil Structure: Microalgal biochar can enhance soil structure and texture. Especially in sandy or clayey soils, it can increase water retention capacity, combining better drainage and water-holding properties. Increase in Plant Nutrient Retention Capacity: Microalgal biochar can increase the soil's capacity to retain plant nutrients. This can enable plants to access necessary nutrients for a longer duration. Enhancement of Water Retention Capacity: Microalgal biochar can increase the soil's water retention capacity, supporting plant water supply during dry periods. Regulation of Soil pH: Microalgal biochar can balance soil pH and provide an optimal pH level for plant growth. Support for Soil Microorganisms: Microalgal biochar can support the development of beneficial microorganisms in the soil, contributing to the health and balance of the soil ecosystem. Carbon Storage: Microalgal biochar can increase carbon storage capacity in the soil by introducing carbon. This can aid in storing carbon from the atmosphere in the soil. Stimulation of Plant Growth: Microalgal biochar can promote plant growth by making nutrients and water in the soil more efficiently available. Improvement of Seed Germination: Microalgal biochar can enhance seed germination and the development of young plants, supporting the overall process of plant cultivation (Ayaz et al., 2021; Zhang et al., 2021).

The aim of this study is to cultivate five different microalgal (*Chlorella* sp., *Chlorella vulgaris*, *Neochloris conjuncta*, *Botyrococcus braunii*, *Scenedesmus obliquus*) strains and to evaluate the potential of the products obtained from the pyrolysis of the resulting biomass at two different temperatures (350 and 600°C) in terms of plant nutrition.

MATERIALS AND METHODS

This study was carried out in the microalgae production greenhouse located in Isparta University of Applied Sciences, Faculty of Agriculture, Research and Application Farm (37°50′28.9″ N, 30°32′16.1″ E). Open ponds were preferred as a cultivation system. Care was taken to ensure that the treatments were the same during cultivation, and a separate open pond was used for each strain. While the total capacity of each open pond was 1.2 tons, the study was carried out with a volume of 1 T (Figure 1). Cs, Cv, Nc, Bb, and So strains were selected in

the study (SAG, 2024; UTEX,2024; CCAP, 2024) (Figure 2). Basal media was preferred as the media for all strains and the pH value was recorded between 6 and 8 during the cultivation phase. The cultivation period lasted 12 days for each strain.

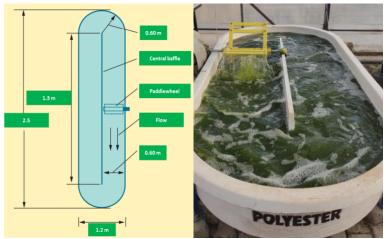


Figure 1. Open channel pond for microalgae cultivation











Figure 2. Microalgal strains in study (a) Cs, (b) Cv, (c) Nc, (d) Bb, and (e) So

Microalgal-based biochar production

Microalgal biomass samples of each strain harvested from open channel ponds were dried in an oven at 65° C until their weights remained constant. In the study, a muffle furnace was used for pyrolysis processes in a laboratory environment, and the pyrolysis duration was set to 2 hours. The dried microalgal biomasses were subjected to pyrolysis process at 350 and 600 °C, each 100 gr.

Harvest of microalgal biomass

At the end of the cultivation periods, microalgal biomass was harvested with an industrial centrifuge. Half of the harvested microalgal biomass was dried in a Memmert oven at 65°C.

Macro and micronutrient elemental analysis

Microalgal microorganisms obtained at the end of cultivation practices micro and macro elements of biomass and biochar-treated sample contents were analyzed by ICP-OES.

Energy analysis

Equation 1 for energy ratio, Equation 2 for energy efficiency, Equation 3 for specific energy, and Equation 4 for net energy gain are considered (Hesampour et al., 2021).

$$Energy\ ratio = Output\ energy\ (MJ)/(Input\ energy\ (MJ)) \tag{1}$$

Energy efficieny = Yield
$$(kg)/Input$$
 energy (MJ) (2)

Specific energy =
$$(Input energy (MJ)/(Yield (kg))$$
 (3)

Net energy gain = Output energy
$$(MJ)$$
 - Input energy (MJ) (4)

CO₂ removal

During the study period, the amount of carbon dioxide consumed in each channel pond system varied depending on the microalgae strain and biomass yield. At the end of the trials, CO₂ removal was calculated using Equation 5 (Slade and Bauen, 2013).

$$CO_2$$
 mitigation = Dry microalgal biomass (kg) X 1.83 kg CO_2 (5)

RESULTS

Micro and macro elemental analysis

Biochar samples were analyzed for micro and macro element contents are given in Table 1. When the biomasses were subjected to pyrolysis at 2 different temperatures and un-treated biomasses were examined, the highest N value was obtained in the sample pyrolyzed at 350 °C in terms of N values, while the highest value in terms of P was obtained in the sample pyrolyzed at 350 °C. In terms of K, the highest value among the samples was obtained from the sample pyrolyzed at 350 °C. When 5 different microalgae strains were analyzed, the highest N value at 350 °C pyrolysis temperature was obtained at 9.64 % in the Bb strain, while the highest N value at 600 °C was obtained at 8.10 % in the Bb strain.

Table 1. Elemental analysis values of microalgal biochars.

Biochar temp. (°C)	Microalgae	Cv	So	Nc	Bb	Cs
350 °C	N (%)	8.96	9.10	7.85	9.64	9.18
	P (%)	4.58	5.00	2.28	6.27	5.23
	K (%)	1.36	1.08	1.99	2.13	3.86
	Ca (%)	22.59	20.92	11.50	12.55	17.80
	Mg (%)	1.70	1.12	3.48	1.47	0.81
	Fe (ppm)	10560.1	11984.1	3830.39	14274.3	9789.89
	Cu (ppm)	31.572	34.341	9.032	44.933	91.32
	Mn (ppm)	8617.69	11255.7	118.465	12513.2	4839.67
	Zn (ppm)	2650.02	2069.33	75.936	2575.08	2021.74
	B (ppm)	467.223	274.147	21.924	273.046	168.823
600 °C	N (%)	7.05	7.85	5.96	8.10	6.98
	P (%)	3.71	4.22	1.75	4.73	3.67
	K (%)	1.06	0.90	1.48	1.53	2.69
	Ca (%)	17.69	17.09	8.66	9.30	12.66
	Mg (%)	1.35	0.94	2.56	1.07	0.54
	Fe (ppm)	8694.55	9896.93	2802.5	10560.9	6991.39
	Cu (ppm)	26.026	26.822	10.079	39.589	69.856
	Mn (ppm)	6642.69	9261.73	85.49	9312.72	3512.89
	Zn (ppm)	2188.45	1730.37	56.041	1957.2	1423.33
	B (ppm)	392.014	230.937	17.603	198.691	135.832

When evaluated in terms of P value, the highest P value was obtained at 6.27 % in the Bb strain at 350 °C pyrolysis temperature, while the highest P value among the samples at 600 °C pyrolysis temperature was obtained in the Bb strain. When the study was evaluated in terms of K value, the highest values among the strains at both temperature values were obtained in the Cs strain (Table 1). When it comes to sustainable and effective agriculture, the pyrolysis process is observed to be effective for the use of microalgae biochar pyrolyzed at 350°C as a biofertilizer and biostimulant in the soil-water-plant cycle.

Energy Analysis

Energy input and output during the cultivation of biochar obtained from microalgal biomass and biochar obtained by pyrolysis at different temperatures As a result of the analysis; energy ratio, energy efficiency, specific energy, and net energy gain balances of microalgal biochar pyrolyzed at 350°C are given in Table 2.

Table 2. Energy input-output values of microalgal biomass pyrolyzed at 350°C.

	Cv	So	Nc	Bb	Cs
Energy ratio	7.772	6.712	7.595	6.712	7.205
Energy efficiency	0.335	0.289	0.327	0.289	0.311
Spesific energy	2.99	3.46	3.05	3.46	3.22
Net energy gain	21.35	18.01	20.79	18.01	19.56

After the biocharification process of microalgal biomass pyrolyzed at 350 °C, the Cv strain has the highest energy efficiency value, while the So and Bb strains have the lowest energy efficiency values. When the same table in terms of net energy gain, again the highest net energy gain was calculated for the Cv strain. Regarding specific energy, the highest value was calculated in the So and Bb strains, while the lowest value was obtained in the Cv strain (Table 2). 600°C pyrolyzed microalgal biochar energy ratio, energy efficiency, specific energy, and net energy gain balances are given in Table 3.

Table 3. Energy input-output values of microalgal biomass pyrolyzed at 600°C.

	Cv	So	Nc	Bb	Cs
Energy ratio	5.633	4.865	5.505	4.865	5.223
Energy efficiency	0.284	0.245	0.277	0.245	0.263
Spesific energy	3.524	4.080	3.606	4.080	3.800
Net energy gain	15.62	13.03	15.19	13.03	14.24

After the biocharification process of microalgal biomass pyrolyzed at 600 °C, the Cv strain has the highest energy efficiency value, while the So and Bb strains have the lowest energy efficiency values. When the same table is analyzed regarding the net energy gain, the highest net energy gain was calculated for Cv strain. Regarding specific energy, the highest value was calculated in the So and Bb strains, while the lowest value was obtained in the Cv strain (Table 3). The energy ratio, energy efficiency, specific energy, and net energy gain balances of crude microalgal biomasses not included in the pyrolysis process are given in Table 4.

Table 4. Energy input-output values of raw microalgal biomass.

	Cv	So	Nc	Bb	Cs
Energy ratio	39.648	34.242	38.747	34.242	36.765
Energy efficiency	1.622	1.401	1.585	1.401	1.504
Spesific energy	0.62	0.71	0.63	0.71	0.66
Net energy gain	52.41	45.08	51.19	45.08	48.50

Energy input and output values of microalgal biomass samples harvested with an industrial centrifuge were calculated. When the table is analyzed in terms of energy efficiency, it is seen that the highest value belongs to the Cv strain, while the lowest energy efficiency value was calculated in the So and Bb strains. While the highest specific energy value was calculated in the So and Bb strains, the lowest value was calculated in the Cv strain, followed by the Nc strain. The highest value as net energy gain was calculated in the Cv strain, followed by the Nc strain. The lowest value was calculated in the So and Bb strains. When the three tables are examined together, it is seen that the highest energy ratio is in microalgal biomasses that are evaluated as raw without processing. Energy ratio, energy efficiency, and net energy gain values decreased with increasing temperature in the pyrolysis process. If the pyrolysis process is to be carried out, it is thought that 350°C temperature will be efficient in terms of energy-biomass relationship compared to other temperatures (Table 4).

CO₂ removal

Thanks to the fact that microalgae consume carbon dioxide as food, it is possible to talk about CO_2 removal in every application. The yield values of the biomass obtained from the applications were calculated. Within the scope of this study, Atmospheric CO_2 removal values were calculated according to the yield values (Figure 3).

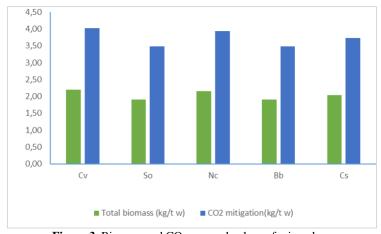


Figure 3. Biomass and CO₂ removal values of microalgae

Although they were cultivated in the same culture nutrient media, differences in biomass yields and CO_2 removal values were detected. The highest CO_2 removal among the treatments was calculated as 4.03 kg/t w in the Cv microalgae strain, while it was calculated that the same strain would remove 209.35 CO_2 /tons year if the cultivation was continued all year round. The lowest CO_2 removal between treatments was determined in So and Bb microalgae strains. CO_2 removal values were also calculated if cultivation continued for 1 year in the same culture nutrient media. CO_2 removal value was determined in the range of 180.80-209.35 kg/t y.

DISCUSSION

Use of microalgal biochar as biofertilizer or biostimulant

Nitrogen and phosphorus are the two main nutrients in the fertilizers. Scenedesmus obliquus was used strain as a treatment material in rose industry wastewater (Uysal, 2022). Scenedesmus obliquus strain was biocharised both untreated and at 2 different pyrolysis temperatures and the difference in terms of K element is quite significant. When they evaluated in terms of the N element, they obtained a significant increase at 350°C pyrolysis temperature. They reported that high pyrolysis temperature increases the energy input, therefore, when analyzed in terms of yield, 350°C pyrolysis temperature is suitable for the study. In this study, if it is necessary to evaluate in terms of macro elements, it is possible to say that 350 °C pyrolysis temperature is more efficient than 600 °C pyrolysis temperature. When analyzed in terms of microelements, it is possible to say that the content of biochar obtained at 350 °C pyrolysis temperature is more efficient (Uysal, 2022). Researchers compared the biostimulant and secondary metabolite properties of microalgae strains. They reported that the Scenedesmus obliquus strain contained auxin, cytokinin, and gibberellin for germination, root, and leaf secondary metabolites for mai bean, cress, and cucumber (Gonzalez-Perez et al., 2022). Drought is known to reduce water turgidity and normal cellular functions by affecting cellular water potential. Many studies have reported reduced photosynthesis, plant growth, and crop productivity under drought conditions (Kambo and Dutta, 2015). Although the drought effect is evident at different stages of the plant life cycle, i.e. seed germination, seedling, vegetative and reproductive stages, several studies have reported the ameliorative effects of biochar on drought-exposed plants (Matovic, 2011; Fahad et al., 2017; Abideen et al., 2020). Researchers have stated that biochar parameters such as higher pH, ash, nitrogen, and extractable inorganic nutrients enhance plant growth under drought stress (Ullah et al., 2021).

Energy analysis

Research reported that microalgal biodiesel and untreated microalgal biofertilizer samples were analyzed for energy input and output and that biofertilizer had the highest values (Uysal, 2022). Also was reported that in microalgae-wastewater applications, after the energy input-output analysis for biomass output, R3 application had the highest energy ratio among the applications with 83.063. It was followed by R2 with 55.201. In terms of energy efficiency, it was reported that the highest value was calculated in the R3 application, while the highest net energy gain was calculated at 40.93 in the R1 application, which did not include the application of GIAS. The always high energy efficiency value of biomass is proportional to the energy requirements of the procedures involved in the processing and conversion of biomass into by-products (Uysal, 2022). It is possible to see similarities in this study. Energy ratio, energy efficiency, net energy gain, and specific energy values were compared at both pyrolysis temperatures. It was observed that the energy analysis values of biochar pyrolyzed at 350°C were higher than those of biochar pyrolyzed at 600°C. It is possible to see this situation more clearly in the raw biomass subject to application without energy input. While specific energy values are low, other energy parameters have the highest values.

CO₂ removal

In a wastewater-microalgae treatment study, it was studied in 3 different application periods and it was reported that the highest removal for CO_2 mitigation in the control treatment, that is, without waste application, belonged to the second treatment and the removal amount was 1.68 kg (Uysal, 2022). In this study, CO_2 removal values were found to be higher even though the same culture medium and even one of the strains was the same. This is explained by the fact that higher biomass was obtained from this study. The biomass value and hence the CO_2 removal value obtained in the wastewater treatment can be attributed to the fact that microalgae are stressed and limit cell growth.

CONCLUSION

It is possible to see that the biofertilizer and biostimulant properties of biocharized bio-mass are further enriched. When evaluated in terms of energy, untreated biomasses have higher energy efficiency values and are therefore preferable. When all the data were evaluated together, it was determined that Cv was the most efficient strain among 5 microalgae strains. When it comes to sustainable and effective agriculture, the use of biochar of microalgae pyrolyzed at 350°C as a biofertilizer and biostimulant in the soil-water-plant cycle is envisaged to be environmentally friendly.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing interests in this study.

Author contribution

The author contributed in the full study.

Funding

No financial support was received in this study.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.12

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 98-107

Inheritance patterns of major phenological traits in pear and breeding effectiveness of parental varieties

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Article History

Received: January 5, 2025 Revised: March 4, 2025 Accepted: March 6, 2025 Published Online: March 10, 2025

Article Info

Article Type: Research Article Article Subject: Horticultural Production

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1613747

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Abstract

This study was conducted to plan targeted breeding programs in pear cultivation and, as a result, genetic parameters of major phenological traits (full bloom time, harvest time, and the duration from full bloom to harvest) were calculated along with the breeding values of parental varieties for these traits. In this context, phenological records of 2,051 hybrid plants obtained from 37 crossing combinations were collected. It was determined that all examined traits were quantitative. The genetic effect on the inheritance of full bloom time was found to be low at 31%, whereas it was higher for harvest time (83%) and the duration from full bloom to harvest (86%). In the development of early-maturing genotypes, the 'Akça' variety stood out both as a maternal parent (204.88 days) and as a pollinator (211.32 days). For late-maturing genotypes, 'Kieffer' (236.11 days) as a maternal parent and 'Ankara' (239.09 days) as a pollinator were prominent. Among the study materials, hybrids of 'Williams' Conference,' which bloomed after the 100th day of the year and completed their physiological development in approximately 150 days, showed promise for breeding lateblooming, late-maturing genotypes. Conversely, the 'Santa Maria×Akça' combination, which bloomed in the same period and required less than 100 days from full bloom to harvest, was significant for developing late-blooming, earlymaturing genotypes. The results of this study provide valuable insights for planning new breeding projects addressing the impacts of global climate change. **Keywords:** Phenology, Heterosis, Inheritance, Hybrid, *Pyrus communis* L.

Cite this article as: Mertoglu, K., Polat, M., Evrenosoglu, Y. (2025). Inheritance patterns of major phenological traits in pear and breeding effectiveness of parental varieties. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 98-107. https://doi.org/10.31015/2025.1.12

INTRODUCTION

With its favorable climatic conditions, Türkiye demonstrates its potential in early, mid, and late-season pear (Pyrus communis L.) production and ranks among the top five pear-producing countries globally (FAO, 2022). Despite this advantageous position in production, the country's export volume is still quite low with 89,302 tons (FAO, 2022). The pear market offers significant economic opportunities because the global trade volume of fresh pears exceeds 3 million tons. Achieving higher export levels in this economically promising species seems feasible by ensuring quality and standardization in production. In this context, breeding programs aimed at developing genotypes that meet the demands of both producers and consumers are essential. The traits targeted in breeding programs are predominantly quantitative, controlled by multiple genes, and exhibit a complex inheritance pattern (Evrenosoğlu et al., 2019; Karaat and Serce, 2020). Consequently, hybrid breeding remains the most widely employed method (Bilgin et al., 2020; Saridas et al., 2021; Kurnaz et al., 2024). However, in hybrid breeding programs, the emergence of desirable traits in new genotypes largely depends on chance, making the process unpredictable (Lyrene, 2018; Paranhos et al., 2022).

Wild species are often favored in breeding programs due to their superior resistance against diverse biotic and abiotic stressors. However, hybridizations involving wild species frequently lead to the expression of undesirable traits in progeny, such as grittiness (caused by stone cells), small fruit size, thorniness, and irregular fruit shapes

(Simionca Marcaşan et al., 2023). To address these issues, subsequent backcrossing with high-quality cultivars is typically required. Despite this approach, the unpredictable inheritance of desirable traits in backcrossed progeny, coupled with the lengthy, labor-intensive, and resource-intensive nature of such breeding efforts, has redirected contemporary breeding strategies toward utilizing cultivars with greater commercial value (Evrenosoğlu et al., 2010). The limited number of pear varieties cultivated globally has further resulted in the reuse of parental lines from previous programs in contemporary breeding initiatives.

To enhance the effectiveness of hybrid breeding programs, researchers must undertake comprehensive genetic studies aimed at improving both the predictability and efficiency of these initiatives. A key component of such efforts is the meticulous evaluation of parental lines for their breeding values, determined based on the traits under investigation. Additionally, the prediction of genetic parameters for each trait and the detailed elucidation of their inheritance mechanisms are indispensable for shaping the direction of future breeding programs. By carefully selecting optimal parental lines during the initial stages, the probability of obtaining progeny with the desired traits can be substantially increased. This strategy not only streamlines the hybrid breeding process but also reduces the time and financial resources required to achieve targeted breeding outcomes. Recent research efforts have increasingly prioritized addressing uncertainties in breeding programs by pinpointing the genetic loci associated with specific traits (Sanchez-Perez et al., 2012; Zhen et al., 2018) and elucidating their inheritance mechanisms (Liu et al., 2024). This line of investigation has not only gained significant prominence but also exhibited a marked upward trend in recent years reflecting its critical importance in advancing the field of plant genetics and breeding (Nyadanu et al., 2017; Evrenosoğlu et al., 2019; Fallah et al., 2022).

In this study, some phenological characteristics of 2051 F₁ pear hybrids obtained from 37 hybridization combinations involving 12 pear varieties were analyzed, and the parental varieties' breeding values were assessed. regarding related traits. Additionally, hybridization combinations that produced heterotic individuals were determined, providing practical recommendations for future pear breeding programs regarding the selection of parental lines and combinations. Furthermore, genetic parameter estimates were calculated for all evaluated traits, providing insights into the efficiency of hybridization in trait development, the genetic malleability of these traits, and their sensitivity to environmental factors.

MATERIALS AND METHODS Material

The study material consisted of 2051 hybrids obtained from 37 distinct hybridization combinations involving 5 maternal and 11 pollinator varieties within the scope of projects aimed at developing fire blight-resistant pear genotypes with high fruit quality parameters (TUBITAK TOVAG 106O719 and TUBITAK TOVAG 110O938) (Evrenosoğlu et al., 2010).

The study material that was located in experimental fields of Eskisehir Osmangazi University, Faculty of Agriculture in Eskişehir, where has a typical continental climate. The climatic characteristics observed during the experimental period at the trial site are presented in Table 1. Due to global climate change, air temperatures in both experimental years were generally observed to be higher than the long-term averages. This increase in air temperature, which enhances the potential for water vapor dissolution in the air, was accompanied by a similar trend in relative humidity. Regarding precipitation, an examination of long-term data reveals consistency across seasonal months; however, during the study period, irregular rainfall and drought periods were observed.

Table 1. Climate Characteristics of the Experimental area.

	Precipitation (mm)		Humic	Humidity (%)			Temperature (°C)		
	2018	2019	LTA	2018	2019	LTA	2018	2019	LTA
December	63.6	74.1	45.1	96.0	89.9	93.6	2.7	2.9	3.6
November	29.6	33.9	29.2	79.2	76.2	80.3	8.4	7.9	7.5
October	41.0	18.3	27.0	75.5	70.1	79.6	14.0	14.2	12.9
September	2.8	4.0	17.0	65.4	62.1	58.4	18.6	18.3	17.3
August	18.0	3.2	12.4	63.5	61.0	54.7	22.9	22.3	21.8
July	39.2	36.4	14.2	65.5	62.4	53.0	22.3	21.3	21.9
June	46.6	36.6	29.9	69.5	67.9	57.2	19.9	20.9	18.9
May	62.2	39.8	41.9	74.8	65.1	60.8	16.8	16.5	14.8
April	12.6	24.8	40.5	61.6	69.3	62.8	13.8	9.5	9.9
March	53.6	9.2	30.3	73.5	64.5	65.1	9.2	6.3	5.3
February	40.5	50.1	32.5	90.7	79.6	92.6	6.6	3.4	4.7
January	31.5	60.2	38.7	95.5	91.0	98.2	2.2	4.3	0.3
Average	36.8	32.6	29.9	75.9	71.6	71.4	13.1	12.3	11.6

LTA: Average climate data between 1929 and 2019, bold lines mean growing periods.

Phenonological observations

The full bloom stage was recorded as the period when 70–80% of flower buds had fully bloomed (Karaçalı, 2012). The determination of whether the fruits had reached harvest maturity was based on criteria such as color development, abscission layer formation, and taste (Mertoğlu and Evrenosoğlu, 2017). The total number of days between the full bloom date and the harvest date was calculated (Karaçalı, 2012).

Genetic Parameter Estimation and Statistical Analyses

The genetic parameters of the traits under consideration were calculated using estimates of variance components. Variance component estimates were obtained using the REML (Restricted Maximum Likelihood) method, based on the mathematical model (1) provided below, and computed through the ASReml software.

$$y = Z_1YIL + Z_2a + Z_3b + Z_4melez + E$$
Here;

$$YIL; \qquad \qquad \sim N(0, A\sigma_{yll}^2)$$
impacts of the chance regarding year,
$$a; \qquad \qquad \sim N(0, A\sigma_b^2)$$
impacts of the chance regarding maternal parents,
$$b; \qquad \qquad \sim N(0, I\sigma_b^2)$$
impacts of the chance regarding pollinators,
$$melez; \qquad \qquad \sim N(0, I\sigma_{melez}^2)$$
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$$E; \qquad \qquad \sim N(0, I\sigma_{melez}^2)$$
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$$V_{A}$$

RESULTS AND DISCUSSION

In the F₁ hybrids obtained from different hybridizations, measurements were taken over two consecutive years, and the results regarding the variance components and genetic parameters of the phenological traits are presented in Table 2. The variance attributed to additive genes for full bloom was proportionally lower within the total phenotypic variance compared to harvest time and the duration from full bloom to harvest. Consequently, the narrow-sense heritability derived from these ratios was estimated as 32% for full bloom time. In contrast, it was 83% and 86% for harvest time and the duration from full bloom to harvest, respectively. These results indicate that environmental factors significantly influence full bloom traits compared to harvest time and the duration from full bloom to harvest (Table 2). This phenomenon can be attributed to the requirement for specific climatic thresholds to initiate flowering. Szabo et al. (2019) and Legave et al. (2013) emphasized that meeting temperature requirements is a critical factor influencing the initiation of flowering, with a reported linear regression relationship between temperature and flowering at $R^2 = 0.55$. In contrast, fruit development is a dynamic process that continues uninterrupted from fertilization to harvest. Assimilation products generated during daytime photosynthesis are transported to the fruits day and night, making harvest time and the duration from full bloom to harvest less susceptible to year-to-year environmental variations compared to full bloom time. Similar findings have been reported in other studies, which also noted that the narrow-sense heritability for full bloom time is lower than that of other phenological traits due to the reduced influence of genotype on this trait (Piaskowski et al., 2018; Hajnajari et al., 2019). The inclusion of data spanning two vegetation periods in this study allowed for a clearer delineation of these trait differences.

The broad-sense heritability, which incorporates the effects of non-additive genes, was estimated at 52%, 92%, and 98% for full bloom time, harvest time, and the duration from full bloom to harvest, respectively. These results indicate that multiple genes are involved in the inheritance of these phenological traits, demonstrating a polygenic inheritance pattern. Similar findings have been reported in studies on other fruit species, including almond (Sanchez-Perez et al., 2007), peach (Rakonjac et al., 2011), sour cherry (Piaskowski et al., 2018), and apple (Hajnajari et al., 2019), where phenological traits were also shown to exhibit polygenic inheritance. In such cases, identifying the chromosomal loci of the genes controlling these traits becomes crucial, with an emphasis on achieving genetic progress through major-effect genes (Sanchez-Perez et al., 2007). The dominance heritability estimates from additive genes were found to be relatively low, at 5%, 2%, and 3% for full bloom time, harvest time, and the duration from full bloom to harvest, respectively. This suggests that epistatic interactions have a greater influence on inheritance compared to dominance effects. A study by Hwang et al. (2015) on Asian pears examined the inheritance of harvest time using 13 varieties and 15 hybrid combinations. Their findings, consistent with our results, reported heritability values generally exceeding 80%.

Table 2. Variance Components and Genetic Parameter Estimates for Phenological Traits.

	Variance	components	3	Genetic parameters				
	V_A	V_D	σ_{YIL}^2	σ_E^2	σ_P^2	h^2	H^2	d^2
NDBH	366.47	50.69	7.38	172.54	425.83	0.86	0.98	0.03
Harvest time	452.43	53.27	142.76	164.61	546.91	0.83	0.92	0.02
Full Bloom	3.72	2.54	12.08	7.42	12.08	0.31	0.52	0.05

NDBH: Number of days from full bloom to harvest, V_A : additive genetic variance, V_D : non-additive genetic variance (dominance and epistasis), σ_{YIL}^2 : variance of year, σ_E^2 : Error variance, σ_P^2 : total phenotypic variance, h^2 : narrow-sense heritability, H^2 : broad-sense heritability, d^2 : Heritability of dominance

Descriptive statistics and breeding values for full bloom time, harvest time, and the duration from full bloom to harvest across parent varieties and years are provided in Table 3. Significant statistical differences were observed among maternal parents, pollinators, and years for all traits examined.

The mean values of hybrids derived from maternal parents for full bloom indicated the following sequence of reaching full bloom: 'Kieffer' (93.39 days), 'Akça' (96.60 days), 'Williams' (98.06 days), 'Santa Maria' (98.13 days), and 'Maggness' (99.79 days). Similarly, the ranking of pollinators for this trait was as follows: 'Conference' (95.51 days), 'Santa Maria' (96.29 days), 'Moonglow' (96.97 days), 'Bursa' (96.97 days), 'Taş' (97.18 days), 'Kieffer' (97.23 days), 'Ankara' (97.35 days), 'Williams' (97.40 days), 'Kaiser Alexandre' (98.57 days), 'Limon' (99.30 days), and 'Akça' (101.19 days). In a study conducted under the ecological conditions of Bingöl-Türkiye, the full bloom times of five varieties ('Akça,' 'Ankara,' 'Deveci,' 'Santa Maria,' and 'Williams') ranged between the 83rd ('Williams') and 119th ('Santa Maria') days of the year across two consecutive years (Osmanoğlu et al., 2013). Similarly, in Serbia, under a harsh continental climate, hybrids reached full bloom between the 105th and 146th days of the year over three years (Gordana, 2019). Another study conducted at the Horticulture Research Institute in Erzincan observed that approximately 15% of genotypes reached full bloom early, 70% during the mid-period, and 15% late (Öz and Aslantaş, 2015). Full bloom variations for both maternal and pollinator varieties showed approximately 10% variation (Table 3), suggesting a low and uniform level of variation for this trait.

When evaluating the effects of maternal parents on ripening, the 'Akça' (204.88 days) stood out as the leading candidate for obtaining early genotypes, while 'Kieffer' (236.11 days) was prominent for late-ripening genotypes.

Other maternal parents, such as 'Santa Maria' (220.33 days), 'Williams' (229.93 days), and 'Maggness' (230.99 days) were identified within this range. The 'Akça' (211.32 days) also stood out as a pollinator for early ripening, while 'Ankara' (239.09 days) was promising for late-ripening generations. Among pollinators, 'Bursa' (219.68 days) and 'Moonglow' (220.39 days) contributed to early ripening, while 'Limon' (234.70 days) and 'Kieffer' (235.33 days) were effective for obtaining late-ripening genotypes. Studies with similar materials indicated that harvest times for the examined varieties and genotypes ranged between July 17 and September 9, reflecting the population's broad variation (Mertoğlu and Evrenosoğlu, 2017; Evrenosoğlu and Mertoğlu, 2020). A study in Bosnia and Herzegovina under ex-situ conditions also reported wide variation, ranging from extremely early ripening to late-season maturity (Zeljkovic et al., 2019).

The coefficients of variation for harvest time were generally between 6-7%, lower than those for full bloom. Similarly, hybrids derived from 15 different crossing combinations showed that the coefficient of variation for harvest time did not exceed 4% (Hwang et al., 2015). In the current study, the coefficients of variation for full bloom ranged from 9-11%, while those for harvest time ranged from 6-8%, suggesting that subsequent generations resemble the parent varieties more closely for harvest time than for full bloom.

The duration from full bloom to harvest followed trends similar to harvest time. The 'Akça' variety contributed to the fastest completion of developmental physiology as both a maternal parent (109.29 days) and a pollinator (111.38 days). Conversely, 'Kieffer' (143.69 days) as a maternal parent and 'Ankara' (142.75 days) as a pollinator contributed to the slowest development. Other maternal parents such as 'Santa Maria' (123.22 days) supported relatively faster development, whereas 'Maggness' (132.46 days) and 'Williams' (132.91 days) contributed to laterseason genotypes. Among pollinators, varieties such as 'Bursa' (123.70 days), 'Moonglow' (124.42 days), and 'Williams' (127.51 days) supported moderate fruit development speed, while 'Limon' (136.39 days), 'Santa Maria' (138.35 days), and 'Kieffer' (139.10 days) contributed to slower fruit development. A study in northern Anatolia reported that promising pear genotypes required 89 to 212 days from full bloom to harvest maturity across years (Öztürk and Demirsoy, 2013). Variation observed for harvest time was higher than for full bloom. The 'Kieffer' variety was promising for developing late-ripening genotypes, as indicated by its low coefficient of variation (4.75%).

During the first year of the study, data from all hybrids indicated that full bloom occurred around the 90th day of the year, while in the second year, it extended to the 110th day. The earlier bloom in 2018 was likely due to higher average temperatures in April of that year (Table 3). Legave et al. (2013) and Szabo et al. (2019) identified temperature requirements as the most critical factor influencing the onset of bloom. The delay in bloom during the second year also affected harvest time, which occurred on average 15 days later (238th day) than in the first year (223rd day). Additionally, the shorter duration from full bloom to harvest in 2018 (129 days) compared to 2019 (135 days) highlights the role of warmer temperatures in accelerating development, whereas cooler conditions prolonged it (El Yaacoubi et al., 2014).

The performances of hybridization combinations for the evaluated phenological traits obtained from 37 hybridizations are presented in Table 4. For full bloom, combinations ranged between the 88^{th} day (Akça × Taş) and the 112^{th} day (Maggness × Moonglow) of the year, while harvest dates occurred between the 194^{th} day (Akça × Taş) and the 246^{th} day (Williams × Conference). Regarding the duration from full bloom to harvest, the hybrid combinations exhibited a distribution between 99.86 days (Santa Maria × Akça) and 144.82 days (Williams × Conference).

In regions where early-season cultivation is emphasized, genotypes that complete their development quickly gain importance. In this context, among the hybridizations the Akça used, especially the 'Santa Maria × Akça' (99.86 days) stands out prominently. Conversely, genotypes that bloom after late spring frosts and do not require a very high accumulated temperature are needed in areas with high altitudes and a predominant continental climate. Within the study material, the 'Williams × Conference' combination, which blooms after the 100th day of the year and has a harvest time exceeding 140 days from full bloom, appears promising for such conditions.

Table 3. Descriptive Statistics and Breeding Values of Parent Varieties for Phenological Traits

		•	s and I	Breedin	ig Value	es of Parent Vari	eties fo	or Phen	ological				
Materna	-	ıll Bloom				Harvest				NDBH			
1	N	Mean±S.D	Mi	Ma	C.V	Mean±S.D	Mi	Ma	C.V	Mean±S.D	Mi	Ma	C.V
			n	X	(%)		n	X	(%)		n	X	(%)
William	9	(0.25)98.06	79.	118	10.6	(5.66)229.93	187	271	8.96	(5.30)132.9	84.	184	14.5
S	3	$^{\mathrm{B}}\pm10.45$	00	.00	6	$^{\mathrm{B}}\pm20.61$.00	.00		$1^{B}\pm19.33$	00	.00	5
	2												
Maggne	4	(1.64)99.79	82.	118	11.0	(7.73)230.99	182	273	6.76	(6.23)132.4	74.	221	12.3
SS	8	$^{A}\pm11.01$	00	.00	4	^B ±15.61	.00	.00		$6^{B}\pm16.41$	00	.00	9
	2												
Akça	4	(-	84.	118	11.6	(-	179	245	7.74	(-	83.	137	12.4
_	2	$0.09)96.60^{C}$	00	.00	5	22.02)204.88	.00	.00		21.94)109.2	00	.00	2
		±11.26				D±15.85				9 ^D ±13.57			
SantaM	3	(-	82.	118	10.8	(-	179	266	8.60	(-	75.	157	14.4
aria	4	$0.12)98.13^{B}$	00	.00	4	5.55)220.33 ^C	.00	.00		5.52)123.22	00	.00	7
	8	±10.63				±18.95				^C ±17.83			
Kieffer	2	(-	82.	118	10.4	(14.18)236.1	184	258	4.75	(15.93)143.	97.	169	8.36
	4	1.69)93.39 ^D	00	.00	8	1 ^A ±11.21	.00	.00		69 ^A ±12.02	00	.00	
	7	±9.79											
	•	=>.,,											
Pollinat	N	Mean±S.D	Mi	Ma	C.V	Mean±S.D	Mi	Ma	C.V	Mean±S.D	Mi	Ma	C.V
or	11		n	X	(%)		n	X	(%)		n	X	(%)
Akça	3	(0.22)101.1	79.	118	10.7	(-	179	259	7.88	(-	74.	221	14.0
7 Hiça	9	9 ^A ±10.86	00	.00	4	21.07)211.32	.00	.00	7.00	21.07)111.3	00	.00	8
	9) ±10.00	00	.00	7	F±16.64	.00	.00		8 ^E ±15.68	00	.00	O
William	1	(1.51)97.40	86.	116	10.6	(6.52)223.91	190	253	6.14	(5.02)127.5	100	157	9.86
S	1	C±10.40	00.	.00	7	DCE±13.76	.00	.00	0.14	$1^{DC} \pm 12.58$.00	.00	7.00
8	3	±10.40	00	.00	,	±13.70	.00	.00		1 ±12.36	.00	.00	
Kieffer	1	(-	82.	117	11.2	(7.08)235.33	187	271	8.19	(7.98)139.1	90.	184	12.8
Kienei	5	0.93)97.23 ^C	00	.00	11.2	B±19.28	.00	.00	0.19	$0^{B} \pm 17.86$	90. 00	.00	4
	4	±10.90	00	.00	1	±17.26	.00	.00		0 ±17.80	00	.00	4
SantaM	4	(0.77)96.29	82.	118	11.3	(-	179	262	6.18	(-	91.	169	10.5
aria	2	DE±10.96	00	.00	8	0.57)233.64 ^B	.00	.00	0.16	1.47)138.35	00	.00	0
arra	9	±10.90	00	.00	0	±14.43	.00	.00		BA±14.52	00	.00	U
Conform	7	(82.	115	10.7		187	269	8.45	± 14.32 (3.71)130.0	83.	162	13.2
Confere		(- 0.78)05.51E		115		(2.96)224.54			0.43				
nce	U	0.78)95.51 ^E	00	.00	2	DC±18.97	.00	.00		$3^{C} \pm 17.20$	00	.00	3
17 '	0	±10.24	0.5	110	0.64	(2.92)227.52	107	266	0.73	(0.74)100.0	0.1	150	12.0
Kaiser	9	(2.09)98.57 B+9.50	85.	118	9.64	(2.83)227.53 C+19.61	187	266	8.62	(0.74)129.9	91.	156	13.2
A 1	1		00	.00	10.5		.00	.00	c 25	6 ^C ±17.19	00	.00	3
Ankara	5	(-	82.	117	10.5	(7.21)239.09	184	271	6.35	(9.54)142.7	91.	175	8.91
	5	2.32)97.35 ^C	00	.00	3	^A ±15.18	.00	.00		5 ^A ±12.72	00	.00	
	3	±10.25	0.4	110	0.00		101	2.52	a	,	100	1.5.5	0.24
Moongl	3	(0.58)96.97	84.	113	9.92	(-	191	262	5.72	(-	100	155	8.24
ow	6	$^{DC} \pm 9.62$	00	.00		3.82)220.39 ^D	.00	.00		4.37)124.42	.00	.00	
_						E±12.61				D±10.25			
Bursa		(0.35)96.97	86.	113	9.67	(-	187	252	8.39	(-	83.	158	16.3
	0	$^{DC}\pm 9.37$	00	.00		$2.51)219.68^{E}$.00	.00		2.83)123.70	00	.00	1
						±18.43				$^{D}\pm20.17$			
Taş	1	(-	83.	118	11.2	(-	191	273	8.36	(-	97.	164	11.8
	4	1.30)97.18 ^D	00	.00	0	2.44)226.43 ^C	.00	.00		1.16)130.26	00	.00	0
	3	$^{\text{C}} \pm 10.88$				± 18.92				$^{\text{C}}$ ±15.38			
Limon	2	(-	87.	116	11.4	(3.82)234.70	215	245	4.02	(3.91)136.3	117	154	7.99
	3	$0.19)99.30^{B}$	00	.00	9	$^{\mathrm{B}}\pm9.43$.00	.00		$9^{B}\pm10.90$.00	.00	
		± 11.41											
Years	N	Mean±S.D	Mi	Ma	C.V	Mean±S.D	Mi	Ma	C.V	Mean±S.D	Mi	Ma	C.V
			n	X	(%)		n	X	(%)		n	X	(%)
2019			96.	118	3.11		182	273	7.91		74.	164	15.4
		110.00^{A}	00	.00		237.39 ^A	.00	.00		134.35 ^A	00	.00	5
2018			79.	99.	3.32		179	271	7.52		86.	221	12.9
		89.23 ^B	00	00		222.59^{B}	.00	.00		128.39^{B}	00	.00	0

89.23^B 00 00 222.59^B .00 .00 128.39^B 00 .00 0 N: Number of plants, NDBH: Number of days from full bloom to harvest, C.V: Coefficient of variation Table 4. Performance of Hybrid Combinations for Phenological Traits

	Ful	l Bloom				Harvest				NDBH			
	N	Mean±S.	Mi	Ma	C.V	Mean±S	Mi	Ma	C.V	Mean±S	Mi	Ma	C.V
		D	n	X	(%)	.D	n	X	(%)	.D	n	X	(%)
Akça×Willia	7	95.14 ^J -	88.	114	11.5	208.57 ^J -	194	224	11.4	114.43 ^G -	104	137	11.9
ms	_	o±11.02	00	.00	8	N±4.34	.00	.00	7	J±4.51	.00	.00	3
Akça×Kieffer	5	93.60 ^L -	84.	111	10.9	203.40 ^L -	199	214	6.43	110.80 ^H -	90.	122	12.2
.1 0		O±10.29	00	.00	9	N±2.87	.00	.00	20.5	J±5.49	00	.00	8
Akça×Santa	1	95.77 ^{F-}	85.	115	11.6	201.08 ^K -	179	245	20.7	106.31 ^{IJ}	91.	131	13.1
Maria	3	K±11.15	00	.00	4	N±5.76	.00	.00	6	±3.65	00	.00	7
Akça×Confere	1	96.73 ^G	86.	115	12.1	205.09 ^K -	187	232	12.2	109.36 ^{IJ}	83.	130	16.5
nce	1 5	^L ±11.73 105.20 ^B ±	00 90.	.00 118	3 12.2	N±3.71 212.80 ^H -	.00 188	.00 245	9 22.0	±4.98 108.60 ^{IJ}	00 99.	.00 136	3 15.5
Akça×Kaiser	3	103.20°± 12.87	90. 00	.00	4	M±9.84	.00	.00	0	±6.93	99. 00	.00	0
Akça ×Taş	1	88.00 ^P ±*	88.	.00 88.	4 *	194.00 ^N	.00 194	.00 194	*	±0.93 107.00 ^{IJ}	107	107	*
AKÇa ^1aş	1	88.00 ±	00	00		±*	.00	.00		±×	.00	.00	
Williams×Akç	2	101.29 ^{DC}	79.	117	10.9	210.38 ^{I-}	187	259	15.5	110.09 ^{IJ}	84.	146	13.1
a	4	±11.04	00	.00	0	M±0.99	.00	.00	4	±0.84	00	.00	4
u	5	±11.01	00	.00	Ü	20.77	.00	.00	•	±0.01	00	.00	•
Williams×Kie	7	94.92 ^{J-}	84.	113	10.5	236.82 ^{A-}	187	271	19.9	142.91 ^B	98.	184	17.1
ffer	4	0±10.05	00	.00	9	F±2.32	.00	.00	2	A±2.00	00	.00	7
Williams×	3	99.03 ^{C-}	84.	113	10.4	229.23 ^B -	200	262	15.9	131.19 ^{A-}	110	155	13.9
Santa Maria	1	^I ±10.37	00	.00	7	G±2.87	.00	.00	9	F±2.51	.00	.00	5
Williams×	1	$101.88^{C} \pm$	88.	115	10.4	245.71 ^A	217	269	16.4	144.82 ^A	108	162	12.9
Conference	7	10.64	00	.00	4	± 4.00	.00	.00	9	± 3.14	.00	.00	6
Williams×	4	99.02 ^C -	89.	116	9.60	232.02 ^{A-}	199	266	16.3	134.00 ^{A-}	102	155	13.4
Kaiser	6	^I ±9.51	00	.00		G±2.41	.00	.00	3	$E \pm 1.99$.00	.00	6
Williams×	4	97.02^{F-}	83.	113	10.2	240.27^{B}	192	271	15.7	144.24 ^A	91.	175	11.7
Ankara	1	$K \pm 9.89$	00	.00	0	^A ±0.77	.00	.00	2	± 0.58	00	.00	5
	6												
Williams×Mo	1	93.82 ^K -	84.	112	8.24	220.88 ^E	199	266	12.6	128.06 ^A -	116	155	8.87
onglow	7	o±7.73	00	.00		$^{J}\pm 3.06$.00	.00	2	$^{G}\pm 2.15$.00	.00	
Williams×Bur	1	95.83 ^{I-}	89.	113	9.60	237.92 ^A -	215	252	12.2	143.08 ^B	112	158	14.1
sa	2	o±9.20	00	.00		D±3.53	.00	.00	2	A±4.08	.00	.00	3
Williams×Taş	7	95.26 ^J	85.	118	10.4	225.49 ^B -	191	257	19.6	131.23 ^A -	106	158	14.2
******** * * *	0	O±10.45	00	.00	5	I±2.34	.00	.00	1	F±1.70	.00	.00	0
Williams×Lim	4	$96.00^{HN} \pm$	87.	105	10.3	231.75 ^A -	215	245	15.5	136.75 ^A -	129	141	5.68
on	•	5.20	00	.00	9	G±7.78	.00	.00	6	D±2.84	.00	.00	12.0
Kieffer×Santa	2	93.41 ^{L-} ⁰ ±9.79	82.	118	10.4	236.11 ^{A-} E±0.71	184	258	11.2	143.69 ^A	97.	169	12.0
Maria	4 7	°±9./9	00	.00	8	±0.71	.00	.00	1	± 0.77	00	.00	2
Santa	4	100.52 ^C -	84.	118	10.9	199.39 ^{M-}	179	228	14.2	99.86 ^J ±2	75.	134	13.4
Maria×Akça	4	E±11.03	00	.00	7	N±2.14	.00	.00	2	.03	00	.00	4
Santa	1	97.59 ^F -	86.	116	10.6	224.83 ^B -	190	253	13.3	.03 128.24 ^{A-}	100	157	12.1
Maria×Willia	0	J±10.43	00	.00	9	I±1.30	.00	.00	6	G±1.19	.00	.00	6
ms	5	110.43	00	.00	,	11.50	.00	.00	O	-1.17	.00	.00	O
Santa	3	101.58 ^{DC}	85.	113	10.8	229.52 ^A -	187	262	22.2	128.94 ^{A-}	94.	155	19.6
Maria×Kieffer	1	±10.98	00	.00	1	G±3.99	.00	.00	4	G±3.53	00	.00	6
SantaMaria×C	2	92.56°±9	82.	110	10.5	219.84 ^E -	194	262	15.5	128.28 ^{A-}	97.	153	15.1
onference	5	.77	00	.00	5	K±3.11	.00	.00	4	G±3.03	00	.00	6
Santa	3	97.60 ^{F-}	85.	116	9.71	223.03 ^C -	187	263	23.7	126.43 ^B -	91.	156	20.2
Maria×Kaiser	0	^J ±9.47	00	.00		J±4.33	.00	.00	4	^H ±3.70	00	.00	6
Santa Maria ×	3	95.07 ^{J-}	82.	112	11.5	227.73 ^B -	195	266	18.6	133.67 ^{A-}	102	157	15.7
Ankara	0	$^{\rm O}\!\!\pm\!10.99$	00	.00	6	$^{H}\pm3.40$.00	.00	2	$E \pm 2.87$.00	.00	4
Santa	1	99.11 ^{C-}	87.	113	10.3	219.28 ^{F-}	191	237	12.9	121.17 ^{D-}	100	145	10.8
Maria×Moong	8	$^{I}\pm 10.30$	00	.00	9	$K \pm 3.05$.00	.00	6	$^{I}\pm 2.55$.00	.00	2
low													
Santa	2	97.46 ^F -	86.	112	9.57	211.86 ^H -	187	231	14.7	115.39 ^F -	83.	141	16.3
Maria×Bursa	8	^J ±1.81	00	.00		$^{M}\pm 2.79$.00	.00	8	J±3.09	00	.00	7
Santa	3	100.21 ^C -	83.	115	11.1	222.00 ^D -	191	253	19.4	122.79 ^C -	97.	149	14.3
Maria×Taş	3	F±1.94	00	.00	5	I±3.39	.00	.00	9	^I ±2.50	00	.00	5
Santa	4	$101.75^{\circ}\pm$	89.	116	12.4	238.75 ^{A-}	232	245	5.85	138.00 ^A -	121	154	13.4
Maria×Limon		6.22	00	.00	5	^C ±2.93	.00	.00		^C ±6.75	.00	.00	9
Maggness×Ak	1	101.22^{DC}	87.	117	10.3	218.18 ^G -	182	248	16.8	118.88 ^E -	74.	221	18.0
ça	1	± 10.48	00	.00	6	$^{L}\pm 1.61$.00	.00	6	$^{I}\pm 1.72$	00	.00	5
	0												

Maggness× Williams Maggness× Kieffer Maggness× Santa Maria	1 4 4 1 3	$\begin{array}{c} 93.00^{\text{M-}} \\ o_{\pm}* \\ 98.45^{\text{C-}} \\ {}^{\text{I}}\!$	93. 00 82. 00 86. 00	93. 00 117 .00 118 .00	* 11.6 3 11.3 3	$\begin{array}{c} 235.00^{\text{A-}} \\ ^{\text{F}}\pm * \\ 240.55^{\text{B}} \\ ^{\text{A}}\pm 1.69 \\ 233.29^{\text{A-}} \\ ^{\text{G}}\pm 1.25 \end{array}$	235 .00 220 .00 196 .00	235 .00 258 .00 261 .00	* 11.2 1 14.6 6	$\begin{array}{c} 143.00^{B} \\ ^{A}\pm\times \\ 143.09^{B} \\ ^{A}\pm1.79 \\ 133.41^{A-} \\ ^{E}\pm1.07 \end{array}$	143 .00 105 .00 99.	143 .00 170 .00 168 .00	* 11.9 0 12.5 2
Maggness× Conference Maggness× Kaiser Maggness× Ankara	8 1 7 1 0 1 0	$\begin{array}{l} 92.71^{\text{NO}} \pm \\ 6.60 \\ 96.10^{\text{HM}} \pm \\ 7.20 \\ 99.24^{\text{C-}} \\ ^{\text{H}} \pm 11.19 \end{array}$	87. 00 89. 00 85. 00	110 .00 115 .00 117 .00	7.12 7.49 11.2 7	$\begin{array}{c} 222.88^{\text{C-}} \\ {}^{\text{J}}\pm1.72 \\ 227.70^{\text{B-}} \\ {}^{\text{H}}\pm4.36 \\ 237.72^{\text{A-}} \\ {}^{\text{D}}\pm0.93 \end{array}$	210 .00 195 .00 184 .00	241 .00 241 .00 262 .00	7.08 13.7 8 9.64	$^{131.18^{A-}}$ $^{F}\pm 1.87$ $^{132.60^{A-}}$ $^{E}\pm 4.84$ $^{139.48^{A-}}$ $^{C}\pm 1.34$	118 .00 103 .00 93. 00	149 .00 153 .00 174 .00	7.72 15.3 1 13.8 5
Maggness× Moonglow Maggness× Taş Maggness× Limon	7 1 3 9 1 5	112.00 ^A ± * 98.28 ^D J±1.76 99.53 ^C G±3.09	112 .00 86. 00 88. 00	112 .00 113 .00 116 .00	* 11.0 2 11.9 8	$\begin{array}{c} 232.00^{\text{A-}} \\ \text{G}_{\pm}* \\ 232.72^{\text{A-}} \\ \text{G}_{\pm}2.44 \\ 234.40^{\text{A-}} \\ \text{G}_{\pm}2.21 \end{array}$	232 .00 202 .00 218 .00	232 .00 273 .00 245 .00	* 15.2 6 8.56	$\begin{array}{c} 121.00^{D\text{-}} \\ ^{I}\pm\times \\ 135.44^{A\text{-}} \\ ^{D}\pm2.54 \\ 135.87^{A\text{-}} \\ ^{D}\pm3.05 \end{array}$	121 .00 101 .00 117 .00	121 .00 164 .00 152 .00	* 15.8 8 11.8 2

N: Number of plants, NDBH: Number of days from full bloom to harvest, C.V: Coefficient of variation

CONCLUSIONS AND RECOMMENDATIONS

All examined traits exhibited polygenic inheritance. High heritability levels were observed for phenological traits, moderate for morphological traits, and low for pomological and chemical traits. The heritability of full bloom time (31%) was found to be significantly lower than that of harvest time (83%) and the duration from full bloom to harvest (86%). With global climate change, the duration and severity of late spring frosts have increased in recent years, making the selection of genotypes suited to specific regions more critical. Therefore, late-blooming genotypes, as well as early or late-maturing genotypes, will undoubtedly gain importance. For late blooming, the parent variety 'Maggness' and the pollinator variety 'Akça' are recommended for breeding programs.

One of the most striking results of the study is the demonstrated importance of gene interactions between parents due to their specific combining abilities. Among the study material, the 'Williams×Conference' hybrids, which bloom after the 100^{th} day of the year and complete their development in approximately 150 days, showed promise for breeding late-blooming, late-maturing genotypes. Similarly, the 'Santa Maria×Akça' combination, which blooms late but requires less than 100 days from full bloom to harvest, was found highly promising for the development of late-blooming, early-maturing genotypes.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors do not have a conflict of interest to declare.

Author contribution

All authors have contributed equally to the article.

Ethics committee approval

This article does not contain any studies with human participants or animals by any of the authors.

Funding

The projects in which the study materials were obtained and inoculated were supported by TUBITAK (TOVAG 1060719 and TOVAG 1100938).

Acknowledgments

This study is a part of the PhD thesis of the first author.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.13

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 108-114

Forage quality and yield of Sal Pasture (Rize, Türkiye)

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Article History

Received: January 9, 2025 Revised: March 4, 2025 Accepted: March 6, 2025 Published Online: March 10, 2025

Article Info

Article Type: Research Article Article Subject: Agro-Ecosystem Function and Prediction

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Available at ttps://dergipark.org.tr/jaefs/issue/90253/1616545







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Abstract

This study investigated the forage quality and yield of Sal Pasture, located in the Çamlıhemşin district of Rize province, within the Eastern Black Sea Region of Türkiye. The study was conducted during July of 2023 and 2024. Plant samples were collected from 12 different selected points using 50x50 cm quadrats, harvesting vegetation at ground level. Subsequent laboratory analyses determined key nutritional parameters. The average fresh yield was 721.50 kg/da, with a statistically significant difference (p<0.05) observed between the two years (748.00 kg/da in 2023 and 695.00 kg/da in 2024). Similarly, dry matter yield also showed a significant inter-annual variation (p<0.05), averaging 158.24 kg/da (164.00 kg/da in 2023 and 152.48 kg/da in 2024). The average crude protein (CP) content was 13.27%, indicating a moderate protein level. Fiber fractions, as measured by acid detergent fiber (ADF) and neutral detergent fiber (NDF), averaged 37.39% and 64.92%, respectively. Digestible dry matter (DDM) averaged 59.77%, and the relative feed value (RFV) was 85.66. Digestible energy (DE) and metabolizable energy (ME) averaged 2.83 Mcal/kg and 2.32 Mcal/kg, respectively. Mineral analysis revealed average concentrations of 0.22% phosphorus (P), 1.43% potassium (K), 1.47% calcium (Ca), and 0.39% magnesium (Mg). The Ca/P ratio averaged 6.68, and the K/(Ca+Mg) ratio averaged 0.77, with a statistically significant difference (p<0.05) observed between years for the latter (0.81 in 2023 and 0.72 in 2024). These findings provide valuable insights into the nutritional potential of Sal Pasture for livestock grazing and highlight the influence of inter-annual variability on forage quality

Keywords: Sal Pasture, Forage Quality, Nutritional Value, Yield

Cite this article as: Catal, M.I. (2025). Forage quality and yield of Sal Pasture (Rize, Türkiye). International Journal of Agriculture, Environment and Food Sciences, 9 (1): 108-114. https://doi.org/10.31015/2025.1.13

INTRODUCTION

Pastures and meadows are essential roughage sources for animal nutrition, playing a crucial role in livestock farming (Aydın and Uzun 2005). These areas provide valuable gene resources for cultivated plants, habitats for wildlife, and essential ecological functions such as biodiversity conservation and erosion control (Comaklı and Menteşe 1999; Carlier et al. 2005). In Türkiye, approximately 70% of livestock farming relies on pastures and meadows, which fulfill a significant portion of the animals' annual roughage requirements, particularly in terms of essential nutrients like crude protein and starch (Gökkuş 1994; Okatan and Yüksek 1997; Çomaklı 2018).

The investigation of pasture and meadow vegetation serves two primary purposes. Firstly, it aims to gather quantitative and qualitative data on pastures and meadows in regions where vegetation characteristics are not well understood. Secondly, it seeks to evaluate the effects of improvement and management practices on the vegetation cover of these areas (Cerit and Altın 1999).

In this context, studies conducted in various regions of Türkiye have shown that the yield and quality characteristics of pastures and meadows vary significantly depending on aspect, altitude, grazing intensity, and other environmental factors. For example, Çaçan et al. (2014) demonstrated significant differences between protected and grazed pastures, with higher dry matter yield (203.70 kg/da), crude protein content (19.69%), ADF content (29.48%), and NDF content (43.31%) in protected areas. In grazed areas, these values were 106.85 kg/da,

15.40%, 37.76%, and 50.86%, respectively. Tanriverdi (2019) found that aspect influences crude protein content and yield in pastures in Muş, with the highest crude protein content (14.37%) observed in the east-facing aspect.

Nadir (2010) studied the forage quality and yield of pastures in Tokat and found that dry matter yield ranged from 244.08 to 276.05 kg/da, and crude protein content ranged from 16.5% to 18.8%. Candan (2014) studied the effect of cutting frequency on pastures in Samsun and found that dry matter yield ranged from 146.09 to 274.19 kg/da. Taşdemir (2015) examined the effect of aspect on pastures in Elazığ and found that crude protein yield ranged from 141.3 to 282.3 kg/da. Öner (2016) examined the effect of altitude on forage quality in Erzurum and found that dry matter yield was 134.83 kg/da in ungrazed areas and 68.21 kg/da in grazed areas. Tutar (2017) investigated the effect of aspect on pastures in Bingöl and found that the highest crude protein content (12.9%) was observed in the south-facing aspect. Severoğlu (2018) investigated the effect of slope on forage quality and found that forage quality decreased with increasing slope.

This study endeavors to assess the forage potential and overall pasture quality of Sal Pasture in Rize, Türkiye, by meticulously analyzing its forage yield and quality attributes. The findings are anticipated to provide valuable insights that can contribute to the sustainable management of livestock activities within the region. Furthermore, this research aims to offer a comprehensive understanding of the pasture's ecological and productive characteristics, which may serve as a foundation for future studies and informed decision-making in regional agricultural practices.

MATERIALS AND METHODS

Study Area: Location, Soil, and Climate Characteristics

This research was conducted in Sal Pasture, located in the Çamlıhemşin district of Rize province, renowned for its natural beauty within the Eastern Black Sea Region. The study site is situated approximately 2000 meters above sea level and approximately 19 km from the district center. The research was carried out during the years 2023 and 2024. The location of the study area is presented in Figure 1, and some photographs of the site are provided in Figure 2. Sal Pasture represents a significant example of the characteristic pasture ecosystem of the region.

Soil samples from Sal Pasture were analyzed to determine key physical and chemical properties. The analysis revealed a saturation percentage of 75.9%, classified as clay loam. The soil pH was measured at 4.67, indicating a strongly acidic reaction. The total salt content was 0.17%, classified as slightly saline. The lime (calcium carbonate) content was 0.12%, indicating low lime content. The organic matter content was found to be 2.09%, classified as medium. Available phosphorus (P_2O_5) was measured at 4.76 kg/da, and available potassium (K_2O) was measured at 30.47 kg/da, both classified as medium. Analysis of long-term meteorological records indicates that the average annual temperature for Rize province is 14.5 °C, with an annual total precipitation of 2301.2 mm (Anonymous 2025).

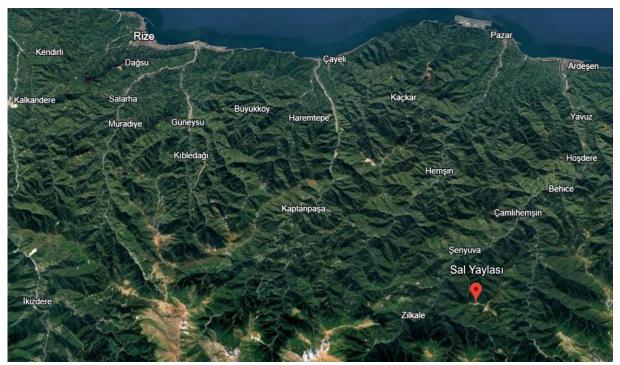


Figure 1. Location of the study area on the map (Google Earth)



Figure 2. Some photos taken from the study area

Methodology

In this study, plant samples were collected from 12 different selected points within the Sal Pasture area during July of 2023 and 2024. Samples were harvested by cutting the vegetation at ground level using 50x50 cm quadrats. Fresh weights of the collected samples were measured in situ using a portable precision balance. Subsequently, the samples were dried at $70\,^{\circ}\text{C}$ for 48 hours to determine their dry weights, which were then converted to yield per unit area (kg/da). The dried plant samples were ground and homogenized using a mill with a 1 mm sieve. The contents of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent protein (ADP), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were analyzed using a Foss NIR Systems Model 6500 Win ISI II v1.5 NIRS instrument. Dry matter intake (DMI), digestible dry matter (DDM), relative feed value (RFV), digestible energy (DE), and metabolic energy (ME) were calculated from ADF and NDF values using the following equations from the literature:

Digestible Dry Matter (DDM) = $88.9 - (0.779 \times \text{MADF})$ (Oddy et al. 1983)

Dry Matter Intake (DMI) = 120 / (%NDF) (Sheaffer et al. 1995)

Relative Feed Value (RFV) = (DDM x DMI) / 1.29 (Sheaffer et al. 1995)

Digestible Energy (DE) = 0.27 + 0.0428 x (%DDM) (Fonnesbeck et al. 1984)

Metabolizable Energy (ME) = 0.821 x DE (Mcal/kg) (Khalil et al. 1986)

Finally, Ca/P and K/(Ca+Mg) ratios were calculated to evaluate the relationships among macro element contents.

Statistical analysis

Data pertaining to the parameters examined in this study were analyzed using analysis of variance (ANOVA) with the JMP 13 statistical software package. Significant differences among years identified by ANOVA were determined using the Least Significant Difference (LSD) multiple comparison test (p<0.05).

RESULTS AND DISCUSSION

Nutrient composition data obtained from the two-year analysis of grass samples collected from Sal Pasture are presented in Table 1. These analyses reveal the overall nutritional quality of the pasture's grasses and potential variations between years.

Table 1. Yield and nutritional value of Sal Plateau pasture

Features Analyzed	1. Year	2. Year	Average
Fresh Yield (FY) (kg/da)	748±18.83 a	695±31.89 b	721
Dry Yield (DY) (kg/da)	164±6.86 a	152±12.55 b	158
Crude Protein (CP) (%)	13.17±0.28	13.36±0.84	13.27
Acid Detergent Fiber (ADF) (%)	37.79±4.07	36.99±2.49	37.39
Neutral Detergent Fiber (NDF) (%)	65.17±4.10	64.66±3.48	64.92
Acid Detergent Protein (ADP) (%)	1.43±0.16	1.41±0.12	1.42
Digestible Dry Matter (DDM) (%)	59.46±3.17	60.08±1.94	59.77
Dry Matter Intake (DMI) (%)	1.84±0.12	1.86±0.10	1.85
Relative Feed Value (RFV)	84.88±10.37	86.44±7.67	85.66
Digestible Energy (DE) (Mcal/kg)	2.81±0.08	2.84±0.14	2.83
Metabolic Energy (ME) (Mcal/kg)	2.31±0.07	2.33±0.11	2.32
Phosphorus (P) (%)	0.21±0.05	0.23±0.06	0.22
Potassium (K) (%)	1.48±0.17	1.37±0.10	1,43
Calcium (Ca) (%)	1.44±0.11	1.50±0.21	1.47
Magnesium (Mg) (%)	0.38±0.08	0.40 ± 0.09	0.39
Ca/P	6.55±1.11	6.82±1.26	6.68
K/(Ca+Mg)	0.81±0.06 a	0.72±0.02 b	0.77

The nutritional characteristics of forage samples collected from Sal Plateau pasture were assessed over two consecutive years, and the results are presented in Table 1. The average fresh yield was determined to be 721 kg/da. A statistically significant difference (p<0.05) was observed in fresh yield between the two years, with the first year exhibiting a higher yield (748 kg/da) compared to the second year (695 kg/da). This inter-annual variation in fresh yield could be attributed to fluctuating environmental factors such as precipitation and temperature. The average dry matter yield, which represents the dry matter available for consumption by grazing animals, was 158 kg/da. Similar to fresh yield, a statistically significant difference (p<0.05) was found in dry yield between the two years, with the first year showing a higher value (164 kg/da) than the second year (152 kg/da).

The average crude protein (CP) content, a key indicator of forage nutritional value, was 13.27%. This value suggests a moderate to good protein content in the Sal Plateau pasture. Fiber content, represented by acid detergent fiber (ADF) and neutral detergent fiber (NDF), averaged 37.39% and 64.92%, respectively. ADF, which is negatively correlated with digestibility, indicates a relatively good digestibility potential of the forage. NDF, which influences feed intake, suggests a moderate intake potential. The average acid detergent protein (ADP) content was 1.42%.

Digestible dry matter (DDM) averaged 59.77%, indicating the proportion of dry matter available for digestion by ruminant animals. Dry matter intake (DMI), an estimate of voluntary feed intake, averaged 1.85%. The relative feed value (RFV), a comprehensive index combining digestibility and intake potential, averaged 85.66. Digestible energy (DE) and metabolic energy (ME) averaged 2.83 Mcal/kg and 2.32 Mcal/kg, respectively, providing valuable information on the energy content of the forage.

The average mineral contents were as follows: phosphorus (P), 0.22%; potassium (K), 1.43%; calcium (Ca), 1.47%; and magnesium (Mg), 0.39%. These mineral concentrations are essential for various physiological functions in grazing animals. The calcium to phosphorus (Ca/P) ratio averaged 6.68, which is within the desirable range for ruminant nutrition. A statistically significant difference (p<0.05) was found between the two years for the potassium to calcium plus magnesium [K/(Ca+Mg)] ratio, with the first year showing a higher value (0.81 ± 0.06) than the second year (0.72 ± 0.02) . This difference could be related to changes in plant species composition or soil nutrient availability between the years.

This study evaluated the nutritional characteristics of forage samples collected from Sal Plateau pasture over two years. The findings were compared with previous research conducted in different regions of Türkiye to provide a broader context and identify potential regional variations in pasture quality.

Regarding dry matter yield (DY), the average value obtained in this study (158.24 kg/da) was considerably lower than the value reported by Kılıç (2018) for Beypinari pasture in Trabzon (827.3 kg/da). This substantial difference in DY could be attributed to several factors, including variations in climatic conditions (precipitation, temperature, solar radiation), soil properties (nutrient availability, soil type), botanical composition (dominant plant species), and grazing management practices between the two locations. Sal Plateau, being a high-altitude pasture, may experience shorter growing seasons and harsher environmental conditions compared to the lower-altitude Beypinari pasture, thus influencing biomass production.

The average crude protein (CP) content in Sal Plateau pasture (13.27%) fell within the range reported in several studies. While it was lower than the values reported by Şahinoğlu (2010) for Bafra pasture (16.33-18.64%), Nadir (2010) for Tokat pasture (16.48-18.81%), Kokten et al. (2010) in Anti-Taurus Mountain rangeland shrubs (5.9-23.1%), Çaçan and Kökten (2014) for Bingöl pasture (16.08%), Aydın and Başbağ (2017) for Karacadağ pastures (19.19%) and Kökten and Tanrıverdi (2020) for Muş pasture (14.37%), it was comparable to the range observed by Güllap (2010) in Erzurum pastures (8.26-13.12%), Parlak et al. (2015) in Çanakkale pastures (9.10-13.18%), Taşdemir and Kökten (2015) in Elazığ pasture (12.2%), Cacan and Kokten (2019) in Bingol pasture (12.8-14.1%). This variability in CP content across different regions highlights the influence of local environmental conditions and plant species composition on forage protein levels.

Fiber components, as measured by acid detergent fiber (ADF) and neutral detergent fiber (NDF), play a crucial role in determining forage digestibility and intake. The average ADF (37.39%) and NDF (64.92%) values obtained in this study were generally higher than those reported by Şahinoğlu (2010) (ADF: 29.82-31.99%; NDF: 46.39-55.21%), Nadir (2010) (ADF: 24.38-26.84%; NDF: 34.59-36.32%), and Aydın and Başbağ (2017) (ADF: 29.78%; NDF: 47.76%). However, the ADF values were similar to those reported by Tutar and Kökten (2019) (34.8-37.4%) in Bingöl. The NDF value was also similar to the upper range of values observed by Tutar and Kökten (2019) (52.5-62.7%) and Güllap (2010) (43.57-50.28%). Higher ADF and NDF values generally indicate lower digestibility and potentially reduced feed intake by grazing animals.

The relative feed value (RFV), a comprehensive index combining digestibility and intake potential, was 85.66 in this study. This value was substantially lower than the RFV values reported by Nadir (2010) (174.96-189.77), Taşdemir and Kökten (2015) (103.0-118.4), Aydın and Başbağ (2017) (137.7) and Kökten and Tanrıverdi (2020) (102.0-112.5), but closer to the values found by Tutar and Kökten (2019) (91.8-109.4) and Cacan and Kokten (2019) (85.0-92.8). This difference in RFV further emphasizes the regional variation in forage quality and its implications for animal performance.

Regarding mineral content, the phosphorus (P) content of Sal Plateau pasture (0.22%) was lower compared to the values reported by Şahinoğlu (2010) (0.40-0.43%), Aydın and Başbağ (2017) (0.34%), Kökten and Taşdemir (2023) (0.34-0.39%) and Saygın and Kokten (2024) (0.26%). Potassium (K) content (1.43%) was lower than the values reported by Şahinoğlu (2010) (2.32-2.60%), Aydın and Başbağ (2017) (2.42%), Çaçan and Kökten (2023) (24.1%), Kökten and Taşdemir (2023) (2.44-3.00%) and Saygın and Kokten (2024) (1.51%). Calcium (Ca) content (1.47%) was higher than the range reported by Şahinoğlu (2010) (0.90-1.33%), Çaçan and Kökten (2023) (1.14%), Kökten and Taşdemir (2023) (1.06-1.20%) and Saygın and Kokten (2024) (1.21%), but comparable to Aydın and Başbağ (2017) (1.09%). Magnesium (Mg) content (0.39%) was slightly higher than the range found by Şahinoğlu (2010) (0.26-0.36%), Kökten and Taşdemir (2023) (0.26-0.33%) and similar to Aydın and Başbağ (2017) (0.31%) and Saygın and Kokten (2024) (0.31%). The K/(Ca+Mg) ratio (0.77) was considerably lower than those reported by Şahinoğlu (2010) (1.61-2.13%), with a statistically significant difference (p<0.05) between the two years of the present study. These differences in mineral content could be related to soil mineral composition, plant species, and environmental factors.

The mineral analysis conducted on the Sal Plateau pasture revealed that the average phosphorus (P) content (0.22%) was insufficient for animal nutrition. Conversely, the average potassium (K) content (1.43%) and magnesium (Mg) content (0.39%) were found to be adequate for meeting animal requirements. The average calcium (Ca) content (1.47%) was also determined to be sufficient. However, the average Ca/P ratio (6.68) indicated an imbalanced mineral proportion, which could potentially lead to bone development issues in animals. The average K/(Ca+Mg) ratio (0.77) suggested a low risk of tetany. Nevertheless, the observed phosphorus deficiency and imbalanced Ca/P ratio highlight the necessity for mineral supplementation or fertilization to improve the mineral composition of the pasture.

In conclusion, the nutritional quality of Sal Plateau pasture, as assessed in this study, exhibited some differences compared to other regions of Türkiye. While CP content was comparable to some studies, DY and RFV were generally lower, suggesting potential limitations in biomass production and overall forage quality. The observed inter-annual variations in certain parameters highlight the importance of considering environmental factors and implementing appropriate grazing management strategies to optimize pasture productivity and nutritional value for grazing livestock.

CONCLUSION

This study comprehensively assessed the forage quality and yield of Sal Pasture in Rize, Türkiye, revealing valuable insights into its nutritional potential for livestock grazing. The observed inter-annual variability in fresh and dry matter yields underscores the influence of climatic factors on pasture productivity. While the crude protein content indicated a moderate nutritional value, the fiber fractions (ADF and NDF) and digestibility parameters (DDM, DE, ME) provided a detailed understanding of the forage's quality. Mineral analyses highlighted specific areas of concern. The phosphorus content was notably low, suggesting a potential deficiency for grazing animals. Conversely, potassium, calcium, and magnesium levels were found to be adequate. The Ca/P ratio indicated a mineral imbalance that could impact animal health, while the K/(Ca+Mg) ratio suggested a low risk of tetany. The

significant inter-annual variation in the K/(Ca+Mg) ratio further emphasizes the dynamic nature of pasture mineral composition. These findings suggest that while Sal Pasture offers valuable forage resources, strategic interventions such as mineral supplementation or targeted fertilization may be necessary to optimize its nutritional value and ensure the health and productivity of grazing livestock. Future research should focus on long-term monitoring of pasture dynamics and the development of sustainable management practices to enhance forage quality and yield in this unique ecosystem.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

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

Funding

This study did not obtain any external funding.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.14

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 115-122

Non-Destructive chlorophyll meters: a comparison of three types of meters for grain yield estimation of durum wheat under semi-arid environments

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Article History

Received: January 27, 2025 Revised: March 3, 2025 Accepted: March 5, 2025 Published Online: March 10, 2025

Article Info

Article Type: Research Article Article Subject: Cereals and Legumes

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1627611







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Abstract

Optimizing management practices to maximize crop yield and efficiency necessitates real-time monitoring of plant growth throughout the growing season. Utilizing spectral indices, such as normalized difference vegetation index, SPAD chlorophyll meter readings, and the CM-1000 chlorophyll meter, can provide quantitative data to aid in making informed management decisions. This study investigated the relationships between spectral indices (NDVI, SPAD, CM-1000) and grain yield in five durum wheat genotypes under semi-arid conditions. Spectral indices were taken at three growth stages: heading, anthesis, and maturity. Our findings revealed significant variations in spectral reflectance values among the genotypes and across growth stages. NDVI values were highest during the early growth stages and declined towards maturity. SPAD values also exhibited a similar trend, peaking at heading and anthesis. Chlorophyll content, as measured by SPAD readings, varied across growth stages, with different genotypes exhibiting peak chlorophyll content at different times. CM-1000 measurements showed significant differences among genotypes at all stages, with 'Fırat 93' and 'Hasanbey' generally exhibiting higher chlorophyll content. Correlation analysis revealed significant positive relationships between NDVI values at different stages, as well as between CM-1000 measurements and grain yield. Conversely, SPAD values showed a negative correlation with grain yield. These findings suggest that CM-1000 measurements could be a valuable tool for selecting high-yielding durum wheat genotypes under semi-arid conditions.

Keywords: NDVI, SPAD, CM-1000 chlorophyll meter, Durum wheat, Growing stages

Cite this article as: Kizilgeci, F., Tebrizli, N., Elis, S., Ozkan, R., Bayhan, M. Yildirim, M. (2025). Non-Destructive chlorophyll meters: a comparison of three types of meters for grain yield estimation of durum wheat under semi-arid environments. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 115-122. https://doi.org/10.31015/2025.1.14

INTRODUCTION

Durum wheat (Triticum durum Desf.) is one of the most important staple crops worldwide, contributing significantly to the global food supply (Kizilgeci et al., 2021). It is a critical crop in semi-arid regions where water availability limits productivity (Grosse-Heilmann et al., 2024). Increasing both the yield and quality of wheat is of great importance in terms of ensuring food security. Various physiological traits such as chlorophyll content and vegetation indices have been shown to correlate with grain yield and quality parameters, making them important tools for selection in wheat breeding programs (Yıldırım et al., 2013; Kendal, 2018; Kizilgeci et al., 2019; Yakushev et al., 2022; Kızılgeçi and Cebeli, 2024). Plant breeders use phenotypic, genotypic, genomic, field tests and physiological characteristics as selection criteria in plant breeding (Sinha and Swaminathan, 1984; Jackson et al., 1996; Cooper et al., 2014; Reynolds and Langridge, 2016; Kızılgeçi et al., 2018). In particular, selection based on physiological characteristics has begun to take an important place in plant breeding studies today. Many tools are used to determine physiological features. In the last decades, new technological advances have generated a

greater interest in monitoring crops, which have been monitored for years through satellite images. Currently, to complement these images, various tools are being used fast and non-invasive way to monitor the health and growth of their crops, factors that will affect the characteristics of the harvest, such as the quantity and quality of the produce. Among these tools are meters capable of measuring physiological variables of the plants, such as the concentration of chlorophyll, the leaf surface, and other variables related to health, which have proven to be very useful; chlorophyll meters for their easy handling, NDVI meters for their information on vegetation vigor, and even estimation of yield, and chlorophyll fluorescence meters which in many cases are useful to detect stress before it can be observed, mainly in the color of the leaf. The Normalized Difference Vegetation Index (NDVI) provides a non-destructive means to monitor crop growth and predict yields (Hassan et al., 2019). NDVI, calculated using near-infrared and red wavelengths, is sensitive to the chlorophyll content and biomass of crops, making it a valuable tool in precision agriculture (Sun et al., 2022). Chlorophyll content is a vital physiological parameter in crop health, as it plays a central role in photosynthesis and energy capture (Martins et al., 2023). In wheat breeding programs, the soil-plant analyses development (SPAD) meter provides a non-destructive and rapid method for estimating chlorophyll content by measuring light absorption by leaves (Kızılgeçi and Cebeli, 2024). Nitrogen status assessment, fertilizer management, crop health monitoring, Early detection of plant stress, and yield prediction. SPAD measurements, particularly during the heading and anthesis stages, are valuable tools for assessing chlorophyll content and predicting yield in durum wheat (Yıldırım et al., 2011; Mohammadi et al., 2022) The significant variation in SPAD values across genotypes and growth stages reflects the differences in chlorophyll dynamics and yield potential under semi-arid conditions. (Kizilgeci et al., 2021). CM 1000 Chlorophyll meter has revolutionized plant health monitoring by providing rapid and accurate measurements in situ. CM 1000 Chlorophyll meter operates by measuring the reflectance of light at two wavelengths: 700 nm (red) and 840 nm (near-infrared). These measurements are used to calculate a chlorophyll index, which correlates with the relative greenness of the leaves. The main objective of this study was to evaluate the relationship between spectral reflectance measuring devices and their performance in yield estimation at different development stages of durum wheat under rainfed conditions.

MATERIALS AND METHODS

The study was conducted at the research station of Teknobiltar company during the 2018-2019 production season (37°55′34″N, 40°15′12″E, 594 m above sea level). The soil of the research site was characterized as clay loam, with low organic matter and phosphorus content and a pH of 7.8. Five bread wheat genotypes were used as plant materials 'Fırat-93', 'Hasanbey', 'Hat-300', 'Sena' and 'Svevo'. The experimental layout was a randomized complete block design with three replications. Fertilizers were applied at sowing at a rate of 60 kg ha⁻¹ nitrogen (N) and 60 kg ha⁻¹ phosphorus (P). An additional 60 kg ha⁻¹ N in the form of urea was applied during the stem elongation stage. Chemical treatments were employed to control diseases, pests, and weeds.

Measurements

NDVI, SPAD, and CM1000 values were measured at three different growth stages: heading, anthesis, and maturity. NDVI was recorded using a Greenseeker, SPAD readings were obtained using a SPAD-502 meter, and chlorophyll content was determined using a CM 1000 device.

Measurements (NDVI, SPAD and CM 1000) were taken between 10:00 and 14:00 when the weather was clear and sunny.

The SPAD meter measures the chlorophyll content in leaves using wavelengths of 650 nm (red light) and 940 nm (infrared light). This device determines the amount of chlorophyll by measuring how much of the light sent into the leaf is absorbed and how much passes through. The SPAD value is usually shown as a number between 0 and 99. High SPAD values indicate high chlorophyll content and generally a healthy plant.

Green Seeker holds the device 60-120 cm above the plant, with the sensor at a fixed height and angle perpendicular to the ground. The sensor emits bursts of red and near-infrared (NIR) light at the plants. As it moves steadily across the field, GreenSeeker continuously measures the reflected light and calculates the NDVI value in real time. NDVI values range from 0.00 (no vegetation or poor vegetation health) to 0.99 (very healthy vegetation).

NDVI were calculated as below;

NDVI = (NIR - RED)/(NIR + RED)

NIR: Near infrared value, RED: red reflectance value.

The FieldScout CM 1000 Chlorophyll meter uses "point-and-shoot" technology to instantly measure ambient and reflected light at 700 nm and 840 nm wavelengths. These measurements are used to calculate the relative chlorophyll index, which indicates the greenness of plant leaves or turf grass canopies. The device measures within a conical viewing area of 12 to 72 inches and reports the chlorophyll index on a scale from 0 to 999.

Statistical Analysis

Data were analysed using one-way ANOVA, followed by Least Significant Difference (LSD) test (5% level) for mean comparisons. Correlation analysis was performed with the JMP 18 clinical based on a randomized complete block design.

RESULTS AND DISCUSSION

NDVI Values Different Growth Stages

NDVI is widely used to assess vegetation health and predict crop yields. The NDVI values varied significantly between the genotypes and the three growth stages (Figure 1). Kızılgeçi and Cebeli (2024) reported that there were statistically significant differences between genotypes in the anthesis and maturity periods of bread wheat, but no significant difference was observed in the heading stage. At the heading stage (NDVI-H), 'Fırat-93' showed the highest NDVI (0.79), followed by 'Hat-300' (0.76). Similarly, at anthesis (NDVI-A), 'Firat-93' (0.76) maintained a leading NDVI value, followed by 'Hat-300' (0.72). However, by maturity (NDVI-M), all genotypes exhibited lower NDVI values, with 'Hasanbey' (0.57) showing relatively high values compared to the others. These results indicate that NDVI values are generally higher during the earlier stages of crop growth, particularly at heading and anthesis, when vegetation is denser, and chlorophyll content is high. By the maturity stage, chlorophyll content declines, leading to lower NDVI values. This trend highlights the importance of early NDVI measurements for predicting final yield. The lower NDVI values observed at maturity (NDVI-M) reflect the natural senescence process, which reduces chlorophyll content as the plant approaches harvest. However, NDVI-M may still provide insights into the health and stability of the crop at the end of its life cycle. NDVI values are affected by many factors, including fertilization, genotype, disease, plant growth stage, abiotic stress and the application of fertilizers (Aparicio et al., 2002; Ashourloo et al., 2014; Mekliche et al., 2015; Kizilgeci et al., 2021).

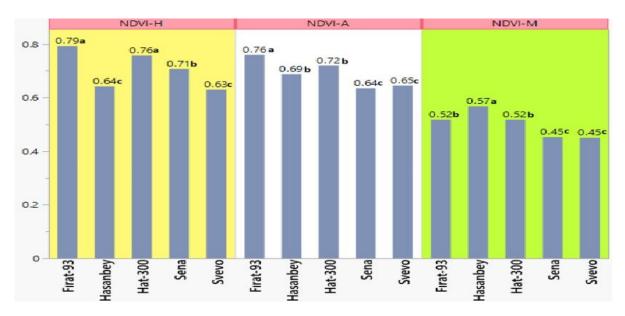


Figure 1. NDVI values measured at different growing stages of durum wheat genotypes NDVI-H: Heading stage, NDVI-A: Anthesis stage, NDVI-M: Maturity stage.

SPAD Values Different Growth Stages

SPAD values varied significantly across genotypes and growth stages (Figure 2). At the heading stage (SPAD-H), 'Hat-300' (53) showed the highest SPAD value, followed by 'Hasanbey' (51). By the anthesis stage (SPAD-A), 'Hasanbey' exhibited the highest SPAD value (47.2), with the other genotypes showing slightly lower values, though still within a close range (42.2–46.5). At the maturity stage (SPAD-M), a notable decline in SPAD values was observed across all genotypes. 'Svevo' and 'Sena' displayed the highest values (46 and 45, respectively), while 'Fırat 93' and 'Hasanbey' exhibited the lowest values at this stage (38.5 and 39.9, respectively). This decrease in SPAD values towards maturity is expected due to the senescence process, which reduces chlorophyll content as the plant approaches harvest. These results suggest that SPAD values are highest during the early reproductive stages (heading and anthesis), when chlorophyll content and photosynthetic activity are at their peak. By the maturity stage, chlorophyll degradation leads to lower SPAD readings. The relatively stable SPAD values observed for 'Svevo' throughout the growth stages suggest that this genotype may exhibit better tolerance to chlorophyll degradation, which could contribute to its final yield stability, particularly under stress conditions. SPAD values at early growth stages provide a reliable indicator for predicting wheat yield and were incorporated into wheat breeding programs for semi-arid regions (Giunta et al., 2002; Le Bail et al., 2005; Kizilgeci, 2020).

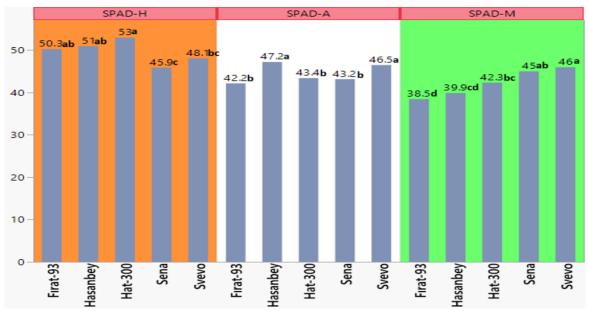


Figure 2. SPAD values measured at different growing stages of durum wheat genotypes SPAD-H: Heading stage, SPAD-A: Anthesis stage, SPAD-M: Maturity stage.

CM 1000 Chlorophyll Meter Values Different Growth Stages

The chlorophyll content, as measured by the CM 1000 chlorophyll meter, varied significantly among the five wheat varieties and across the three growth stages CM 1000 readings at the heading stage showed significant variation between the wheat varieties. Kızılgeçi and Cebeli (2024) reported that they determined significant differences among genotypes for CM 1000 chlorophyll meter values measured during the heading and anthesis periods, excluding the maturity period. 'Fırat-93' exhibited the highest chlorophyll content, followed by 'Hasanbey'. At anthesis, 'Fırat-93' again showed the highest chlorophyll content. The remaining varieties had lower chlorophyll values. At the maturity stage, the chlorophyll values decreased in all varieties, indicating a reduction in photosynthetic activity as the plants approached harvest. 'Fırat-93', 'Hasanbey', and 'Hat-300' showed relatively similar values (338, 338, and 331, respectively), while 'Sena' (325) and 'Svevo' (289) exhibited the lowest chlorophyll content. The observed decline in chlorophyll content from heading to maturity is consistent with the natural senescence process in plants as they allocate resources towards grain filling rather than leaf maintenance. The results also indicate that the wheat varieties exhibit different chlorophyll dynamics, which could be utilized in breeding programs to select from varieties with higher chlorophyll retention and, potentially, improved productivity under varying environmental conditions.

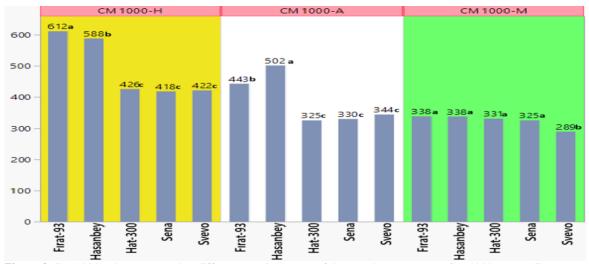


Figure 3. CM 1000 values measured at different growing stages of durum wheat genotypes CM 1000-H: Heading stage, CM 1000-A: Anthesis stage, CM 1000-M: Maturity stage.

The Correlation Analysis

Physiological traits and yield are essential for the successful selection of high-performing cultivars in crop breeding programs. Understanding the relationships between these traits allows breeders to make informed decisions regarding the selection of genotypes with superior performance under specific environmental conditions. Correlation analysis provides insights into the degree of association between different traits, offering critical information about how one trait may influence another.

Correlation analysis of spectral reflectance instruments of durum wheat genotypes measured in different stages is given in Figure 4. The correlation between NDVI-H and NDVI-A, CM 1000 was positive and significant. NDVI-A showed a positive and significant with NDVI-M, SPAD-H, CM 1000-H, CM 1000-A and CM 1000-M. A positive and significant correlation between SPAD-H and CM 1000-M was detected. The correlation between CM 1000-H and CM 1000-A, CM 1000-M, yield was observed. The correlation between CM 1000-A and CM 1000-M was also significant and positive. A negative and significant correlation was observed between NDVI-H and SPAD-A.

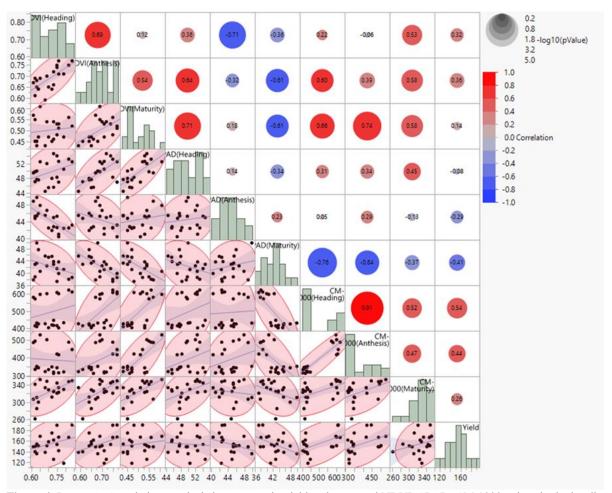


Figure 4. Pearson's Correlations analysis between grain yield and measured NDVI, SPAD, CM 1000 values in the heading, anthesis, and maturity stages.

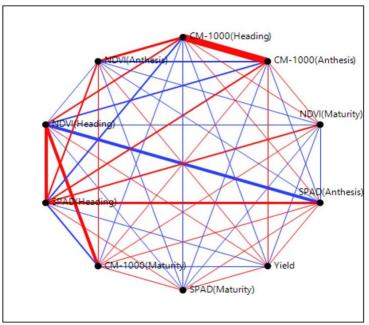


Figure 5. Partial correlation diagram.

When Figure 5 is analyzed according to the partial correlation diagram, red lines indicate a positive correlation and blue lines indicate a negative correlation. As the line thickness between the analyzed traits increases, it shows that the relationship between them is high. A positive correlation was observed between grain yield and the CM 1000 chlorophyll meter and NDVI measurements taken during the heading, anthesis and maturity periods. However, a negative correlation was evident between SPAD measurements and grain yield (Figure 4 and Figure 5). The correlation results were similar to the results reported by Kızılgeçi and Cebeli (2024).

CONCLUSION

This study was carried out to evaluate the measurement performances of the devices by measuring the leaf chlorophyll pigment content of five durum wheat genotypes with three chlorophyll meters. Significant variations were observed among the genotypes for measuring all the chlorophyll meters. Genotypes exhibited the highest chlorophyll content changed throughout the growth stages, as indicated by SPAD measurements. CM1000 measurements, which are used to determine the amount of chlorophyll on a canopy basis, the varieties Fırat 93 and Hasanbey appeared to have higher chlorophyll content than the other varieties during all of the measurement periods. This suggests the CM1000 may be a reliable tool for identifying high-chlorophyll genotypes, minimally affected by growth stage.

Similarly, NDVI values measured in different periods showed high correlation among themselves, supporting that superior genotypes can be determined stably without being affected by growth stages. A positive and significant correlation was found between CM 1000 measurements at different growth stages and grain yield, suggesting that this parameter could be a reliable indicator of high-yielding cultivars. Conversely, the SPAD values showed a negative correlation with grain yield, indicating that this parameter may not be the most suitable predictor of yield potential in durum wheat under the conditions of this study. These findings provide valuable insights for wheat breeding programs, highlighting the potential of using physiological traits, particularly chlorophyll content, as selection criteria for improving grain yield in durum wheat.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

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

Author contribution

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

Acknowledgments

The authors would like to thank Teknobiltar R&D for providing research facilities and support during the field experiment.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.15

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 123-131

Nutrient dynamics in apple: Analyzing macro and micronutrient distribution in leaves and fruits

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Article History

Received: January 29, 2025 Revised: March 4, 2025 Accepted: March 6, 2025 Published Online: March 10, 2025

Article Info

Article Type: Research Article Article Subject: Horticultural Production

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1629151

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Abstract

Apple cultivation is a key component of sustainable agriculture, significantly contributing to global fruit production. This study aimed to analyze the macro and micronutrient contents in apple leaves and fruits and to evaluate their relations with each other. The research was conducted in Denizli, Türkiye, using the Scarlet Spur apple cultivar grafted onto MM 111 rootstock, with a planting density of 4.5 × 2.5 m. Nutrient concentrations were measured using ICP-AES and spectrophotometric techniques. The results showed that nitrogen (0.50%–0.63%) and potassium (0.10%–0.94%) were the most abundant macronutrients in fruit, whereas calcium (0.04%–0.06%) and magnesium (0.06%–0.07%) were lower. Among micronutrients, iron (7.40–9.20 ppm) and boron (98.35–115.55 ppm) were found in higher concentrations, while zinc (2.07-2.44 ppm) and copper (1.70–1.80 ppm) were relatively low. Leaf tissues exhibited higher nutrient concentrations than fruit, with nitrogen (2.41%-2.56%), potassium (1.66%-1.83%), and calcium (1.49%–1.63%) being dominant. Strong negative correlations were observed between nitrogen and calcium in fruit (r = -0.99), while calcium and magnesium in leaves showed a strong positive relationship (r = 0.99). These results suggest that proper nutrient management is essential to improve fruit quality and optimize yield. The study emphasizes the necessity of balanced fertilization strategies and highlights the potential of apples as a rich dietary source of essential minerals. Future research should focus on optimizing fertilization practices and understanding the environmental factors influencing nutrient uptake.

Keywords: Fruit quality, Nutrient uptake, Macro and micronutrients, Sustainable agriculture

Cite this article as: Mertoglu, K., Kirca, L. (2025). Nutrient dynamics in apple: Analyzing macro and micronutrient distribution in leaves and fruits. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 123-131. https://doi.org/10.31015/2025.1.15

INTRODUCTION

Fruit cultivation plays a crucial role in agricultural production, being of immense economic and ecological importance. Türkiye provides ideal environment for fruit cultivation owing to it's diverse climatic conditions and rich soil structure (Karadeniz et al., 2013; Şenyurt et al., 2015). This favorable situation has resulted in horticultural production making up a significant share of Türkiye's overall agricultural production (Kaplan, 2016; Ağaoğlu et al., 2019).

According to FAO (2023) data, global apple production reached approximately 97.3 million tons. China produces 49.6 million tons on its own, followed by the United States (5.15 million tons), Türkiye (4.60 million tons), Poland (3.89 million tons), and India (2.87 million tons). These figures reveal that the top five appleproducing countries together contribute nearly 68% of total global production. With its favorable ecological conditions and significant production potential, Türkiye is the world's third largest apple producer. It is the highest producer in Europe and Asia after China.

In modern fruit cultivation, one of the fundamental pillars of sustainable agricultural production is the strategic management of plant nutrition. This is critical for optimizing yield and quality parameters. In modern fruit production, the provision of essential nutrients in a balanced manner to support the plants vital functions is of paramount importance (Bayram & Büyük, 2021). Nutrients required by plants are classified into macronutrients and micronutrients depending on the quantities needed. Macronutrients, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), are needed in large quantities, while micronutrients, including iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B), and molybdenum (Mo), are required in trace amounts (Zincircioğlu, 2018). Each of these nutrients plays a specific role in plant metabolism, and deficiencies of these elements can lead to characteristic symptoms (Yıldız, 2012; Kacar et al., 2013). Previous studies have reported that nitrogen deficiency, which is a key component of photosynthesis, slows down vegetative growth and causes chlorosis in leaves (Şenel, 2019). Similarly, calcium deficiency, which is essential for cell wall stability and fruit quality, can lead to shortened storage life and physiological disorders (Jaime-Guerrero et al., 2024).

The nutrient requirements of apple trees are crucial for both fruit quality and yield. During their growth and development, apple trees require various macro and micronutrients, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). Researches has shown that determining the concentrations of these nutrients in the leaves of apple trees is vital, as it affects the trees' nutritional status and consequently their yield (Erdal, 2005; Bayram & Büyük, 2021). A study conducted in apple orchards in the Isparta region revealed deficiencies in phosphorus (P), calcium (Ca), potassium (K), and manganese (Mn) in the leaves of the trees (Erdal, 2005). These deficiencies can negatively impact the health of apple trees and fruit quality.

Apple is a nutritious fruit and the nutrient content of its fruits plays an important role in terms of health (Dumanoglu et al., 2018). Apples contain various antioxidant compounds such as phenolic compounds, ascorbic acid (vitamin C), vitamin E, and β -carotene (Özel et al., 2020). These bioactive compounds are associated with positive health outcomes, including the prevention of several chronic diseases such as cancer, diabetes, and cardiovascular diseases (Nizamlioğlu, 2022). In addition, the nutritional profile of apples is increasingly important in light of growing consumer preferences for healthier diets.

Nutrient element analyses play a pivotal role in developing fertilization programs for apple trees. These analyses help identify the specific nutrients required by the trees, thus allowing for the more effective planning of fertilization practices (Çimrin et al., 2000; Küçükyumuk & Erdal, 2014). Foliar applications of iron and zinc fertilizers have been shown to positively influence the nutrient content of both the leaves and fruits of apple trees, contributing to improved tree development (Çimrin et al., 2000; Baysall & Erdal, 2015). Furthermore, boron fertilization has been found to increase nutrient element concentrations in apples, highlighting its potential benefits in fruit production (Baysal & Erdal, 2015).

Iron and zinc are critical micronutrients in human nutrition that significantly enhance the nutritional value of apples. Despite the consumption of 20–25 mg of iron through the normal daily diet, only 1–2 mg of this iron is absorbed by the small intestine (Güleç, 2018). Therefore, the iron content and bioavailability of foods are crucial. Zinc, the second most abundant trace element in the human body after iron, is essential for the function of over 300 enzymes (Akdeniz et al., 2016). Zinc is found primarily in the bones, muscles, hair, and skin, with an estimated total body content of 2 g in adults. Adequate zinc intake is essential for maintaining a strong immune system and nervous system (Gombart et al., 2020; Marcos, 2021). Zinc plays a crucial role in human nutrition, being an essential trace element that also has significant physiological effects on both plants and animals.

The primary aim of this study is to (1) determine the levels of macro and micronutrients in the leaves and fruits of apple trees, (2) explore the relationships between these elements, and (3) provide a comprehensive analysis of the dynamics of nutrient elements in apple cultivation.

MATERIALS AND METHODS

The study was conducted in 2023 in the Çivril district of Denizli, Türkiye. The plant material consisted of the Scarlet Spur apple cultivar, grafted onto MM111 rootstock, which was planted in 2017 with a spacing of 4.5×2.5 m between and within rows. The research site is located at an altitude of 840 meters and features a transitional climate between the Mediterranean and Continental climates.

The formation of the abscission layer, coloration, and taste were taken into consideration as criteria for harvesting the fruits (Akkurt et al., 2024). Leaf samples were taken from the newest leaves, which had finished growing in July (Mertoğlu et al., 2024). The samples were decontaminated through sequential washing in a detergent solution, followed by tap and distilled water rinsing to remove any external contaminants. Subsequently, the samples were dried at a controlled temperature of 70°C until a constant weight was achieved. Dried samples were then homogenized by grinding to a particle size of less than 0.5 mm for uniformity in analysis. The powdered samples were subjected to acid digestion using a nitric acid (HNO₃) and perchloric acid (HClO₄) mixture in a 3:1 volume ratio, as described by Kacar (1972). Elemental analysis included the determination of K, Mg, Ca, Fe, Mn, Cu, and Zn, which were quantified using an inductively coupled plasma atomic emission spectrometer (ICP-AES; Varian Liberty Series II, Varian Inc., Palo Alto, CA, USA). P content was determined through the Barton reagent method using a UV/VIS spectrometer (Shimadzu 1208, Shimadzu, Kyoto, Japan) according to Barton (1948). Total nitrogen content was analyzed using the micro-Kjeldahl method (Lees, 1971).

The study was established according to the randomized plot experimental design with five replications. In this study, all statistical analyses and data visualizations were performed using RStudio (2024.12.0+467). Descriptive statistics (minimum, maximum, mean, standard deviation, and coefficient of variation) for nutrient elements were calculated using the 'stats' and 'dplyr' packages. Pearson correlation analysis (p<0.05) was applied to determine relationships between elements, and the 'corrplot' package was used. Data visualizations, including box-plots, scatter plots, and density curves, were generated using the 'ggplot2' and 'GGally' packages. For data organization and manipulation, the 'tidyverse' and 'reshape2' packages were utilized (Zar, 2013).

RESULTS AND DISCUSSION

The minimum, maximum, mean, standard deviation, and coefficient of variation (C.V.%) values for the nutrients in apple fruit and leaves are presented in Table 1. Regarding the fruit, the nitrogen (N) content ranged from 0.50% to 0.63%, with a mean value of 0.58%. Phosphorus (P) content ranged from 0.08% to 0.09%, with a mean of 0.09%; potassium (K) ranged from 0.10% to 0.94%, with a mean of 0.64%; calcium (Ca) ranged from 0.04% to 0.06%, with a mean of 0.05%; and magnesium (Mg) ranged from 0.06% to 0.07%, with a mean of 0.06%. Among micronutrients, iron (Fe) ranged from 7.40 to 9.20 ppm (mean 8.57 ppm), copper (Cu) ranged from 1.70 to 1.80 ppm (mean 1.76 ppm), manganese (Mn) ranged from 2.76 to 2.97 ppm (mean 2.89 ppm), zinc (Zn) ranged from 2.07 to 2.44 ppm (mean 2.24 ppm), and boron (B) ranged from 98.35 to 115.55 ppm (mean 104.97 ppm). The highest coefficient of variation was observed for K (%73.31), while the lowest for Cu (%3.12).

In the apple leaves, the macronutrient nitrogen content ranged from 2.41% to 2.56%, with a mean of 2.50%. Phosphorus content ranged from 0.21% to 0.22%, with a mean of 0.21%; potassium ranged from 1.66% to 1.83%, with a mean of 1.74%; calcium ranged from 1.49% to 1.63%, with a mean of 1.58%; and magnesium ranged from 0.50% to 0.60%, with a mean of 0.57%. Among micronutrients, iron content ranged from 79.00 to 110.50 ppm (mean 92.67 ppm), copper ranged from 8.40 to 9.40 ppm (mean 8.97 ppm), manganese ranged from 38.30 to 44.70 ppm (mean 41.93 ppm), zinc ranged from 16.30 to 20.00 ppm (mean 17.97 ppm), and boron ranged from 81.80 to 108.40 ppm (mean 97.60 ppm). The highest coefficient of variation was observed for Fe (%17.44), and the lowest for P (%2.71).

We detected was higher than the values reported by Ahmed et al. (2024) in the fruit, the potassium content for the Golden and Starking apple cultivars (0.39% and 0.57%, respectively). Our phosphorus content was in agreement with their findings (Golden: 0.09%, Starking: 0.10%). However, our calcium content was significantly lower than their reported value of 0.12% for both cultivars. Similarly, our magnesium content was higher than the values reported for Golden (0.03%) and Starking (0.03%).

Regarding micronutrients, our iron content was higher than the values reported by Ahmed et al. (2024) for Golden (4.80 ppm) and Starking (7.80 ppm). Our copper content was similar to the values in the literature (Golden: 1.91 ppm, Starking: 2.73 ppm), while our manganese content was higher than their findings (Golden: 0.96 ppm, Starking: 2.13 ppm). Our zinc content was lower than the literature values (Golden: 6.76 ppm, Starking: 7.35 ppm), but boron was significantly higher than the reported values (Golden: 6.74 ppm, Starking: 6.66 ppm).

The nitrogen content we detected was higher than the values reported in the literature (0.1-0.3%) (Kurešová et al., 2019; Yıldız et al., 2022). The nutrient element contents in the leaf samples were generally consistent with the ranges reported by Özel et al. (2020) and Sas-Paszt et al. (2014).

Nava et al. (2018) was reported that the coefficients of variation for nutrient elements can differ due to environmental factors. Similarly, in our study, the highest variation coefficient in the fruit was found for potassium, and in the leaves for iron. These differences can be attributed to the mobility of nutrient elements within the plant and their sensitivity to environmental factors. These variations could be influenced by cultivar characteristics, ecological conditions, soil composition, and cultivation techniques (Nemeskéri et al., 2015). The notably high levels of nitrogen, potassium, magnesium, and boron in the fruit, and the low calcium and zinc levels, may be linked to regional soil characteristics and fertilization programs (Richardson et al., 2021). These differences can also be attributed to factors such as the soil nutrient content, irrigation water, harvest time, location, temperature, light intensity, and fruit types, as well as the different parts of the fruit. The high variation coefficients for potassium in the fruit (%73.31) and iron in the leaves (%17.44) suggest that these elements' contents are more influenced by environmental factors.

Figure 1 illustrates the correlations between nutrient elements in apple fruit. Upon examining statistically significant relationships (p<0.05), several noteworthy correlations were identified: a very strong negative correlation between N and Ca (r=-0.99), a perfect positive correlation between N and Fe (r=1.00), a very strong negative correlation between P and Cu (r=-0.99), a perfect positive correlation between P and Cu (r=-0.99), a perfect positive correlation between K and Cu (r=1.00), and a very strong negative correlation between Fe and Ca (r=-0.99). These findings indicate that in the fruit, N changes inversely with Ca and directly with Fe, while P exhibits an inverse relationship with both K and Cu, K has a direct relationship with Cu, and Fe changes inversely with Ca. However, no statistically significant correlations were observed between other element pairs.

Table 1. Descriptive Statistics for Macro and Micro minerals in Apple Fruits and Leaves

Plant part	Variable	Min.	Max.	Mean	StDev	C.V. %
Fruit	N (%)	0.500	0.630	0.583	0.07	12.40
	P(%)	0.081	0.094	0.086	0.01	8.14
	K (%)	0.100	0.944	0.637	0.47	73.31
	Ca (%)	0.040	0.063	0.049	0.01	25.69
	Mg (%)	0.055	0.066	0.060	0.01	9.28
	Fe (ppm)	7.400	9.200	8.567	1.01	11.81
	Cu (ppm)	1.700	1.800	1.763	0.06	3.12
	Mn (ppm)	2.760	2.970	2.893	0.12	4.01
	Zn (ppm)	2.070	2.440	2.237	0.19	8.39
	B (ppm)	98.350	115.550	104.967	9.26	8.82
Leaf	N (%)	2.410	2.560	2.503	0.08	3.25
	P(%)	0.210	0.220	0.213	0.01	2.71
	K (%)	1.660	1.830	1.740	0.09	4.91
	Ca (%)	1.490	1.630	1.580	0.08	4.94
	Mg (%)	0.500	0.600	0.567	0.06	10.19
	Fe (ppm)	79.000	110.500	92.667	16.16	17.44
	Cu (ppm)	8.400	9.400	8.967	0.51	5.72
	Mn (ppm)	38.300	44.700	41.933	3.29	7.84
	Zn (ppm)	16.300	20.000	17.967	1.88	10.45
	B (ppm)	81.800	108.400	97.600	13.99	14.33

When analyzing the correlations of nutrient elements in apple leaves, the only statistically significant (p<0.05) relationship was found to be a very strong positive correlation (r=0.99) between Mg and Ca. This indicates that the concentrations of Mg and Ca in the leaves tend to increase or decrease together. Although no other relationships were statistically significant, strong positive correlations were found between B and Ca (r=0.990), B and Mg (r=0.98), Cu and Mg (r=0.96), Cu and K (r=0.95), Zn and P (r=0.94), Cu and Ca (r=0.94), Zn and Mn (r=0.92), and Mn and N (r=0.915). Furthermore, a negative correlation was identified between Fe and N (r=-0.98), but it was not statistically significant at the 0.05 level.

In previous studies, correlations between nutrient elements have been observed that align with some of our findings. For instance, Nava et al. (2018) reported a positive correlation between Ca and Mg in Fuji apples, which is consistent with the very strong positive correlation found between Mg and Ca in our leaf tissue samples. Bozkurt et al. (2001) also identified a high correlation (r=0.80) between Ca and Mg in apple leaves from the Van region. In a study on apricot (Çelik, 2019), a positive relationship between Fe and Ca and K was reported. Similarly, Ceylan et al. (2004) observed a negative correlation (r=-0.59) between N and K in kiwi fruit leaves, whereas this relationship was not statistically significant in our study.

Moreover, in a study by Çelik (2019) on apricot leaves, a positive correlation between K and Cu minerals was identified, which also aligns with some of the findings in our study. A further comparative study on different fruit species (Golden apple, Starking apple, pear, and quince) revealed a significant and strong positive relationship between P and Mg (p<0.05, r>0.70) (Ahmed et al., 2024). In the same study, significant and strong positive correlations were also found between Fe and B, which differs from the relationships observed in our study. These variations might stem from differences in the fruit species being studied, as each type of fruit has distinct mechanisms for nutrient uptake and transport.

The contrasts between these studies and ours highlight the complex nature of nutrient element interactions and suggest that these relationships are not universal but vary depending on factors such as fruit species, variety, ecological conditions, and even the specific plant part (leaf versus fruit). The discrepancies in nutrient element correlations across studies indicate that the mechanisms governing nutrient dynamics are influenced by a combination of genetic, environmental, and physiological factors. As such, further research is necessary to fully understand the intricacies of these interactions and their implications for plant nutrition.

Based on the findings of this study and those of previous research, it is evident that the relationships between nutrient elements can vary significantly depending on fruit types, varieties, environmental conditions, and plant parts. Therefore, when designing plant nutrition programs or conducting agricultural research, it is crucial to consider these diverse factors. Adapting nutrient management strategies to specific conditions will ultimately lead to efficiency and sustainability in agricultural practices, improving both crop yield and quality.

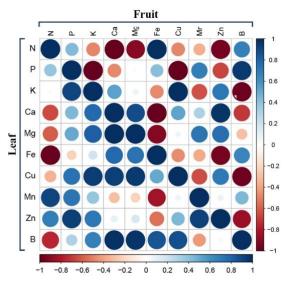


Figure 1. Correlation Analysis of Macro and Micro Minerals in Fruit and Leaf

The distribution of macro nutrient contents in apple fruit and leaf samples is presented in Figure 2. Upon analyzing the figure, it becomes evident that nitrogen (N) exhibits the highest concentration, ranging from approximately 1.5% to 2.5%, with a broad distribution. Following nitrogen, potassium (K) shows a concentration range of 1% to 1.8%, also demonstrating a relatively wide distribution. Calcium (Ca) is distributed between 0.5% and 1.5%, while magnesium (Mg) content varies from 0.2% to 0.6%. The element with the lowest concentration is phosphorus (P), which has a narrow distribution between 0.1% and 0.2%. Outlier values are represented by the points seen in the box-plot.

The findings from the study by Ahmed et al. (2024) report potassium content in the Golden and Starking apple varieties as ranging from 3585-3930 mg/kg and 3533-5671 mg/kg, respectively. This observed variation may be attributed to factors such as sampling time, variety characteristics, and the analytical methods used. Additionally, Nour et al. (2010) highlighted the substantial variation in nutrient element contents across different apple varieties, reinforcing the influence of these factors on the results.

This variability in nutrient content across different apple varieties emphasizes the need to consider a wide range of environmental and genetic factors when interpreting nutrient analysis results. Furthermore, such differences might indicate that localized soil properties, climate conditions, and agricultural practices play a significant role in shaping the nutrient composition of apple fruits.

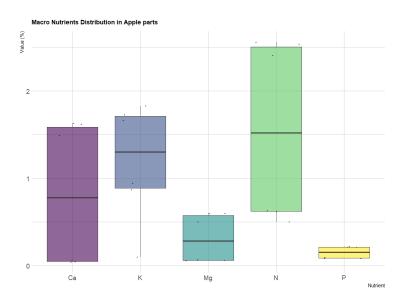


Figure 2. Distribution of Macro Minerals (Ca, K, Mg, N, P) in Apple Fruit and Leaves (%).

The distribution of micronutrient contents in apple fruit and leaf samples is illustrated in Figure 3. Upon examining the distribution of micronutrients, it is apparent that boron (B) exhibits the highest concentration,

ranging from 80 to 120 ppm. This is closely followed by iron (Fe), which shows a broad distribution spanning from approximately 10 to 90 ppm. Manganese (Mn) content fluctuates between 5 and 45 ppm, while zinc (Zn) is found to range from 5 to 20 ppm. Copper (Cu) displays the lowest concentration, ranging between 1 and 10 ppm. In the box-plot graph, the points represent outliers. Notably, the distribution of boron and iron stands out for its broader spread compared to the other elements. The findings for zinc and copper in our study largely align with those reported by Ahmed et al. (2024) (Zn: 6.76-22.85 ppm, Cu: 1.91-11.80 ppm), suggesting that the transport and accumulation of these elements in the plant may be relatively stable. Habte et al. (2017) also suggested that the distribution of elements in fruits is strongly influenced by genetic structure and physiological processes.

In their research, Nava et al. (2018) found that leaf tissues contained higher concentrations of nutrients than fruit tissues in Fuji apples, a result that aligns with our findings. In our study, we observed that the concentrations of both macro and micronutrients in leaf tissues were significantly higher than those in fruit tissues.

When comparing the distribution of nutrient elements in both the fruit and leaf samples, it is evident that the leaves generally exhibit higher concentrations. This is likely due to the fact that leaves play a crucial role in storing nutrients essential for photosynthesis and various metabolic processes (Radojčin et al., 2021). Furthermore, Ahmed et al. (2024) reported that element distribution varies across different parts of the fruit (skin, flesh, seeds), with the highest accumulation generally occurring in the seeds.

While the distribution of nutrient elements in apple fruit and leaves in our study partially aligns with the literature, we also identified some key differences. These variations may be attributed to factors such as cultivar-specific traits, growing conditions, soil composition, climate variables, and differences in analytical methods. It is important to note that environmental factors, including soil fertility, irrigation, and timing of harvest, can significantly influence the nutrient content in both fruit and leaf tissues. Therefore, understanding the underlying causes of these discrepancies can facilitate the optimization agricultural practices and improve nutrient management strategies for apple cultivation.

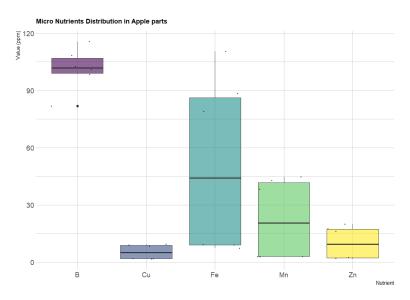


Figure 3. Distribution of Micro Minerals (B, Cu, Fe, Mn, Zn) in Apple Fruit and Leaves (ppm).

The bivariate distribution plots and density curves illustrating the relationships between nutrient elements in apple fruit and leaf tissues are presented in Figure 4. As depicted in the figure, the scatter plots for the relationships between macroelements show not only the individual distributions of each element (on the diagonal) but also the correlations between them. Nitrogen (N) exhibits a broad distribution ranging from 0.5% to 2.5%, whereas phosphorus (P) has a more concentrated distribution, ranging from 0.08% to 0.20%. Potassium (K) shows variability between 0.5% and 1.5%, calcium (Ca) is distributed between 0% and 1.5%, and magnesium (Mg) spans from 0.2% to 0.6%. Of particular note are the pronounced relationships between nitrogen (N) and calcium (Ca), as well as between phosphorus (P) and potassium (K). In the scatter plots, blue points represent leaf samples, while red points represent fruit samples. The density curves on the diagonal highlight the distribution characteristics of each element, providing further insight into their concentration patterns within the tissues. These visualizations underscore the variability and associations of macroelements, which are key for understanding nutrient dynamics in apples.

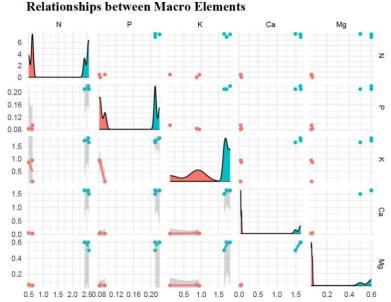


Figure 4. Bivariate Relations and Distributions of Macro Minerals in Tissues.

The bivariate relationships between micronutrient elements in apple fruit and leaf tissues are presented in Figure 5. These distribution plots illustrate the relationships between the micronutrients, with Fe ranging from 30-90 ppm, Cu from 2.5-7.5 ppm, Mn from 10-40 ppm, Zn from 5-20 ppm, and B spanning 90-110 ppm. It is of particular note is the clear linear trend observed in the relationships of B with other elements, indicating a consistent correlation pattern across samples. A negative correlation is observed between Fe and both Cu and Mn, suggesting that as one of these elements increases, the other tends to decrease. Conversely, a positive correlation between Zn and B is observed, implying that higher concentrations of one are associated with higher levels of the other. The blue points in the plots represent leaf samples, while the red points represent fruit samples, allowing for a clear distinction between tissue types. The density plots along the diagonal provide additional insight into the distribution characteristics of each micronutrient, showing how the concentration of each element varies within the dataset. Notably, the distribution of the B element displays a bimodal (dual-peak) pattern, suggesting that there may be two distinct subpopulations or environmental factors influencing its distribution. These results underline the complex interactions between micronutrients in apples and the potential for varying nutrient dynamics between different tissues.

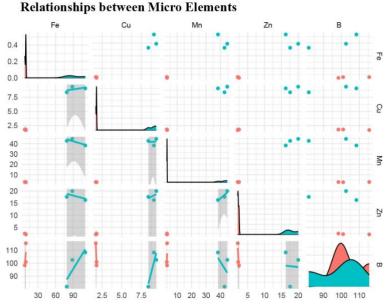


Figure 5. Bivariate Relations and Distributions of Micro Minerals in Tissues.

CONCLUSION

This study provides crucial insights into the distribution and interactions of macro and micronutrients in apple trees, emphasizing their impact on fruit quality and yield. The findings revealed significant variations in nutrient content between leaves and fruits, with leaf tissues generally exhibiting higher concentrations. Notably, nitrogen levels in fruit ranged from 0.50% to 0.63%, while in leaves, they were significantly higher (2.41%-2.56%). Potassium, essential for fruit quality, showed a wide variation in fruit (0.10%-0.94%) compared to leaves (1.66%-1.83%). A concerning observation was the relatively low calcium (0.04%-0.06%) and zinc (2.07-2.44 ppm) levels in fruit, which may negatively affect storage life and disease resistance. The strong negative correlation between nitrogen and calcium (r=-0.99) indicates a potential imbalance that could impact fruit firmness and shelf life. These results underscore the importance of precise nutrient management to maintain optimal fruit quality and maximize productivity. Appropriate fertilization programs should be implemented based on regular soil and leaf analyses to balance apples' low calcium and zinc levels to harvest high-quality fruits and manage post-harvest period according to different ecologies.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no conflict of interest.

Author contribution

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

Funding

No financial support was received for this study

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.16

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 132-143

Characterization of PGPR from rhizospheric soil of some vegetable crops cultivated at Sylhet district of Bangladesh

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Article History

Received: September 25, 2024 Revised: March 5, 2025 Accepted: March 9, 2025 Published Online: March 12, 2025

Article Info Article Type: Research Article Article Subject: Phytopathology

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Available at :://dergipark.org.tr/jaefs/issue/90253/1554973







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Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are rhizosphere-dwelling microorganisms which hold a great deal of potential for both plant growth stimulation and disease prevention. The characterization of PGPR will aid in the advancement and deployment of biocontrol agents. In this present work, rhizospheric soils were collected from several locations of Sylhet Agricultural University in order to obtain plant growth promoting rhizobacteria. Nineteen bacterial samples were extracted from a variety of fifteen distinct vegetable crops, viz. tomato, brinjal, beans, okra, cabbage, cauliflower, pumpkin, amaranth, malabar spinach, bitter gourd, ridge gourd, spiny gourd, sponge gourd, wax gourd, and snake gourd. These isolates were examined morphologically, biochemically, and screened for plant growth stimulating capability as well as their efficacy in combating the plant pathogen Fusarium oxysporum through antifungal activity. Among the isolates, only Lysinibacillus macroides (RB2), Lysinibacillus fusiformis (RB6) and Acinetobacter baumannii (RB15 and RB17) showed antifungal and growth promotion potentials. Therefore, the present study indicates that the vegetable rhizosphere contains potential rhizobacteria which could be utilized to enhance plant development and reduce disease incidence on vegetable

Keywords: PGPR, vegetable crops, Lysinibacillus macroides, Lysinibacillus fusiformis and Acinetobacter baumannii.

Cite this article as: Jui, S.S., Hasan, R., Ema, I.J., Nasim, H.T., Islam, M.M. (2025). Characterization of PGPR from rhizospheric soil of some vegetable crops cultivated at Sylhet district of Bangladesh. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 132-143. https://doi.org/10.31015/2025.1.16

INTRODUCTION

Bangladesh, as a developing nation, relies heavily on its agro-economy, where vegetable production is an important agricultural activity that contributes to the country's food and nutritional needs. In this country, the market demand for vegetables comes in second place to that of rice (Mim et al., 2019). However, the average yield of vegetable crops is influenced by plant pathogenic microorganisms. Many diseases that occur throughout the crop growing season cause a significant loss (Azad et al., 2014). Therefore, people use a variety of tactics, including the application of a variety of chemicals, in order to fight against plant diseases while increasing agricultural production. Yet, the extended usage of these agrochemicals has significant adverse consequences for the ecosystem and people's health (Li et al., 2020). Contemporary public apprehensions regarding the detrimental impacts of agrochemicals have led to a growing curiosity about thoroughly understanding the cooperative relationships between plants and soil microorganisms. This has led to a pressing need for widely known biological agents (Mahanty et al., 2017). Utilizing plant growth promoting rhizobacteria appears to be a promising way to address this challenge (Gupta et al., 2015; Chauhan et al., 2021).

Plant growth promoting rhizobacteria (PGPR) are a collective of soil microorganisms thriving within the rhizosphere along with the plant root surface and benefit plant's overall health. Bacterial species, notably Acinetobacter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Gluconacetobacter, Lysinibacillus, Pseudomonas, Rhizobium, and Serratia are widely acknowledged as PGPR (Vessey 2003; Rokhbakhsh-Zamin et al., 2011; Bhattacharyya and Jha 2012; Huang et al., 2017; Naureen et al., 2017).

PGPR stimulates plant development by several mechanisms, categorized as direct and indirect. Direct mechanisms refer to processes which provide nutrients or generate growth stimulants in order to directly encourage plant development. In contrast, those processes that protect plants against infection or allow plants to grow properly under stressful conditions are termed indirect mechanics (Goswami et al., 2016). Direct mechanisms comprise the potential to nitrogen-fix, solubilize insoluble phosphate, sequester iron, and generate phytohormones (including auxins, cytokinins, and gibberellins), while the capacity to synthesize antibiotics, enzymes, or to generate systemic resistance within plants are examples of indirect mechanisms (Gouda et al., 2018; Meena et al., 2020).

Additional research has recognized the plant growth promoting (PGP) and antagonistic potential of PGPR. Bacillus subtilis obtained from Solanum lycopersicum (tomato) plants showed antifungal activity against Alternaria alternata, Penicilium digitatum, and Fusarium oxysporum, as well as improved both qualitative and quantitative growth parameters in Solanum lycopersicum and Solanum melongena plants (Sarbadhikary and Mandal, 2017). Pseudomonas aeruginosa, Bacillus subtilis, and Acenetobacter pitti exhibited antagonistic activity against Rhizoctonia solani, the pathogen responsible for foot rot disease in vegetable crops (Kumari et al., 2018). Enterobacter cloacae, Chryseobacterium jejuense, and Klebsiella pneumonia, all isolated from tomato rhizosphere were found to have antagonistic and PGP activities (Abdeljalil et al., 2016). Pseudomonas aeruginosa isolated from tomato rhizosphere inhibited the mycelial development of Fusarium oxysporum and Alternaria solani (Paramanandham et al., 2017).

Biofertilizers containing PGPR offer a more economical, sustainable, and productive alternative compared to chemical fertilizers. Moreover, they are highly effective and accessible to marginal farmers (Basu et al., 2021). Therefore, PGPR inoculants may be utilized to ensure the consistency of long-term agricultural production, presenting a more widely recognized substitute for intensive agriculture in numerous regions worldwide (Kenneth et al., 2019). Consequently, there is an immediate need to find viable PGPR strains for our farmers.

In line with this objective, the study aimed to isolate, characterize, and identify PGPR from vegetable crops rhizosphere, focusing on their PGP attributes and antagonistic activity. In this research work, nineteen bacterial isolates were collected, and their morphological and biochemical characteristics were examined. In addition, the isolates underwent screening for PGP properties, and selected representatives were molecularly characterized.

MATERIALS AND METHODS

The study site and cultivars

The research was conducted in the central laboratory of the Microbiology and Immunology Department, Faculty of Veterinary, Animal, and Biomedical Sciences, Sylhet Agricultural University, located in Sylhet, Bangladesh, from January 2021 to June 2021. Fifteen vegetable crops, e.g., tomato, brinjal, bean, okra, cabbage, cauliflower, pumpkin, amaranth, malabar spinach, bitter gourd, ridge gourd, spiny gourd, sponge gourd, wax gourd, and snake gourd were collected from different locations of SAU (24°54' to 36°36" N and 91°54' to 02°36" E). The climate in the region is characterized by subtropical monsoons. The majority of agricultural soils in the Sylhet region belong to the loamy sand textural class, acidic with pH values of 4.9-6.1 containing the organic matter of 0.5-2.45% and EC of 0.26-1.17 dsm-1 (Hossain and Sattar, 2002).

Collection and preservation of rhizospheric soil

Nineteen samples were taken from two-month-old healthy vegetable crop plants. The rhizosphere around the vegetable crops was dug out to a 0-15 cm depth and then uprooted with sufficient rhizosphere soils without hampering the secondary and tertiary roots. The plant roots and rhizospheric soil were kept in plastic bags at 4° C.

Isolation of PGPR from rhizospheric soil

To isolate rhizobacteria, fresh roots weighing 2–5 g were washed and then surface sterilized with 5% NaOCl. The root samples underwent pulverization after being washed three times with sterile distilled water. Then, using both soil suspension and root samples, a serial dilution was prepared and it was carried out up to ten times. A portion of this solution (0.1 ml) was spread on nutritional agar (NA) medium and incubated for 24 hours at 37°C for bacterial culture. Then a single colony was isolated from the culture and re-streaked onto new plates, where it was cultured in the same way. Isolated colonies were selected for single isolation based on several features, such as shape, color, and margin. This was then cultured in nutrient broth slants to generate pure culture.

Morphological Characterization

Isolates cultured on NA plates were observed for morphological analysis. Several properties of colonies were recorded, including size, shape, elevation, margin, surface, pigmentation, etc., as indicated by Somasegaran and Hoben (2012). Light microscopy was used to observe cell size. Gram reaction and motility tests were performed. In vitro assessments were conducted for the abiotic stress tolerance activities of the isolates in different temperature ranges (10, 28, 37, and 45° C) and salt concentrations (5% and 10% NaCl).

Biochemical characterization

Bergey's Manual of Systematic Bacteriology was followed in the biochemical characterization process (Bergey et al., 1994). Routine biochemical tests such as citrate utilization activity, catalase activity, KOH solubilization,

indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, and carbohydrate fermentation test (glucose, sucrose, maltose, and mannitol) were carried out for the PGPR isolates.

Tests for plant growth promoting (PGP) activities

Phosphate solubilizing ability

All 19 isolates were placed on the Pikovskaya agar plates and incubated at 28°C. After 24 hours of growth, the plates were inspected to see if the bacterial colonies had a halo zone surrounding them, an indication of their ability to dissolve tri-calcium phosphate (Pikovskaya, 1948).

Indole-3-Acetic Acid (IAA) production

IAA production was analyzed by following Salkowski's method (Ehmann, 1977). The isolates were cultured in yeast malt dextrose broth (YMD broth) for four days at 28°C, followed by centrifugation at 3000 rpm for 30 minutes. Subsequently, 1 ml of the resulting supernatant was incorporated with 2 ml of Salkowski's reagent, and the mixture was stored in the dark for 30 minutes. Controls included reagents mixed with distilled water. After that, the treated reagents were checked for turning red.

Production of ammonia

Peptone water was utilized to assess the ammonia production capacity of test isolates. Freshly cultured isolates were placed in every test tube containing 10 ml of peptone water, which were subsequently incubated at 37°C for 48 to 72 hours. Then each tube was filled with 0.5 ml of Nessler's reagent and checked for the formation of a brown to yellow color (Cappuccino and Sherman, 2013).

Production of Hydrogen Cyanide (HCN)

Isolates were grown on NA medium supplemented with 4.4 g per liter of glycine. A Whatman filter paper No. 1 was put on top of the plate after being soaked in a solution containing 2% sodium carbonate and 0.5% picric acid. Following the sealing of the plates with parafilm, they underwent incubation at 36±2°C for four days. Afterward, the filter paper was observed for a color transition from orange to brown (Lorck, 1948).

In Vitro Screening for Antagonism

The antagonistic behavior was assessed against Fusarium oxysporum, isolated from naturally infected tomato plants that showed typical Fusarium wilt symptoms, adopting the dual culture method (Skidmore and Dickinson, 1976). In this approach, isolates were cross-streaked on one side of PDA plates, while a five-day-old mycelial disc of the plant pathogen was placed on the other side. The plates were then incubated in the range of 28±2°C for a period of 5 days. The percentage of the fungal mycelial inhibition by the bacteria was determined using the following formula (Noumavo et al. 2015):

Percentage of growth inhibition (%) = $\frac{VI - V2}{V1} \times 100$

Where, V1= the diameter of the fungus growth (control),

V2= the diameter of the fungus growth in the dual culture plate.

Molecular characterization

Based on plant growthpromoting characteristics and antifungal properties, four bacterial isolates (RB2, RB6, RB15, and RB17) were selected for molecular characterization. This identification was accomplished by amplifying 16S rRNA using the PCR primers: 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (CGG TTA CCT TGT TAC GAC TT). The extracted bacterial DNA was utilized for PCR reaction. PCR reaction and sequencing were performed by the commercial service provider Invent Technologies Ltd., Dhaka, Bangladesh. The obtained sequences were compared with the GeneBank database using BLAST (Basic Local Alignment Search Tool), and the percentage similarity was subsequently determined. A phylogenetic tree was constructed by using the software package MEGA (Molecular Evolutionary Genetic Analysis) following the neighbor joining method (Saitou and Nei, 1987) with bootstrap values (Felsenstein, 1985) based on 1,000 replications.

Statistical Analysis

The inhibitions of fungal growth by the bacterial isolates were observed, and the mean along with standard deviation of these inhibitions were estimated. The statistical analysis of the data was performed using the Agricole package within the R software. Significant differences among the means were determined utilizing Fisher's least significant (LSD) test, considering a significance level of $P \le 0.05$.

RESULTS AND DISCUSSION

Strain isolation and morphological characterization

The bacterial strains isolated from vegetable crops rhizosphere were named as RB1, RB2, RB3, RB4, RB5, RB6, RB7, RB8, RB9, RB10, RB11, RB12, RB13, RB14, RB15, RB16, RB17, RB18, and RB19. Table 1 represents the result of the colony morphology and microscopic findings of PGPR isolates. From morphological analysis it was found that the bacterial isolates were fast-growing with a wide range of colony form. The isolates formed colonies that were typically circular, flat elevation, smooth surface, odorless, and varied in color. The colonies' diameter ranged from 0.7 to 1.6 mm. Under a microscope, ten rod-shaped isolates (RB2, RB3, RB5, RB6, RB7, RB11, RB12, RB14, RB18, and RB19), commonly known as bacilli, were discovered. Seven isolates (RB1, RB4, RB8, RB9, RB10, RB13 and RB16) were found to be cocci or spherical in form and two (RB15 and RB17)

were found to be short rod. There were 9 gram-positive isolates (RB2, RB3, RB6, RB7, RB11, RB12, RB13, RB14, and RB18) and 10 gram-negative isolates (RB1, RB4, RB5, RB8, RB9, RB10, RB15, RB16, RB17 and RB19) among the nineteen isolates. Except for RB15 and RB17, all isolates were motile. Morphological study revealed that the isolates varied greatly concerning their size, shape, elevation, color, margin, surface, odor and gram staining which were confirmed by other studies (Chen et al., 2009; Hardiansyah, 2020).

Table 1. Morphological features of colonies of PGPR isolates.

Isolates Shape		Size	Elevation	Surface	Color	Odor	Cell	Motility	Gram
		(mm)					Shape		Staining
RB1	Round	0.8	Raised	Smooth	Off white	Odorless	Round	Motile	-
RB2	Round	1.2	Flat	Rough	Yellowish	Odorless	Rod	Motile	+
RB3	Oval	0.9	Flat	Rough	Yellowish	Odorless	Rod	Motile	+
RB4	Round	0.9	Raised	Smooth	Off white	Odorless	Round	Motile	-
RB5	Oval	0.7	Flat	Rough	Off white	Odorless	Rod	Motile	-
RB6	Oval	1.0	Raised	Smooth	Yellowish	Odorless	Rod	Motile	+
RB7	Oval	1.4	Flat	Rough	Off white	Odorless	Rod	Motile	+
RB8	Round	1.0	Raised	Smooth	Yellowish	Odorless	Round	Motile	-
RB9	Round	0.7	Flat	Smooth	Off white	Odorless	Round	Motile	-
RB10	Round	1.2	Raised	Smooth	Off white	Odorless	Round	Motile	-
RB11	Round	1.0	Raised	Rough	Off white	Odorless	Rod	Motile	+
RB12	Irregular	1.5	Flat	Rough	White	Odorless	Rod	Motile	+
RB13	Round	0.9	Flat	Smooth	Off white	Odorless	Round	Motile	+
RB14	Round	1.4	Flat	Rough	White	Odorless	Rod	Motile	+
RB15	Round	0.8	Raised	Smooth	White	Odorless	Short rod	Non-motile	-
RB16	Round	0.8	Flat	Rough	White	Odorless	Round	Motile	-
RB17	Round	1.4	Raised	Smooth	White	Odorless	Short rod	Non-motile	-
RB18	Round	1.6	Flat	Rough	Off white	Odorless	Rod	Motile	+
RB19	Round	1.2	Raised	Smooth	White	Odorless	Rod	Motile	

NB: '+' indicates positive growth; and '-' indicates No growth

Growth of PGPR at abiotic stress tolerance activities (different temperature ranges and NaCl concentrations)

Temperature and salinity are two important limiting factors that have an impact on agricultural yield and plant development (Tsegaye et al., 2019). Under varied temperature conditions (i.e., 10, 28, 37, and 45° C), the test isolates grown in NA plates for 24 hours exhibited heavy to no growth, as shown in table 2. In 5% NaCl, 8 isolates (RB1, RB5, RB7, RB9, RB11, RB12, RB14, and RB17) were able to grow while the remaining 11 isolates didn't show any growth. No isolate developed growth at 10% salt concentration. The majority of isolates exhibited positive growth at 5% salt concentrations and temperatures of 28°C and 37°C. Table 2 shows the growth potential of isolates at various temperatures and NaCl concentrations.

Table 2. Growth Performance of PGPRs at abiotic stress tolerance activities

Isolates	Tempera	ture (°C)	·	·	Salt concer	Salt concentration (%)		
isolates	10	28	37	45	5	10		
RB1	-	++	+	+	+	-		
RB2	+	++	++	++	-	-		
RB3	-	+	++	-	-	-		
RB4	+	+	+	+	-	-		
RB5	-	++	++	+	+	-		
RB6	+	++	++	++	-	-		
RB7	+	++	++	+	+	-		
RB8	-	++	+	+	-	-		
RB9	-	++	+	+	+	-		
RB10	-	++	++	+	-	-		
RB11	+	++	++	-	+	-		
RB12	+	++	++	++	+	-		
RB13	+	+	++	++	-	-		
RB14	-	+	++	++	+	-		
RB15	+	+	+	+	-	-		
RB16	-	++	+	-	-	-		
RB17	-	+	+	+	+	-		
RB18	+	++	++	+	-	-		
RB19	+	+	++	+	-	-		

NB: '++' indicates heavy growth; '+' indicates positive growth; and '-'indicates No growth

Biochemical Characterization

As for biochemical analysis, the isolates demonstrated a high level of diversity where most of them gave positive results for the Citrate utilization test, Catalase test, KOH solubility test, MR test, VP test, and Carbohydrate fermentation tests. Only one isolate (RB16) was positive for the Indole test. Similar biochemical tests were performed by Asrafujamman et al. (2009) and Tsegaye et al., (2019). The outcomes of all these biochemical tests are presented in Table 3.

Table 3. Biochemical characterization of PGPR isolates.

Isolates	Citrate	Catalase	КОН	Indole	MR	VP	Glucose	Sucrose	Maltose	Mannitol
RB1	-	+	-	-	+	-	+	+	+	+
RB2	+	+	+	-	-	-	+	+	+	+
RB3	-	+	+	-	-	+	+	+	-	+
RB4	+	+	-	-	+	-	+	-	+	-
RB5	-	+	-	-	-	+	+	+	+	+
RB6	+	+	+	-	-	-	+	+	-	+
RB7	-	+	+	-	-	+	-	+	+	+
RB8	+	+	-	-	+	-	+	+	+	-
RB9	-	+	-	-	-	+	+	+	+	-
RB10	+	+	-	-	-	-	+	-	-	+
RB11	-	+	+	-	-	-	+	+	+	+
RB12	+	+	+	-	+	+	+	+	-	+
RB13	-	+	+	-	+	+	+	+	+	+
RB14	-	+	+	-	+	-	+	+	+	+
RB15	+	-	-	-	+	+	+	-	+	-
RB16	-	+	-	+	+	-	-	+	+	+
RB17	+	-	-	-	+	+	+	+	+	-
RB18	+	+	+	-	+	-	+	+	-	+
RB19	+	+	-	-	+	-	+	+	+	+

NB: '+' corresponds to positive response; '-' corresponds to negative response, MR= Methyl Red, and VP= Voges Proskauer

Characterization for plant growth promoting properties

All 19 isolates were examined for their PGP properties (ammonia synthesis, IAA generation, HCN production, and phosphate solubilization). Phosphorus is a vital element for optimal plant development. However, a significant amount of the phosphorus in soil exists in an insoluble form, preventing direct uptake by plants. Some PGPR help to convert them into soluble form by secreting organic acids and phosphatases, thereby enhancing its availability to plants (Souchie et al., 2005; Dash et al., 2017). In the current study, Out of the nineteen rhizobacterial isolates, only six formed a halo zone surrounding the colonies. As a result, these six isolates (RB1, RB2, RB6, RB15, RB16, and RB17) were reported as positive in the phosphate solubilization test, whereas the other thirteen were found to be negative. Other studies have also documented the phosphate solubilization ability of PGPR strainsderived from different vegetable crops (Bechtaoui et al., 2019; Liu et al., 2015; Baliah et al., 2016; Paiter et al., 2019; Mei et al., 2021).

Another crucial PGP characteristic of rhizobacteria is the generation of phytohormones (Patten and Glick, 2002). IAA, an important phytohormone, has a beneficial impact on plant root system elongation and development, contributing in water and nutrient intake as well as the activation of cambial cell division (Grobelak et al., 2015; Kusumawati et al., 2017). Eight isolates (RB1, RB2, RB6, RB10, RB12, RB15, RB17, and RB18) were capable of producing IAA which was evident by their distinctive reddish to pinkish appearance in the solution. Similar results were found by other researchers (Dias et al. 2013; Meliani et al., 2017; Yousef, 2018; Mike-Anosike et al., 2018; Kalimuthu et al., 2019; Cavalcante et al., 2020).

The generation of ammonia was evidenced when the broth culture became brown with the addition of Nesler's reagent. All nineteen bacterial isolates produced ammonia under test conditions, a phenomenon known to have an indirect influence on plant health. This study's findings on the ammonia generating ability of rhizobacteria are aligned with other prior studies (Goswami et al., 2015; Moustaine et al., 2017; Mazumdar et al., 2018; Singh et al., 2019).

Six bacterial isolates (RB2, RB6, RB10, RB15, RB16, and RB17) generated HCN. It was demonstrated by an alteration in filter paper's color from yellow or orange to brown. HCN is a well-known secondary metabolite that has been associated with pathogen control in soil (Kesaulya et al. 2015). Other researchers also reported HCN producing PGPR strains (Vaikuntapu et al., 2014; Kesaulya et al., 2015; Marakana et al., 2018; Abd El-Moaty et al., 2018; Agustiyani et al., 2022). Table 4 represents the performance of PGPR in growth promotion activities.

Effects of antagonistic activity of rhizobacteria

To assess antagonistic activity, dual cultures were performed using the test isolates against *Fusarium oxysporum*. Nine of the nineteen isolates showed some level of antagonism, while the maximum and minimum inhibitions were observed for RB2 (44.74%) and RB12 (23.68%), respectively. Rhizobacteria can limit the growth

of phytopathogens through different ways, such as competing for nutrients and space, generating bacteriocins, enzymes, antibiotics, and siderophores (Jing et al. 2007). Other research also found the antagonistic behavior of rhizobacteria (Manasa et al., 2017; Ali et al., 2020; Attia et al., 2020; Pellegrini et al., 2020).

Table 4.Performance of PGPR in growth promotion activities.

Isolates	Phosphate solubilization	IAA production	Ammonia production	HCN production
RB1	+	+	+	-
RB2	+	+	+	+
RB3	-	-	+	-
RB4	-	-	+	-
RB5	-	-	+	-
RB6	+	+	+	+
RB7	-	-	+	-
RB8	-	-	+	-
RB9	-	-	+	-
RB10	-	+	+	+
RB11	-	-	+	-
RB12	-	+	+	-
RB13	-	-	+	-
RB14	-	-	+	-
RB15	+	+	+	+
RB16	+	-	+	+
RB17	+	+	+	+
RB18	-	+	+	-
RB19	-	-	+	-

NB: '+' indicates positive growth; and '-'indicates No growth

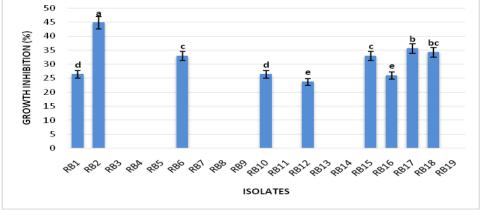


Figure 1. Antagonistic activity of PGPR against Fusarium oxysporum

Molecular Characterization and Identification

In accordance with morphological and biochemical findings, nine out of the nineteen isolates belonged to five different genera, namely *Acinetobacter sp.*, *Bacillus sp.*, *Enterobacter sp.*, *Lysinibacillus sp.*, and *Pseudomonas sp.* (Huang et al., 2017; Pramanik et al., 2018; Singh et al., 2020; Jyolsna et al., 2021). Of them, Isolates RB2, RB6, RB15, and RB17 were molecularly characterized (Figure 2). The outcome indicated that RB2 and RB6 had the highest similarity with *Lysinibacillus macroides* and *Lysinibacillus fusiformis* respectively while RB15 and RB17 both showed similarity with *Acinetobacter baumannii*. Identification of different *Lysinibacillus spp.* as PGPR was also recorded by other studies (Vendan et al., 2010; Singh et al., 2013; Jyolsna et al., 2021; Ahsan and Shimizu, 2021; Passera et al., 2021; Jha and Mohamed 2023; Pantoja-Guerra et al., 2023). *Acinetobacter* has been reported as PGPR by previous studies such as Rokhbakhsh-Zamin et al. (2011), Padmavathi et al. (2015), kumari et al. (2018), Santosa et al. (2018), Leontidou et al. (2020), Singh et al. (2020) and Mujumdar et al. (2023). Phylogenetic analysis was done to evaluate the evolutionary relationships of RB2, RB6, RB15, and RB17 with other species, as illustrated in Figure 3. According to this tree, isolates RB15 and RB17 were discovered to be closely related to two distinct strains of *Acinetobacter baumannii*, with bootstrap values of 99% and 67%, respectively, while isolates RB2 and RB6 demonstrated a close relationship with *Lysinibacillus macroides* and *Lysinibacillus fusiformis*, with bootstrap values of 50% and 17%, respectively.

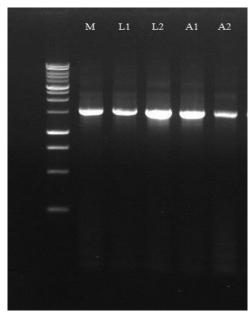


Figure 2. Agarose gel electrophoresis of the DNA amplified with primers 27F and 1492R from genomic DNA of bacterial strains.

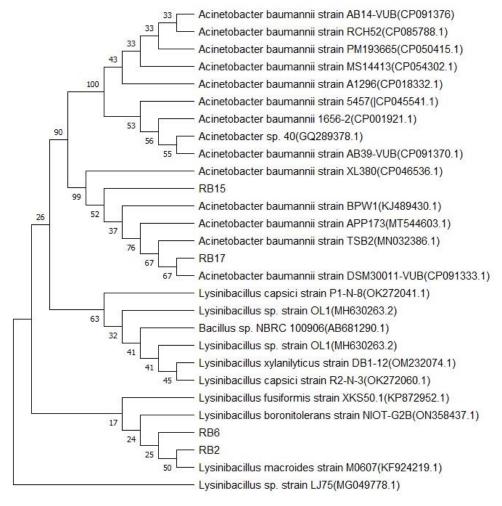


Figure 3: Phylogenetic tree formed by using the neighbor-joining method based on 16S rRNA showing the relationship of isolates RB2, RB6, RB15 and RB17 to related isolates.

CONCLUSION

The application of PGPR has become an environmentally friendly strategy to enhance crop yields by promoting plant growth by means of diverse mechanisms, encompassing the maintenance of nutritional status, activation of disease resistance in plants, and nutrients dissolution for easier plant uptake (Santoyo et al., 2021). This research aimed to identify potential PGPR from vegetable rhizosphere and 19 bacterial isolates were obtained with this purpose. The collected isolates were cultured in NA agar media and observed for morphological, biochemical and molecular characteristics. PGP attributes and antagonistic activity of the isolates were also evaluated. Among the nineteen bacterial isolates studied, four isolates (RB2, RB6, RB15 and RB17) were found most potential considering PGP properties and antagonistic effect against *Fusarium oxysporum*. These isolates were identified as *Lysinibacillus macroides* (RB2), *Lysinibacillus fusiformis* (RB6), and *Acinetobacter baumannii* (RB15 and RB17) based on 16S rRNA gene sequence analysis. The study findings therefore support the hypothesis that these four isolates possess the potential to act as PGPR and can inhibit *Fusarium oxysporum* induced diseases in vegetable crops. The strains RB2, RB6, RB15, and RB17 collectively show promise as candidates for the production of biopesticide and biofertilizer for field application. Further research is needed to evaluate their effectiveness as biofertilizers. In addition, their antagonistic activity against various other pathogens should be examined to ensure a broader range of disease management for sustainable agriculture.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

No political conflict of interest was reported by the authors.

Author contribution

Saima Sadia Jui made a draft of the design of work, data collection, acquisition & analysis of the dataset, and wrote a draft of manuscript. Md. Monirul Islam made the plan of actions to execute and revised the design and analysis critically, and made the interpretation of the results. Rakibul Hasan carefully observed the design and analysis, also revised the manuscript. Israt Jahan Ema revised the manuscript and made valuable suggestions. Hasan Tareq Nasim helped in data collection. All authors read and approved the final manuscript.

Funding

This research was funded by University Grants Commission of Bangladesh. The Ministry of Science and Technology (Most) of Bangladesh also contributed to this work by providing National Science and Technology (NST) fellowship to the first author.

Acknowledgments

We gratefully acknowledge the department of microbiology and immunology, faculty of veterinary, animal, and biomedical sciences, Sylhet Agricultural University, Sylhet, for their laboratory assistance.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.17

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 144-156

The impact of agricultural activities on climate change in BRICS countries and Türkiye

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Article History

Received: January 13, 2024 Revised: March 7, 2025 Accepted: March 10, 2025 Published Online: March 11, 2025

Article Info

Article Type: Research Article Article Subject: Sustainable Agricultural Development

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Available at tps://dergipark.org.tr/jaefs/issue/90253/1618856

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Abstract

Agricultural activities have a significant impact on climate change due to greenhouse gases such as methane, CO2 and nitrous oxide. Agriculture in the BRICS (Brazil, Russia, India, China and South Africa) countries and Türkiye plays a crucial role in global production and contributes to feeding the population, ensuring food security and fighting hunger. Agriculture also has an important impact on environmental sustainability and climate change, as agricultural activities contribute directly to CO₂ emissions. In this sense, agriculture is not only a locomotive for the economic development of the BRICS countries and Türkiye, but also important for controlling environmental degradation and ensuring sustainable growth. Therefore, the study examine the long-run effects of agricultural production, chemical fertilizers used to increase agricultural productivity, the mechanization in agriculture and the rural population on CO₂ emissions for six countries including BRICS and Türkiye for the period 1961-2019 using the PMG-ARDL model. The estimated long-run coefficients show that agricultural mechanization and fertilizer use increase CO2 emissions, while agricultural production and rural population reduce emissions. It was also concluded that the expansion of agricultural land has no significant impact on CO₂ emissions in the long run. The results of the Granger causality test by Dumitrescu and Hurlin (2012) also show that CO2 emissions are not Granger cause of agricultural land and agricultural production, but mechanization, fertilizer use and rural population have a causal effect on CO₂ emissions. The results suggest that policy makers should adopt a balanced and environmentally friendly measures to the agricultural sector in order to ensure environmental sustainability and reduce the negative impacts of agricultural activities.

Keywords: BRICS Countries, Türkiye, PMG-ARDL, CO₂, Agricultural Production, Mechanization in Agriculture

Cite this article as: Kaya, T. (2025). The impact of agricultural activities on climate change in BRICS countries and Türkiye. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 144-156. https://doi.org/10.31015/2025.1.17

INTRODUCTION

The relationship between agricultural production and climate change is of great importance for building a sustainable ecosystem (Hinz et al., 2020; Alamu, 2024). In the 21st century, where the global population has surpassed eight billion and millions of people are struggling with hunger, the balance between agricultural production and environmental sustainability places significant pressure on the economic and social structures of countries (Liu et al., 2021; Senay and Tepecik, 2024). Chemical fertilizers used to increase agricultural productivity, green spaces converted into agricultural lands, mechanization preferred for labor savings, and the rapidly growing population stand out as major factors accelerating climate change (Hedayetullah et al., 2015). While countries encourage fertilizer use and mechanization to feed their populations and boost agricultural productivity, they continue practices that harm the global ecosystem, such as converting thousands of hectares of green areas into farmland each year. In this process, although chemical fertilizers increase the productivity of agricultural soils, the greenhouse gas emissions caused by these fertilizers raise atmospheric carbon levels, leaving adverse environmental impacts (Sezen and Küçük, 2021). Furthermore, mechanization in agriculture increases

energy consumption and fossil fuel use, further elevating CO₂ emissions (Sah and Devakumar, 2018; Aguilera et al., 2019; Lu et al., 2024). Additionally, imbalances between rural and urban populations lead to the destruction of natural habitats and the loss of biodiversity in regions where agriculture is intensively practiced. When all these activities are considered within the context of agricultural production, they underscore the critical necessity of implementing sustainable agricultural policies to combat climate change effectively.

Agriculture encompasses a wide range of activities, including crop cultivation, livestock farming, irrigation, and the use of fertilizers and pesticides. It has demonstrated significant advancements in productivity, particularly with the adoption of modern equipment. The integration of tractors, harvesters, and other machinery into agricultural processes has increased productivity but also led to higher CO_2 emissions due to fossil fuel consumption (Smith et al., 2014; Guan et al., 2023). Additionally, the conversion of forests and other natural ecosystems into farmland releases stored carbon into the atmosphere, thereby accelerating climate change (Houghton, 1995; Zhang et al., 2024). This process significantly contributes to global warming, particularly through the loss of biodiversity and the reduction of carbon sinks. The environmental impacts of agriculture are not limited to mechanization and land-use changes but are also evident in practices such as fertilizer application. The production of nitrogen-based synthetic fertilizers, for instance, is an energy-intensive process that often relies on fossil fuels. When applied to soils, these fertilizers trigger microbial activities that release greenhouse gases like CO_2 and nitrous oxide (N_2O). According to reports by the Intergovernmental Panel on Climate Change (IPCC) in 2017, the agricultural sector accounts for 10-12% of global anthropogenic greenhouse gas emissions, playing a significant role in the release of not only CO_2 but also other greenhouse gases (Change, 2019).

When examining the relationship between agricultural production and climate change, as well as the impact of agriculture on greenhouse gas emissions, the BRICS countries emerge as a key group to consider (Tian et al., 2020). Countries such as Brazil, China, and India are global leaders in agricultural production. Including a country like Türkiye, which ranks among the top ten globally, creates a valuable sample for exploring the relationship between agricultural activities and climate change. In this context, BRICS countries and Türkiye play a crucial role in global food security and economic development due to their diversity and capacity in agricultural production. However, agricultural expansion and intensive fertilizer use in the Amazon rainforest contribute significantly to CO₂ emissions (Galford, 2010). Russia, with its vast arable lands, is one of the most influential producers of wheat and barley. Nonetheless, increasing mechanization and changes in land use have elevated its agriculture-related emissions (Lambin et al., 2001; Guan et al., 2023). India, the world's most populous country, is a major producer of rice, wheat, and sugarcane. Its extensive use of fertilizers and diesel-powered irrigation pumps results in a significant carbon footprint (Pathak et al., 2014). Similarly, China relies heavily on mechanization and fertilizer use in its agricultural practices, particularly in the production of rice and maize, leading to substantial greenhouse gas emissions (Wang et al., 2022). Although South Africa has a smaller agricultural sector compared to other BRICS countries, it contributes to CO2 emissions through mechanization and fertilizer use while also facing environmental challenges such as land degradation and desertification (Nyambo et al., 2020). Türkiye, with its diverse agricultural sector encompassing cereals, fruits, and livestock, accounts for a significant share of national CO₂ emissions, exacerbated by mechanization and land-use changes (Evrendilek and Ertekin, 2002). The agricultural production in these countries not only serves as a source of food but also plays a vital role in economic development. For instance, Brazil's soybean exports and India's rice exports are critical components of their agricultural economies (Mekouar, 2021). However, intensive farming practices aimed at meeting the food demands of growing populations contribute to environmental degradation and increased CO2 emissions, underscoring the need for sustainable agricultural solutions (Tilman et al., 2011; Bhattacharyya et al., 2021; Bhatia et al., 2022). Policies that promote conservation tillage, precision agriculture, and the use of renewable energy are of paramount importance in this regard (Pretty and Bharucha, 2014).

Adopting sustainable practices in the agricultural sectors of the BRICS countries and Türkiye is essential not only for maintaining economic growth but also for reducing the agricultural carbon footprint. Such efforts represent a significant step toward achieving sustainable development goals. When examining the relationship between agricultural production and climate change, as well as the impact of agriculture on greenhouse gas emissions, the BRICS countries emerge as a key group to consider (Tian et al., 2020). Countries such as Brazil, China, and India are global leaders in agricultural production. Including a country like Türkiye, which ranks among the top ten globally, creates a valuable sample for exploring the relationship between agricultural activities and climate change. In this context, BRICS countries and Türkiye play a crucial role in global food security and economic development due to their diversity and capacity in agricultural production.

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In addition to examining the scope and potential of agricultural production, this study provides a detailed analysis of how the agricultural sector in BRICS countries and Türkiye affects carbon dioxide CO2 emissions with the aim of mitigating climate change impacts arising from agricultural activities. Drawing upon data from the 1961-2019 period for the six countries in question, the study evaluates the effects of such variables as CO₂ emissions, the level of mechanization in agriculture, the total size of agricultural land, the share of rural population in total population, and the amount of synthetic fertilizer used in agriculture. By employing the panel ARDL(Autoregressive Distributed Lag) method, both short-run and long-run effects of these variables on CO₂ emissions were identified, thus presenting a comprehensive overview of the dynamics of agriculture-driven emissions over both the short and long term. Additionally, panel causality analysis was conducted to determine the direction of the relationship between agricultural sector variables and CO₂ emissions, enabling a deeper understanding of the nature and scope of their mutual interactions. With its comprehensive dataset spanning nearly sixty years for BRICS countries and Türkiye, the study made a significant contribution to the literature. In particular, the assessments of agriculture's environmental impacts offer valuable insights for developing policy recommendations related to sustainable growth targets and climate change mitigation. Moreover, the analysis of short-run and long-run coefficients helps identify strategic measures to reduce the agricultural sector's effects on the climate, while the causality findings provide critical information about the direction of the long-term relationship between these variables.

Literature review

Agricultural production accounts for approximately one-quarter of total anthropogenic emissions worldwide, making it a significant contributor to greenhouse gas (GHG) emissions (Tubiello et al., 2013; Güler et al., 2019; Malgaz and Atalay, 2022). The BRICS countries and Türkiye, with their diverse agricultural sectors, are no exception. Agricultural practices in these regions often involve extensive land-use changes that may lead to increased emissions (Bennetzen et al., 2016; Cousins et al., 2018; Rahman et al., 2021; Apergis et al., 2023). Furthermore, the intensification of agricultural activities—particularly fertilizer use—contributes significantly to GHG emissions. Since the production of chemical fertilizers predominantly relies on fossil fuels, it generates GHG emissions both during manufacturing and throughout their application (Rosa and Gabrielli, 2022; Ouikhalfan et al., 2022; Çam, 2023; Liu et al., 2023). In particular, the production of nitrogen-based fertilizers leads to the release of nitrous oxide (N₂O), a potent greenhouse gas, thereby amplifying the impact of agricultural activities on climate change (Zemanek et al., 2020; Tubiello et al., 2021; Fu et al., 2021; Gyedu and Tang, 2024). In this context, the BRICS countries and Türkiye contribute to climate change through the heavy use of fertilizers (Asgharipour et al., 2016; Cam, 2024).

The expansion of agricultural land frequently results in the degradation of natural ecosystems that function as carbon sinks. Rapid urbanization and industrialization in the BRICS countries have driven extensive land-use changes, further complicating the relationship between agriculture and CO_2 emissions (Haseeb et al., 2017; Parajuli et al., 2019). For instance, in China, the conversion of farmlands for urban development has led to higher emissions from both the agricultural and construction sectors (Shafiq and Zafar, 2023; Liu et al., 2023). This situation exacerbates the environmental impacts of agricultural expansion, creating major challenges in efforts to combat climate change (Baloch et al., 2019). Similarly, deforestation for agricultural expansion in Brazil has been a key driver of CO_2 emissions, underscoring the growing need for sustainable land management practices (Chel and Kaushik, 2011; Tian et al., 2020). Consequently, adopting sustainable practices in the agricultural production processes of the BRICS countries and particularly Türkiye is critical, not only for reducing environmental impacts but also for ensuring food security (Kara et al., 2021).

Another factor accelerating climate change is the use of agricultural machinery and technology (Raghutla and Chittedi, 2020). Dependence on fossil fuels for machinery, transportation, and processing increases the carbon footprint of agricultural activities (Tukhtamurodov, 2024). In the BRICS countries, energy consumption in

agriculture is closely tied to economic growth, with higher energy use often associated with rising emissions (Saidmamatov et al., 2023). This link is especially evident in nations such as China and India, where rapid economic development has led to heightened energy demands in the agricultural sector (He et al., 2020). The interconnections among agriculture, energy consumption, and CO₂ emissions grow even more complex when considering technological innovations. Advances in agricultural technology can potentially reduce emissions by enhancing production efficiency (Atasel et al., 2022). However, the adoption of such technologies varies across the BRICS countries and Türkiye, influenced by factors such as economic development, infrastructure, and policy frameworks (Chishti and Sinha, 2022). While some countries have made significant strides in implementing sustainable agriculture, others continue to rely heavily on traditional methods that lead to higher emissions (Nazir et al., 2024; Ozdemir, 2024). In addition to technological factors, the economic context of the BRICS countries and Türkiye plays a pivotal role in shaping agricultural practices and their environmental consequences (Tilman et al., 2011; Nnaji and Ogboghro, 2024). Economic growth often results in increased agricultural output, which if not managed sustainably—can drive higher CO₂ emissions (Tan, 2023). Balancing economic development with environmental sustainability is particularly challenging in regions where agriculture is a primary economic driver (Mandimby, 2024). Policymakers must therefore consider the long-term effects of agricultural activities on CO₂ emissions and climate change, promoting strategies that improve efficiency while minimizing environmental impacts.

METHODS

PMG-ARDL Method

In the analysis that includes BRICS countries and Türkiye, the relationship among CO₂, agricultural machinery usage, chemical fertilizer usage, the share of agricultural land in total land area, and rural population was estimated using the PMG-ARDL method (Appiah et al., 2018; Attiaoui and Boufateh, 2019). The amount of carbon emissions is expressed as follows:

$$CO_{2it} = f(MKN_{it}, GBR_{it}, ALN_{it}, AGDP_{it}, KNFS_{it})$$
 (1)

where MKN denotes the number of machines per 100 km² of agricultural land; GBR is the total amount of chemical fertilizer used per hectare; ALN represents the share of land used for agriculture in the total land area; AGD refers to the share of agricultural production in GDP; and KNFS indicates the share of the rural population in the total population. Finally, the dependent variable CO₂ denotes the total amount of carbon dioxide (in billion tons) emitted by a given country in one year.

$$CO_{2it} = \alpha_i + \sum_{j=1}^{p} \theta_{ij} CO_{2it-j} + \sum_{j=1}^{q} \rho_{ij} X_{it-j} + \epsilon_{it}$$
 (2)

Here, p and q are the lag orders of the explanatory variables. α_i is the intercept term, while ϵ_{it} represents the error term. X_{it} is the matrix of explanatory variables specified in Equation (1). The general form of the panel ARDL model is presented above. However, the cointegration relationship among variables were identified using the error correction model. In this context, an error correction coefficient is lying between -1 and 0 and statistically significant indicates a long-term relationship among the variables (Pesaran et al., 1999, 2001).

$$\Delta CO_{2it} = \alpha_i + \gamma_i (CO_{2it-1} - \emptyset_i X_{it-1}) + \sum_{j=1}^{p-1} \theta_{ij} \Delta CO_{2it-j} + \sum_{j=1}^{q-1} \rho_{ij} \Delta X_{it-j} + \epsilon_{it} \quad (3)$$

In this equation, Δ denotes the difference operator. θ_{ij} and ρ_{ij} are short-term coefficient vectors, while \emptyset_i is the vector of long-term coefficients. Following the appropriate coefficient adjustments, the model below is obtained:

$$\Delta CO_{2it} = \varphi_0 + \varphi_1 \sum_{i=1}^n \Delta CO_{2it-1} + \varphi_2 \sum_{i=0}^p \Delta X_{it-1} + \epsilon_{it}$$
 (4)

In this equation, p and n represent the lag lengths of the explanatory variables, and φ_1 is the error correction term.

Dumitrescu and Hurlin Causality Test

The Dumitrescu and Hurlin (2012) Causality Test is particularly effective in detecting causal relationships among variables in panel data sets with a large time dimension. Although it is especially successful for high-dimensional panel data, it also generates efficient results in smaller panel data sets through its test statistics. Dumitrescu and Hurlin (2012) define the causality relationship as follows:

$$Y_{i,t} = a_i + \sum_{k=1}^{m} \partial_i^{(k)} Y_{i,t-k} + \sum_{k=1}^{n} \beta_i^{(k)} X_{i,t-k} + \varepsilon_{i,t}$$
 (5)

Here, $Y_{i,t}$ is the dependent variable, $X_{i,t}$ represents the explanatory variables, both of which were assumed to be stationary, and $\varepsilon_{i,t}$ is the error term that has a zero mean and constant variance. The test uses the null hypothesis stating that all β_i coefficients are equal to zero against the alternative hypothesis that at least one of the β_i coefficients is nonzero:

$$H_0: \beta_i = 0$$
 $\forall_i = 1, ..., N$
 $H_1: \beta_i \neq 0$ $\forall_i = 1, ..., N$

Dumitrescu and Hurlin (2012) demonstrate the power of this method by producing semi-asymptotic test statistics, given by:

$$Z_{N,T}^{Hnc} = \sqrt{\frac{N}{2K}} \left(W_{N,T}^{HnC} - K \right) \tag{6}$$

$$Z_{N,T}^{Hnc} = \sqrt{\frac{N}{2K}} (W_{N,T}^{Hnc} - K)$$

$$Z_{N,T}^{Hnc} = \frac{\sqrt{N} [W_{N,T}^{Hnc} - N^{-1} \sum_{i=1}^{N} E(W_{i,T})]}{\sqrt{N^{-1}} \sum_{i=1}^{N} \text{Var}(W_{i,T})}$$
(6)

Both test statistics follow a chi-square distribution for panel data sets with a large time dimension, providing robust results (Çelik and Ünsür, 2020).

DATA and ANALYSIS

In this analysis, the following variables for the BRICS countries and Türkiye were employed for the 1961-2019 period: CO₂ emissions which used as dependent variable, the share of agricultural production in total output, the proportion of land used for agriculture within total land area, the share of the rural population, the number of agricultural machines per 100 km², and the amount of fertilizer used per hectare (kg) which utilized as explanatory variables. The observation period was set to 1961-2019 because data for some variables were not available for 2020 and thereafter. As part of the econometric analysis, a cross-sectional dependence test and a unit root test were performed on the data. Cross-sectional dependence is crucial in determining which unit root test should be employed and which econometric model is most suitable. When cross-sectional dependence is absent, firstgeneration panel unit root tests produce reliable results; however, if dependence is present, second-generation unit root tests should be applied. The results from the unit root tests, contingent on the degree of stationarity within the data, directly guide the selection of an appropriate econometric model. Econometric models generally assume that variables are stationary over time. Nonetheless, flexible models such as panel ARDL allow the dependent variable to be stationary at first difference (I(1)), while permitting explanatory variables to be stationary at different orders (I(0) or I(1)), provided that the dependent variable meets this requirement. In this regard, assumptions about the dependent variable play a decisive role in model selection. Consequently, the panel ARDL model stands out as a preferred method because it can accommodate variables exhibiting stationarity at different levels and simultaneously estimate both long-term and short-term relationships.

Table 1. Summary Statistics

Variable	Unit	Mean	Standard Deviation	Minimum	Maximum
CO_2	Metric tonnes	20.039	1.317	16.669	23.095
MKN	Machines used in 100 km ²	0.800	1.442	0.007	7.904
GBR	Per hectare /kg	4.018	1.078	0.742	6.013
ALN	Percentage	46.575	21.899	13.155	83.534
AGDP	Percentage	14.732	13.066	1.927	53.326
KNFS	Percentage	48.873	20.330	13.176	83.292

Note: The data were obtained from Our World in Data (2024) (access: https://ourworldindata.org/) and the World Bank databases (2024).

Table 1 presents the summary statistics for the variables used in the analysis. It can be observed that the average carbon emissions in the BRICS countries and Türkiye amount to approximately 20 billion tons. During the sample period, the minimum carbon emissions for these six countries were calculated as 16.669 billion tons, whereas the maximum value reached 23.095 billion tons. Another crucial variable potentially related to carbon emissions is the number of tractors per 100 km² (MKN), which averages 0.8. The minimum value of this variable is 0.007, while its maximum is 7.904 tractors. Another indicator of agricultural capacity, the amount of fertilizer used per hectare (GBR), averages 4.018 kg across the BRICS countries and Türkiye between 1961 and 2019, with the maximum fertilizer application reaching 6.013 per hectare/kg during this period. ALN, a critical indicator of land use, represents the share of agricultural land within total land area. Accordingly, on average, 46.57% of total land in Türkiye and the BRICS countries is allocated to agriculture, varying between 13.155% and 83.534% over the period in question. AGDP reflects the share of agricultural output in total gross domestic product (GDP), indicating the economic importance of agriculture. For the countries analyzed, this rate averages 14.732%, with a maximum value of 53.326%. In addition, KNFS, which denotes the proportion of rural population within the total population, shows that on average, 48.873% of the populations in the BRICS countries and Türkiye reside in rural areas. Over

the sample period, the rural population share ranges from 13% to 83%. These findings demonstrate that the BRICS countries and Türkiye hold significant environmental and agricultural importance, and that agriculture remains a cornerstone of their economic and social structures.

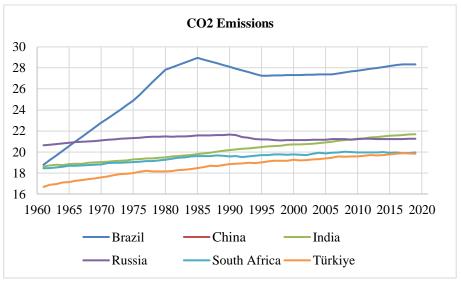


Figure 1. CO₂ Emissions

Figure 1 illustrated the total CO_2 emissions of the BRICS countries and Türkiye from 1961 to 2019. According to this figure, Russia exhibits the most stable trajectory in terms of CO_2 emissions. While Russia's CO_2 emissions remained relatively flat until the late 20th century, they entered a downward trend thereafter. Over the same period, carbon emissions in Türkiye and India have shown a continuous upward trend. South Africa has maintained relatively consistent emissions, whereas China's emission trajectory is similar to that of Türkiye and India.

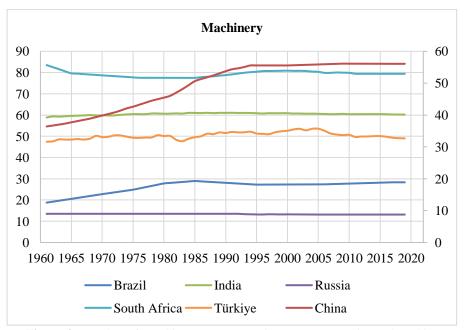


Figure 2. Number of Machines per Country in BRICS Countries and Türkiye

According to Figure 2, the agricultural lands of these countries have generally remained stable, except in the case of Brazil. China and Russia, particularly between 1960 and 1985, experienced a substantial increase in the utilization of agricultural land. In both countries, this trend has leveled off over the past thirty years.

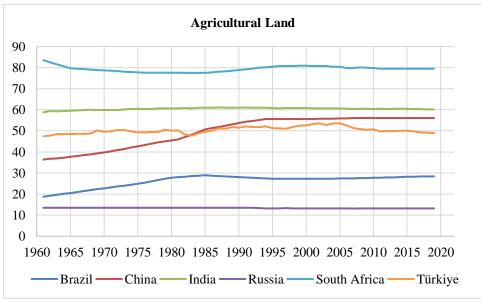


Figure 3. Agricultural Lands in BRICS Countries and Türkiye

Figure 3 shows the number of tractors and agricultural machines per 100 square kilometers. South Africa has exhibited almost no progress in agricultural mechanization (Logarithms of the variables have been taken to remove the unit effect.). By contrast, India and China have made the greatest strides in increasing the use of agricultural machinery. During this period, Türkiye has also shown significant progress.

Table 2. Cross-Sectional Dependence Test (Pesaran, 2004; Pesaran, 2015)

Variable	Pesaran (2004)		Pesaran (2015)	
v arrable	CD-test	P value	CD-Test	P Value
CO ₂	19.640	0.0000	25.245	0.0000
MKN	14.130	0.0000	12.437	0.0000
GBR	17.990	0.0000	21.238	0.0000
ALN	7.950	0.0000	12.76	0.0000
AGDP	24.660	0.0000	12.759	0.0000
KNFS	24.410	0.0000	12.444	0.0000

The cross-sectional dependencies of the variables were examined using the tests proposed by Pesaran (2004) and Pesaran (2015). The results presented in Table 2 indicate that all variables exhibit cross-sectional dependence. Given the presence of cross-sectional dependence, a second-generation panel unit root test was employed to determine whether any of the variables including the dependent variable contain a unit root.

Table 3. CIPS Unit Root Test

	Pesaran (2	007) Panel Unit Roc	ot test (CIPS)		
Variable	Lag	Intercept	Prob.	Intercept & Trend	Prob.
CO ₂	1	-1.056	0.1450	1.231	0.8910
MKN	2	-0.492	0.3110	-0.591	0.2770
GBR	1	-3.165	0.0010	-2.708	0.0030
ALN	1	-1.198	0.1160	0.637	0.7380
AGDP	1	-3.818	0.0000	-2.768	0.0030
KNFS	1	-2.413	0.0080	0.894	0.8140
D. CO ₂	1	-10.727	0.0000	-10.201	0.0000
D.(MKN)	1	-9.697	0.0000	-8.946	0.0000
D.(ALN)	1	-4.013	0.0000	-3.579	0.0000

Note: The D operator indicates the first difference of the variable.

Table 3 presents the results of the CIPS (Cross-Sectionally Augmented IPS) unit root test proposed by Pesaran (2007), which accounts for cross-sectional dependence. This test examines the stationarity of variables in panel data by considering inter-unit dependence and is estimated under two different specifications: one with only an intercept and one with both an intercept and a trend. Rejecting the null hypothesis in either model is sufficient to

conclude that a variable is stationary. The primary hypothesis of the CIPS test is "The variable contains a unit root (Ho)," and failing to reject this hypothesis indicates that the variable is non-stationary. The analysis determined that the dependent variable, CO2 contains a unit root. For the model with only an intercept, the test statistic was calculated as -1.056; for the model with both an intercept and a trend, it was 1.231. In both cases, the results indicate that the CO2 variable is not stationary at its level. Because the application of the panel ARDL model requires the dependent variable to be stationary at the first difference, the first difference of CO2 (D. CO2) was taken. In both the intercept-only and intercept-and-trend models, the test statistics exceeded the critical values and had p-values less than 0.10, confirming that the variable is stationary at the I(1) level. In addition, the stationarity analysis for the other variables showed that while MKN contains a unit root at its level, it becomes stationary after difference once. Similarly, ALN is non-stationary at its level but achieves stationarity after taking the first difference. By contrast, the remaining variables appear to be stationary at the I(0) level. These findings suggest the existence of a long-term relationship between the dependent variable CO2 and the explanatory variables, indicating that the necessary preconditions for estimating a panel ARDL model are met. Consequently, the results confirm that the data are suitable for a long-term relationship analysis, taking into account the stationarity levels of the variables.

Table 4. ARDL Model Coefficient Estimates

Long-Run Coefficients	Coefficient	Std. Error	Z-Value	Prob.
MKN	0.1146	0.061	1.880	0.0600
GBR	0.2626	0.058	4.500	0.0000
ALN	-0.0071	0.104	-0.070	0.9460
AGDP	-0.3049	0.076	-3.990	0.0000
KNFS	-0.3126	0.093	-3.340	0.0010
Short-Run Coefficients	Coefficient	Std. Error	Z-Value	Prob.
ECT (-1)	-0.1358	0.042	-3.210	0.0010
CO_2	0.0016	0.001	1.800	0.0720
MKN	-0.0799	0.089	-0.900	0.3700
GBR	-0.0069	0.004	-1.920	0.0550
ALN	-0.1511	0.405	-0.370	0.7090
AGDP	1.9561	1.639	1.190	0.2330
KNFS	2.7735	0.847	3.270	0.0010
Constant	2.7735	0.847	3.270	0.0010

Table 4 provides a detailed overview of both the short-run and long-run coefficients of the panel ARDL model, as well as an assessment of the long-run relationship among variables via the error correction term (ECT). The error correction term signifies the speed at which the system returns to its long-run equilibrium, and its being negative and statistically significant indicates the presence of a long-term relationship. According to the results presented in the table, the estimated ECT is -0.1358 and is statistically significant. This finding suggests that approximately 13% of the impact of a shock to CO_2 emissions dissipates within one year. However, the complete elimination of the shock's effect requires about 7.5 years, implying that the reversion to equilibrium in economic systems following changes in CO_2 emissions is a time-consuming process, and that environmental policies can have long-term impacts.

Regarding the long-run coefficients, there is a strong positive and statistically significant relationship between agricultural machinery usage and CO₂ emissions. This outcome can be attributed to the predominant reliance of agricultural machinery on fossil fuels, one of the largest sources of anthropogenic greenhouse gas emissions. Based on the estimated coefficients, an increase of one agricultural machine per 100 km2 of land leads to an approximate 0.1146-unit rise in CO₂ emissions. While the use of agricultural machinery enhances production efficiency, it also raises environmental costs at a corresponding rate. Chemical fertilizer usage has a similarly positive effect on CO₂ emissions. The production and consumption of chemical fertilizers contribute to the release of harmful gases, in part because their manufacturing process heavily relies on fossil fuels, such as natural gas—one of the primary inputs in synthetic fertilizer production and a major source of greenhouse gas emissions. The estimated coefficients show that an increase of one kilogram of fertilizer per hectare leads to an average annual rise of 0.2626 units in CO₂ emissions. These results highlight the importance of environmentally friendly agricultural practices and sustainable fertilizer usage policies. Although the coefficient for agricultural land use is negative, it is not statistically significant, indicating no discernible long-run relationship with CO₂ emissions. Theoretically, agricultural land use might be expected to increase CO2 emissions, since tilling the soil can release stored greenhouse gases, and expanding farmland generally reduces green areas. However, the lack of significance in this study may stem from regional differences, data limitations, or other confounding factors. On the other hand, a negative long-run relationship was found between agricultural production and CO₂ emissions. In the context of the BRICS countries and Türkiye, agricultural production appears to be comparatively more environmentally friendly. The findings suggest that a 1% increase in the share of agricultural production in GDP reduces CO_2 emissions by 0.3% in the long run. This outcome underscores the need to promote sustainable agricultural policies and the adoption of green technologies.

Lastly, a significant negative relationship was identified between the share of the rural population in total population and CO_2 emissions. According to the estimated coefficients, a 1% increase in the rural population reduces CO_2 emissions by 0.31% in the long run. This finding suggests that a rural lifestyle exerts a more limited environmental impact compared to urban life. Given that urban populations typically generate greater greenhouse gas emissions due to higher energy consumption, greater reliance on public transportation, and more intensive industrial activities, the favorable effect of a larger rural population on CO_2 emissions becomes understandable. Taken collectively, these findings highlight the substantial role of economic structures, energy consumption, and environmental policies in determining CO_2 emissions. In particular, promoting sustainable practices in the agricultural sector and supporting rural development policies provide meaningful insights for environmental sustainability.

Table 5. Dumitrescu and Hurlin (2012) Granger Causality Test

Dumitrescu and Hurlin (2012) Granger Causality Test										
	MKN	Prob.	GBR	Prob.	AGDP	Prob.	KRNFS	Prob.	ALN	Prob.
Z-bar	10.498	0.0000	19.988	0.0000	0.870	0.3844	12.544	0.0000	3.191	0.0014
Z-bar Tilde	2.016	0.0439	4.519	0.0000	-0.524	0.6002	2.555	0.0106	0.088	0.9298

The Dumitrescu and Hurlin (2012) Granger Causality Test was applied using two different test statistics to evaluate the relationship between CO₂ emissions and the explanatory variables. The results offer important insights into these causality relationships. First, the findings indicate that agricultural machinery usage is a Granger cause of CO₂ emissions. In other words, agricultural machinery contributes significantly to greenhouse gas emissions, and this effect runs from machinery usage to CO₂ emissions. In another relationship, the impact of fertilizer usage on CO2 emissions was examined, and the null hypothesis was rejected under both test statistics, implying that fertilizer usage leads to changes in CO2 emissions. These results underscore the importance of fertilizer usage as a factor increasing greenhouse gas emissions in the BRICS countries and Türkiye. Similarly, in line with the PMG-ARDL model, a meaningful relationship was identified between the rural population and CO₂ emissions, showing that CO₂ emissions are the Granger cause of the rural population (Table 5). This finding reveals that life and economic activities in rural areas make a notable contribution to CO2 emissions. On the other hand, the effect of agricultural land area and agricultural production on CO₂ emissions was not statistically supported. While the Dumitrescu and Hurlin (2012) Granger Causality Test indicates that one of the test statistics for agricultural land is significant, overall it suggests that the causality relationship between agricultural land, agricultural production, and CO₂ emissions is inconclusive. These findings emphasize the complex interplay between CO₂ emissions and various agricultural activities, calling for further research to explore these relationships in greater depth.

CONCLUSION

BRICS countries and Türkiye hold a significant place in global agricultural production, playing a critical role in feeding a growing population, ensuring food security, and combating hunger. Although the agricultural sector is a key driver of economic development, it also has substantial implications for environmental sustainability and efforts to address climate change. This study, which examines the impact of agricultural activities on CO₂ emissions, sheds light on both long-term dynamics and causality relationships, offering important findings regarding their environmental and economic dimensions.

The PMG-ARDL model analysis indicates that agricultural mechanization and chemical fertilizer usage increase CO_2 emissions. This result highlights the direct environmental impacts of agricultural technologies and inputs. On the other hand, agricultural production and the rural population were found to reduce CO_2 emissions, suggesting that the agricultural sector can contribute to low-carbon growth. The lack of a significant long-term effect of expanding agricultural land on CO_2 emissions reflects the complexity of environmental impacts associated with land use.

Findings from the Dumitrescu and Hurlin (2012) Granger Causality Test reveal that machinery usage, fertilizer consumption, and the rural population have a causal influence on CO_2 emissions, whereas agricultural production and agricultural land do not appear to be causes of CO_2 emissions. Taken as a whole, these results underscore the need for a balanced approach to managing agricultural activities in terms of both economic development and environmental sustainability.

The adverse effects of agricultural mechanization and chemical fertilizer usage on CO₂ emissions underscore the necessity of supporting these practices with more environmentally friendly technologies. Improving agricultural technologies sustainably can simultaneously reduce carbon emissions and enhance agricultural productivity. In this context, policies such as using renewable energy sources for agricultural machinery and promoting organic fertilizers instead of chemical ones could be implemented. Additionally, the emissions-reducing

impact of agricultural production shows that this sector can be further developed through the right support policies. Encouraging environmentally friendly agricultural practices would contribute to rural development while reducing the carbon footprint. Educational and awareness campaigns aimed at increasing the environmental consciousness of the rural population, as well as integrating local communities into sustainability initiatives through rural development projects, are important in this regard.

The absence of a clear long-term effect of expanding farmland on CO₂ emissions suggests that land-use policies should focus more on quality rather than quantity. By rehabilitating unproductive farmland or using more efficient production methods on existing agricultural lands, environmental impacts can be minimized.

In conclusion, this study provides a valuable framework for understanding the environmental and economic dimensions of agricultural production in BRICS countries and Türkiye, and for guiding this sector toward a sustainable future with the right policies. Such policies must support economic development while also aiming to reduce carbon emissions. In particular, for nations striving to meet their carbon emissions targets by 2050, advancing mechanization in agriculture while transitioning to cleaner energy sources is crucial to achieving netzero production goals. Although fertilizer application is essential for agricultural productivity, due to its environmental impacts, natural farming practices and reduced fertilizer use are key to lowering carbon emissions. Finally, meeting the social and cultural needs of the rural population—thus preventing overcrowding in urban centers—appears critical for controlling carbon emissions and ensuring a sustainable ecology.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

TK designed the study and performed it. Wrote the paper and reviewed it.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.18

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 157-165

Determination of yield and quality characteristics of some sugar beet (Beta vulgaris L.) varieties by different analytical methods

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Article History

Received: January 29, 2025 Revised: March 7, 2025 Accepted: March 10, 2025 Published Online: March 12, 2025

Article Info

Article Type: Research Article Article Subject: Sustainable Agricultural Development

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Available at

tps://dergipark.org.tr/jaefs/issue/90253/1629041

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Abstract

Sugar beet is known globally as one of the most important sources of sucrose. Sugar beet, which provides raw materials to many industries, creating an important employment opportunity in the regions where it is cultivated. In this study, yield and quality parameters of eight different sugar beet varieties were determined by different analytical methods. The experiment was carried out in 2024 at the experimental field of the Faculty of Applied Sciences, Muş Alparslan University, utilizing a randomized block design with three replications. Following a seven-month vegetative period, yield and quality analysis of the harvested beets were carried out, allowing for the determination of relationships between variety and traits. Statistically significant and important differences were found among the sugar beet varieties in terms of the parameters analyzed. Notably, the Lamberta variety came to the forefront in terms of storage root yield parameters (root weight, root length, single plant weight). Consequently, this variety displayed the highest average root yield compared to other varieties. While the Agatella variety demonstrated high averages for dry matter content and polar sugar content, it exhibited lower storage root and sugar yields. These findings suggest a negative correlation between sugar content and storage root yield and sugar yield. Overall, the Lamberta variety stood out in terms of root yield, while the Annamira variety stood out in terms of sugar yield. As a result of the research, sugar beet varieties varied between root diameters of 9.11-15.41 cm, root lengths of 15.34-18.43 cm, root weights of 646-2892 g, dry matter content of 20.87-24.40%, polar sugar content of 16.68-19.41%, root yields of 5196-8229 kg/da, and sugar yields of 908-1348 kg/da. According to the "which-where-won" model of GGE biplot analysis, the studied traits were clustered under 3 mega environments.

Keywords: Sugar beet, Yield traits, GGE biplot, ANOVA, Beta vulgaris L.

Cite this article as: Baran, N., Yalinkilic, N.A. (2025). Determination of yield and quality characteristics of some sugar beet (Beta vulgaris L.) varieties by different analytical methods. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 157-165. https://doi.org/10.31015/2025.1.18

INTRODUCTION

Sugar beet is a root crop cultivated in temperate climates and known as a source of sucrose. Sucrose accumulates most abundantly in the transversely expanding root of vegetatively mature plants. Sugar beet (Beta vulgaris L.) is a plant belonging to the genus Beta in the Caryophyllales order of the Amaranthaceae family. Sugar beet possess either a diploid (2n=2x=18) and triploid (2n=3x=27) chromosome structure. Most of the hybrid varieties grown economically have diploid or triploid chromosome structure (Peto and Boyes, 1940). Most currently cultivated varieties of foutcrossed sugar beet are hybrids developed through cytoplasmic male sterility (CSM) (Mikami et al., 2011).

The continuous growth in the world population and advancements in technology have elevated the importance of sugar as key food product. Globally, sugar cane and sugar beet are the primary plant-based sources of sucrose. Sugar beet, which serves as a raw material for many industries due to its versatile applications, is a strategic crop in the agricultural systems of approximately 52 countries in the world (Stevanato, 2019). It is generally planted in the spring season, and its vegetative period varies between 5-9 months, depending on regional ecological factors (Zicari et al., 2019). Roots of sugar beet are rich in carbohydrates and hemicellulose, as well as having a high soluble sugar content. The pulp produced from the roots after sugar extraction is used as a valuable animal feed (Cardenaz-Fernandez et al., 2017). Molasses is also used in the production of bioethanol, vinegar, ethyl alcohol, sourdough, pharmaceuticals, and cosmetics (Pavlečić et al., 2010; Šantek et al., 2010; Yalınkılıç et al., 2024). The readily fermentable sucrose content in sugar beet facilitates the fermentation of various products (Gunes et al., 2004).

In Türkiye, sugar beet has an important place in the agricultural systems. It contributes both to the agricultural industry and animal nutrition with its by-products. Approximately 19 million tons of sugar beet production is carried out on an area of 3 million ha (TUIK, 2023). The key provinces with the highest production are Konya, Yozgat, and Kayseri, respectively (Anonymous, 2023). A primary objective of sugar beet farming is to maximize root yield per unit area. Additionally, achieving high sugar content in the roots is crucial for successful sugar beet production (Yalinkilic et al., 2024). Identifying varieties that exhibit high root yield and sugar content and are well-suited to the region's ecological conditions is crucial for meeting the demands of both producers and sugar factories (Ozcan, 1993).

In scientific studies, visual representation of the performance of examined traits or genotypes is crucial, and it provides valuable insights into genotype characteristics (Yan and Tinker, 2006; Baran, 2025). In recent years, GGE biplot analysis has been known as one of the most important methods for the visual interpretation of bidirectional data (Akcura, 2011; Andırman & Baran 2023).

This study was carried out to compare the yield and quality traits of eight genetic monogerm sugar beet varieties using different statistical methods and to determine the varieties with superior performance in terms of the traits examined.

MATERIALS AND METHODS

This study was carried out at the Research and Experimental field of the Faculty of Applied Sciences, Muş Alparslan University, in 2024 with three replications according to the randomized blocks experimental design. Soil properties of the experimental area are given in Table 1.

Table 1. Soil properties of the experimental area

Depth (cm)	Composition Class	In Water Saturated Soil EC	In Water Saturated Soil pH	Lime (%)	Organic Matter	Available Phosphorus
	Class	(dSm ⁻¹)	Saturated Son pri	(,0)	(%)	(P ₂ O ₅) (kg da ⁻¹)
0-30	Clay-loamy	0.61	7.76	1.61	2.21	2.20

The soil in the test area have a clayey-loamy texture. The pH value is 6.61, the available phosphorus is 2.20 kg/da, and organic matter content is 2.21% (Table 1).

The study used genetic monogerm sugar beet varieties such as Orthega, Preziosa, Allanya, Agatella, Lamberta, Annamira, Ludmilla and Anchana. These varieties were developed by KWS company in Germany and are widely used in Türkiye. The general characteristics of the sugar beet varieties constituting the plant material of the study are given in Table 2.

Table 2. Characteristics of sugar beet varieties used as material in the experiment

Variety Name	Origin	Scientific Name	Distinguishing feature
Orthega	Germany	Beta vulgaris L.	Suitable for machine harvesting. High root and sugar yield.
Preziosa	Germany	Beta vulgaris L.	It has very high root and sugar yield.
Allanya	Germany	Beta vulgaris L.	It is a variety with very high root and sugar yield.
Agatella	Germany	Beta vulgaris L.	It has high sugar content.
Lamberta	Germany	Beta vulgaris L.	It is easy to uproot as it is smooth shaped and has high root yield.
Annamira	Germany	Beta vulgaris L.	It has high root yield and good sugar content.
Ludmilla	Germany	Beta vulgaris L.	It has high polar sugar content and is suitable for machine harvesting.
Anchana	Germany	Beta vulgaris L.	Suitable for machine harvesting. It has high root yield.

The trial area was plowed about 25-30 cm with a plow in the fall season and the necessary field preparations were completed in the spring. Sowing began on April 18 for the 2024 sugar beet growing season, and harvesting was completed on October 20. The study, which was carried out in three replications using a random blocks experimental design, the distance between rows was kept into 20 cm intervals and the distance between rows into 45 cm intervals. The length of each plot, consisting of four rows was designed as 4 meters and the distance between the blocks was designed as 2 meters intervals. In the experiment, 25 kg of compound beet fertilizer (13-18-15+2MgO+10SO3+ME) was applied per decare as base fertilizer, and the other part of nitrogen was applied as 25 kg per decare at the second plowing. Necessary maintenance procedures were applied to the experimental area

throughout the growing season. During the harvest period, observations were taken from the middle two rows of the plots. Root weight (g), root length (cm), root diameter (cm), single plant weight (g) (root + leaf) of twenty randomly selected plants were measured. Root yield was calculated in kg/ha by cutting the leaves of the plants in each plot, cleaning the roots, and weighing their weights. Sugar yield was calculated by multiplying root yield and sugar content and dividing the result by 100 (Ozceylan, 1986). After 20 beet roots taken randomly from each plot were cleaned and ground, the sample beet juice was cooled to 20 °C and dry matter ratios were calculated as Brix in a refractometer. For the determination of polar sugar content, beet samples were pulped according to the cold digestion method, and samples weighing approximately 24-26 g were mixed in 178.2 ml of 0.3% aluminum sulfate solution, then filtered and read on a polarimeter (Kavas and Leblebici, 2004).

Climate data for 2024 are given in Table 3. Rainfall after sowing sugar beet seeds is an important factor for seed germination and plant growth. When the sugar beet growing period of the region is examined, it is seen that the highest rainfall occurs in April and May. Rainfall in April and May meets the early water needs of the plant and does not require farmers to irrigate additionally. Monthly mean air temperature during the sugar beet growing season was similar to the long-term annual mean temperature. In this respect, the highest temperatures occurred in July and August. In addition, the average temperature being around 10 $^{\circ}$ C in April had a positive effect on the germination of sugar beet.

Table 3. Meteorological data of Mus province for the 2024 season

Months	Average Precipitation	n (mm)	Average Temp	erature (°C)	Average Relative	Humidity (%)
	Years			Years		Years
	1964-2024	2024	1964-2024	2024	1964-2024	2024
	(Multi-year)		(Multi-year)		(Multi-year)	
April	101.6	48.8	9.3	11.6	62.1	54.9
May	69.9	126.4	14.8	13.5	58.7	64.3
June	26.5	6.4	20.2	21.1	45.2	43.4
July	7.6	7.2	25.0	25.8	33.9	23.2
August	5.6	0.4	25.1	26.4	30.9	17.3
September	15.6	40.2	20.2	21.4	35.5	25.6
October	62.5	43.8	12.8	15.2	56.0	45.4
November	87.4	61.4	4.8	6.7	68.1	73.7
Total	376.7	334.6	-	-	-	-

Source: General Directorate of Meteorology -2024

The data obtained from the study were analyzed using JMP (13.0.1 pro) and the GenStat statistical package program (GenStat 2009), and the results were interpreted by two-way ANOVA and GGE biplot models. The groups and inter-group differences among cultivars for the traits analyzed in the study were evaluated according to the LSD multiple comparison test ($p \le 0.01$ and $p \le 0.05$).

RESULTS AND DISCUSSION

The results of the analysis of variance of the sugar beet varieties used as material in the study are given in Table 4. It was determined that there were statistically significant differences between the varieties in terms of the traits examined in the study, but there was no significant difference between the replications.

Table 4. Analysis of Variance (Mean Squares) of sugar beet varieties for the examined traits

SV	DF	RD	RL	RW	SPW	DMR	PSR	RY	SY
Variety	7	41.6648**	3.59869*	1621576*	1900358*	6.49**	3.0298**	3583508**	93621**
				*	*				
Repetition	2	11.32928	6.86620	8230	1641	3.21682	1.74174	4810	584.7
Error	14	0.43652	0.22831	2686	8326	0.33786	0.19175	3529	2436
Total	23								
CV (%)		0.833	2.796	2.876	4.497	2.633	2.460	0.886	4.326

CV: Coefficient of Variation, **DF**: Degree of Freedom, **SV**: Coefficient of Change, **RD**: Root Diameter, **RL**: Root Length, **RW**: Root Weight, **SPW**: Single Plant Weight, **DMR**: Dry Matter Ratio, **PSR**: Polar Sugar Ratio, **RY**: Root Yield, **SY**: Sugar Yield, *,**: Significant at 5% and 1% level, respectively.

The mean values and groups of yield and yield characteristics of sugar beet varieties are given in Table 5 and Table 6. Sugar beet varieties showed statistically significant differences from each other in terms of all traits examined. When the performances of the varieties were evaluated in terms of root diameter, it was determined that this value varied between 15.41 cm and 8.40 cm and Annamira was the superior variety in terms of root diameter (Table 5). In our study, the varieties with high root diameter also stood out in terms of root yield (Table 6) Hoffmann (2017) reported that there is a close and significant relationship between root diameter and yield parameters in sugar beet and that it is possible to estimate yield by looking at root diameter. Okut and Yildirim (2004) stated that root diameter trait is one of the important developmental criteria for sugar beet and this trait can

be affected by ecological factors, cultivation technique and variety differences. When previous studies on the subject were evaluated, Sahiner and Demir (2020) reported that root diameter varied between 11.95 cm and 12.63 cm, Altunbay (2014) between 10.59 cm and 8.76 cm, Catal and Akinerdem (2013) between 7.4 cm and 8.5 cm, Kulan et al. (2013) between 12.37 cm and 10.93 cm. The root length values of sugar beet varieties varied between 15.34 cm and 18.43 cm and the Lamberta variety showed the highest value in terms of this trait. Preziosa and Agatella varieties had the lowest root length values. Tosun (2014) reported that the root length value among sugar beet varieties varied between 24-20.3 cm; Ozbay (2018) reported that root length values ranged between 21.8-6.5 cm. In the studies on the subject, many researchers stated that root length can be affected by both environmental factors and the genetic structure of the variety used (Leducke, 1956; Hozayn et al., 2013).

Table 5. Mean values and groups of agronomic characteristics of sugar beet varieties

Varieties	Root diameter (cm)	Root length (cm)	Root weight (g)	Single plant weight (g)
Orthega	11.23 ^D	17.54 ^B	1438 ^E	1638 ^E
Preziosa	9.11 ^F	15.34 ^C	949 ^F	1413 ^F
Allanya	10.41^{E}	17.48^{B}	1449 ^E	1688^{E}
Agatella	8.40^{G}	15.45 ^C	646^{G}	772 ^G
Lamberta	14.28^{B}	18.43 ^A	2892^{A}	3200^{A}
Annamira	15.41 ^A	17.48^{B}	2032^{D}	2244^{D}
Ludmilla	12.34 ^C	17.39 ^B	2486^{B}	2797^{B}
Anchana	14.30^{B}	17.58 ^B	2221 ^C	2473 ^C
LSD _(0.05)	0.309	0.836	42.317	52.507

Values with different letters indicate significant groups at 5% significant level.

Statistically significant differences were determined among the sugar beet varieties in terms of root weight and the varieties were distributed in different groups in this respect. The highest value was obtained from the Lamberta variety with 2892 g, followed by the Ludmilla and Anchana varieties with 2486 g and 2221 g, respectively. The lowest root weight value was obtained from Agatella (646 g). Root weight in sugar beet has a significant effect on root yield per unit area (Sklenar et al., 1998). In the study, Agatella and Preziosa varieties were weaker than other varieties in terms of root diameter, root length and root weight. It is seen in Table 4 that the cultivars that stood out in terms of root weight and root length also had high values in terms of root weight. This supports the results of the researchers (Benjamin and Sutherland 1989; Badiu et al., 1996; Tsialtas and Maltaris, 2010) who argued that root diameter and root length have a significant effect on root weight. In similar studies on the subject, Sanli et al. (2015) reported that root weight varied between 790 and 693.3 g; Sanghera et al. (2016) reported that there were significant differences between varieties in terms of root weight and this value varied between 1630 g and 820 g; Fasahat et al. (2021) reported that the average root weight among sugar beet genotypes was 898.8 g.

Table 5 shows that there were statistically significant differences among sugar beet varieties in terms of single plant weight (leaf + root). The highest value was obtained from the Lamberta variety, followed by the Ludmilla and Anchana varieties, respectively. The lowest value for single plant weight was obtained from the Agatella variety. The same table shows that single plant weight varied between 772 g and 3200 g, with significant variations among the varieties for this trait. Basalak and Karadoğan (2022) reported that the highest leaf weight was 1055.7 kg/da, root weight was 6947.0 kg/da, biological weight was 7896.3 kg/da and polar sugar yield was 1248.0 kg/da.

Table 6. Mean values and groups of yield and sugar content of sugar beet varieties

Varieties	Dry matter content (%)	Polar sugar content (%)	Root yield (kg/da)	Sugar yield (kg/da)		
Orthega	20.87 ^E	16.68 ^C	6304 ^D	1069 ^{DE}		
Preziosa	22.82 ^{BC}	17.99 ^B	5393^{E}	987^{EF}		
Allanya	21.61 ^{DE}	16.97 ^C	6305^{D}	$1044^{\rm E}$		
Agatella	24.40^{A}	19.41 ^A	5196 ^F	908^{F}		
Lamberta	21.55^{DE}	17.42 ^{BC}	8229 ^A	1296^{B}		
Annamira	22.69^{BC}	$17.84^{\rm B}$	7621 ^B	1348 ^A		
Ludmilla	21.31 ^{DE}	16.91 ^C	7225 ^C	1144 ^{CD}		
Anchana	22.32 ^{CD}	17.91 ^B	7157 ^C	1229 ^{BC}		
LSD _(0.05)	1.016	0.766	48.505	40.305		

Values with different letters indicate significant groups at 5% level.

Sugar beet roots contain an average of 22-24% dry matter, of which 75% is sugar, 25% is water-insoluble cell wall compounds and 5% is non-sugar compounds (Hoffmann, 2005). In the study, the dry matter content of the varieties varied between 24.40% and 20.87% and the Agatella variety stood out in terms of this feature (Table 6). However, the varieties were distributed in different groups in terms of dry matter content and the Orthega variety

was behind the other varieties with the lowest dry matter content. In the studies conducted on the subject, Sahiner (2020) reported that dry matter content varied between 20.89-23.20%, Rashidi and Abbasi (2011) reported 20.3-23.9%, and Altunbay (2014) reported 21.52-23.96%. Lauer (1995) stated that there is a positive correlation between dry matter content and sugar yield and that beets with high dry matter content also stand out in terms of sugar yield.

The sugar content of sugar beet roots is the most important factor determining the economic value of the plant (Xiao et al., 2021). Sugar beet yield and sugar content are mainly affected by genotype, ecological factors and growing conditions (irrigation, fertilization) (Xie et al., 2022). In this study, polar sugar content values of sugar beet varieties varied between 16.68% and 19.04%. In addition, the Annamira variety showed the highest performance in terms of sugar content, while the Orthega variety was weaker than the other varieties in terms of the aforementioned trait (Table 5). It was noteworthy that varieties with high dry matter content also had high polar sugar content. Agatella and the Annamira varieties had higher values in terms of both dry matter content and polar sugar content compared to other varieties. Azam Jah et al. (2003) reported that polar sugar content varied between 14.4% and 15.8% among sugar beet genotypes, while El-Karouri and El-Rayah (2006) reported that sugar beet varieties had polar sugar content between 12.0% and 15.7%.

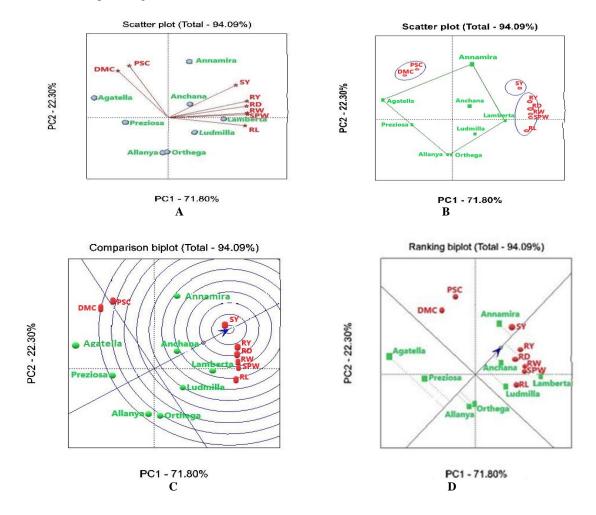


Figure 1- A, B, C, D. Genotype-Trait Relationships of Different Sugar Beet Varieties. RD: Root Diameter (cm), RL: Root Length (cm), RW: Root Weight (g), SPW: Single Plant Weight (g), DMC: Dry Matter Content (%), PSC: Polar Sugar Content (%), RY:Root Yield (kg/da), SY: Sugar Yield (kg/da).

There was a statistically significant difference between sugar beet varieties in terms of root yield (Table 5). The root yields of sugar beet varieties varied between 5192 and 8229 kg/da and it was determined that there was a wide variation among the varieties in terms of this trait. The variety with the highest root yield was Lamberta, followed by Annamira, Ludmilla, and Anchana varieties. The lowest value in terms of the aforementioned trait was obtained from the Agatella variety. Sugar yield values of the varieties varied between 908 and 1448 kg/da. The variety with the highest sugar yield was Annamira, while Lamberta ranked second. Agatella variety lagged behind the other varieties in terms of sugar yield. Sugar yield and root yield are two of the most important parameters for growers (Hoffmann et al., 2009). When the studies conducted by researchers on the subject were

examined, Rychcik and Zawiślak (2002) stated that root yield varied between 5880 and 6090 kg/da among sugar beet varieties; El-Karouri and El-Rayah (2006) stated that it was between 7150 and 8100 kg/da. Erciyes et al. (2016) stated that there is a strong relationship between root yield and sugar yield and the main objective of sugar beet farming is to obtain a high sugar yield from a unit area. Şanlı et al. (2023) stated in their study with seven sugar beet varieties in Isparta that root yield varied between 6680-9745 kg/da and polar sugar ratios varied between 14.5-18.6%. Genotypes with high root yield and sugar content also have high sugar yields per unit area (Hassani et al., 2018). Hoberg et al. (2016) emphasized in their study that environmental factors have a significant effect on sugar yield.

Through the biplot technique, the relationships between genotypes and traits can be examined with graphs obtained from mean values from different angles. The GT biplot plot shows the relationship between two traits, the relationship of one trait with other traits, or the relationship of genotypes with each other according to the traits using the angles between the trait vectors (Yan et al., 2000; Yan and Tinker, 2006; Baran et al., 2022). In this study, the performance of eight different sugar beet varieties in terms of the traits examined were presented with biplots.

According to the scatter biplot method, PC1 (1st principal component) accounted for 71.80%, PC2 (2nd principal component) accounted for 22.30% and 94.09% of the total variation. Figure 1-A graph visualizes the relationship between the sugar beet varieties included in the study and the yield and quality traits of these varieties. In the graph, as the angle view between the vectors representing the traits narrows, positive and high correlation is indicated, and as the angle view widens, weak correlation is indicated. In this case, it can be said that most of the yield and yield parameters have positive and high correlation. In addition, a strong and positive correlation was observed between dry matter content and polar sugar content, while a weak correlation was observed between polar sugar content and root yield. Genotypes positioned near some traits represent good results according to the parameters they are positioned. In this context, the Agatella variety stood out in terms of dry matter content and polar sugar content, while the Lamberta variety showed high performance in terms of root diameter, root weight, single plant weight and root yield. The Annamira variety showed high performance in terms of root yield and sugar yield. In Figure 1-B, the genotypes showing the highest values for one or more traits were identified by using the polygon view of the biplot. Thus, the sugar beet varieties in the center of each sector represent the variety or varieties with the highest performance in that sector and related traits. In the study, the biplot was divided into four sectors. It is seen that the Lamberta variety, located in the middle of the second and third sectors, represents high averages in terms of yield and yield characteristics. In addition, the Agatella variety in the fourth sector stood out in terms of quality characteristics such as dry matter content and polar sugar content. With the comparison biplot created over the average data, it was tried to determine the suitability of sugar beet varieties according to the ideal center. In the graph, the area in the coordinate plane indicated by the blue arrow is accepted as the center point. In this direction, the center indicated by the blue arrow is the most ideal region. Varieties can be categorized according to their distance and proximity to this region (Mohammadi, 2019). Thus, it is seen that the Anchana variety is located in the ideal center. This shows that the Anchana variety exhibits high performance in terms of the parameters examined in the study. In addition, the stability of the varieties in terms of all traits with the Ranking biplot graph is presented in Figure 1-D. The varieties Anchana and Annamira were the closest to the stability line drawn representatively and these varieties were more stable than the other varieties in terms of all the traits examined. Many researchers have evaluated the performance of sugar beet varieties and genotypes through biplot analysis (Hassani et al., 2018; Mostafavi et al., 2018; Taleghani et al., 2023; Abu-Ellail et al., 2024).

CONCLUSION

Statistically, the sugar beet varieties used as material in the study differed significantly from one another in terms of the traits examined. These differences were presented with graphs created by the GGE biplot method. By GGE biplot analysis, the eight traits examined in the study were divided into three mega clusters. One of these clusters included polar sugar content and dry matter content, the second cluster included sugar yield, and the third cluster included root diameter, root length, root weight, single plant weight, and root yield. In the study, when the projections of the varieties according to the point where they were located were evaluated, it was determined that the Agatella variety showed superior performance in terms of polar sugar ratio and dry matter ratio, the Annamira variety showed superior performance in terms of sugar yield, and the Lamberta variety showed superior performance in terms of storage yield and root yield. As a result, it was concluded that GGE biplot graphs will be useful in determining the performance of varieties in terms of the traits examined and in selecting suitable varieties for a particular trait. In addition, it would be more useful to expand the scope of similar studies by conducting them in multiple years and in different locations.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no conflict of interest.

Author contribution

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

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.19

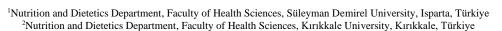
Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 166-173

Changes in the nutritional status of health care workers during and after the **COVID-19** pandemic

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Article History

Received: February 5, 2025 Revised: March 7, 2025 Accepted: March 10, 2025 Published Online: March 12, 2025

Article Info

Article Type: Research Article Article Subject: Food Sciences

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Available at

ttps://dergipark.org.tr/jaefs/issue/90253/1633507







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Abstract

This study examined the changes in the nutritional status of healthcare workers during and after the COVID-19 pandemic. The sample of this descriptive prospective study consisted of 597 volunteer healthcare workers in Isparta, who were selected by a random sampling method. The questionnaire comprised questions aimed at determining the healthcare workers' general information and nutritional status. Nutritional changes were assessed using a scale that ranged from "I eat less than usual" to "I eat more than usual" and "No change" on a chart containing 21 food items. The Beck Depression Inventory (BDI) was used to determine the emotional state of the healthcare workers. The statistical significance level was accepted as 0.05. BDI scores of healthcare workers were analysed, the depression status was severe during COVID-19 but decreased to mild-moderate levels after COVID-19 (p<0.001). The mean water consumption of healthcare workers after COVID-19 was higher than that occurred during COVID-19 (p<0.05). It was observed that the use of nutritional supplement by healthcare workers after COVID-19 was lower than that occurred during COVID-19. This rate decreased after COVID-19 (p<0.001). It was determined that there was a statistical decrease in the consumption of red meat, fish, meat products, honey, molasses, jam, chocolate and candy, pastries, cakes, cookies, fast food, carbonated drinks and energy drinks by healthcare workers after the COVID-19 pandemic (p<0.001). Although the COVID-19 pandemic has increased the nutritional awareness of healthcare workers and led them to eat healthy, factors such as intense working conditions, feelings of insecurity against COVID-19, and stress have negatively affected their nutritional habits.

Keywords: COVID-19 pandemic, Healthcare workers, Nutrition

Cite this article as: Baygut, H., Cakir, B. (2025). Changes in the nutritional status of health care workers during and after the COVID-19 pandemic. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 166-173. https://doi.org/10.31015/2025.1.19

INTRODUCTION

The new type of coronavirus causing respiratory tract infection (SARS-Cov-2) pandemic, which was declared as 'COVID-19' pandemic by the World Health Organisation on February 11, 2020, started in China and affected the whole world (Şeker, et al., 2020). The global emergency ended on May 5, 2023 and it was reported that there were more than 767 million confirmed cases and more than 6.9 million deaths in the world as of 2023 (WHO, 2023).

The COVID-19 pandemic, which deeply affected healthcare systems and societies in many countries, led to an increase in psychological distress and a decrease in quality of life, especially in healthcare workers (Epifanio, et al., 2023). During the pandemic, healthcare workers confronted a myriad of challenges, including substantial workloads, profound fatigue, the potential for personal infection or the transmission to loved ones, the distressing experiences of colleagues' sickness or death, and the loss of numerous patients. Despite these formidable circumstances, healthcare workers endeavoured to manage the psychological ramifications of these distressing circumstances while maintaining their professional obligations (Mehta, et al., 2021). Furthermore, challenges such as the inability to consume nourishment, drink water, and use the toilet were encountered by healthcare workers

to mitigate the risk of infection during periods of high workload, which made this process even more difficult for healthcare workers (Polat and Coşkun., 2020).

Nutritional and other lifestyle factors have been identified as modifiable contributors to depression. However, research has indicated that depression can increase susceptibility to both overnutrition and malnutrition by influencing the quantity and quality of food intake (Esquivel, 2021). Despite the prevalence of health recommendations, including the adoption of a nutritionally balanced diet, maintenance of a healthy body weight, engagement in regular physical activity, and adequate sleep, individuals experiencing high food insecurity during the pandemic placed a higher priority on acquiring sustenance, rather than on the prevention of starvation. Consequently, individuals frequently purchased processed, long-shelf-life, and cost-effective foods, despite the potential for restricting access to essential foods and beverages (Naja and Hamadeh., 2020; Oliveira, et al., 2020). Healthy lifestyle behaviours such as maintaining healthy eating habits and participating in physical activity have been associated with higher quality of life among healthcare workers and indirectly affect the improvement of quality of care and professional performance (Shenkman, et al., 2023). The COVID-19 pandemic had profound effects on the psychological health of healthcare workers and caused nutritional problems as a result of working under intense stress (Oliveira, et al., 2020).

In this study, it was aimed to examine the changes in the nutritional status of healthcare workers during and after the COVID-19 pandemic.

MATERIALS AND METHODS

This descriptive prospective study comprised 597 volunteer healthcare workers working in Isparta, who were selected by a random sampling method. As a result of the statistical G*power analysis, it was determined that at least 594 healthcare workers should be included in the study within the 95% confidence interval. Healthcare workers included in the study during the COVID-19 pandemic were reached also after the COVID-19 pandemic (at least three months after the end of the pandemic) and the same questionnaire was applied face-to-face again. The Ethics Committee approval was obtained and The Helsinki Declaration Protocols (World Medical Association) were followed in the study. Before starting the study, healthcare workers were informed about the study, and a written consent form was obtained from those who voluntarily wanted to participate in the study. Healthcare workers employed in departments not involved in the management of the pandemic were excluded from the study.

The questionnaire form consisted of questions to determine the general information (marital status, disease status, and smoking status) and nutritional status of healthcare workers. Nutritional changes were evaluated using a scale that ranged from 'I eat less than usual' to 'I eat more than usual' and 'No change' on a chart including 21 food items. The Beck Depression Inventory (BDI), which was developed by Beck et al. in 1988 and the validity and reliability of which were established by Ulusoy et al. in 1998, was applied to determine the emotional state of healthcare workers. The BDI, which was used to determine the frequency of depression symptoms experienced by healthcare workers, is a self-report Likert-type scale consisting of 21 items. The items in the scale are scored between 0 and 3 (0=none, 1=mild, 2=moderate, and 3=severe). Severity is evaluated as '0-9=minimal', '10-16=mild', '17-29=moderate', and '30-63=severe' (Beck, et al., 1988; Ulusoy, et al., 1998).

The data were analysed using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA). In data evaluation, the Dependent Sample T Test was used to determine whether there was a difference between water consumption measurements during and after COVID-19, and the McNemar Test was used to determine whether there was a difference between dietary patterns during and after COVID-19. The statistical significance level was accepted as 0.05.

RESULTS AND DISCUSSION

It was observed that there was a statistically significant difference in the disease status, smoking status, and BDI scores of healthcare workers during and after COVID-19 (p<0.05). While there was no significant change in the disease rates of healthcare workers after COVID-19 compared to the disease rates during COVID-19, smoking rate in women decreased after COVID-19. When the BDI scores of healthcare workers were analysed, the depression status was severe during COVID-19 but decreased to mild-moderate levels after COVID-19 (Table 1).

The impact of the COVID-19 pandemic on the health of the general population and healthcare workers has been examined by various researchers (Umbetkulova, et al., 2024; Blasco-Belled, et al., 2022). In a systematic review of longitudinal studies by Umbetkulova et al. (2024), it was found that there the mental health of healthcare workers (women, young people, nurses, frontline workers, those with long working hours and those who were worried about catching the disease) declined over time in 12 publications out of 18, and in 6 studies, there was a positive trend (supportive environment, access to psychological resources, provision of adequate personal protective equipment and insomnia, etc.) associated with various mental health problems (anxiety, depression, insomnia, etc.) has been reported (Umbetkulova, et al., 2024). In addition to stress

factors and demographic variables during COVID-19, lifestyle factors such as nutrition, physical activity, smoking, and alcohol consumption have also been reported to be important for mental health (Jin, et al., 2024; Maffoni, et al., 2021). In this study, it was determined that the disease status, smoking status, and depression status of healthcare workers were affected during and after COVID-19, and there were significant changes in water consumption, nutritional supplement use status, reason for using nutritional supplement, and the number of snacks consumed. Nutrition, lack of physical activity, smoking, and excessive alcohol use are intervenable risk factors of chronic diseases (Hacker, 2024). In this study, when the presence of obesity, type 2 diabetes mellitus, hypertension, hyperlipidaemia, ulcer-gastritis, intestinal diseases, and iron deficiency anaemia related to nutrition of healthcare workers was questioned, it was observed that they all increased after the COVID-19 pandemic period compared to the COVID-19 period. In another study conducted during COVID-19, it was found that 80.5% of healthcare workers did not have any chronic diseases (Ertal, 2021). It was posited that the mental and physical problems experienced due to the COVID-19 pandemic increased the incidence of these diseases in healthcare workers.

Avoiding smoking and minimising stress are also recommended (Gençalp, 2020). In this study, it was found that smoking behaviour was affected during and after COVID-19 and smoking behaviour decreased in women (p<0.05). A body of research has indicated that the COVID-19 pandemic has exerted a disparate impact on smoking behaviour, manifesting as either an increase or a decrease in smoking prevalence among individuals (Bar-Zeev, et al., 2023; Papakala, et al., 2023). It was reported that there was an increase in the stress level of 59% of the healthcare workers during COVID-19 and this increase also increased the smoking rate and 35% of the thencurrent smokers smoked more cigarettes (Bar-Zeev, et al., 2023). In another study, it was reported that there was a significant decrease in the smoking habits of healthcare workers, and this decrease was significantly lower among those who experienced high/severe job-related burnout (Papakala, et al., 2023). It is imperative to raise awareness among healthcare workers and the general public regarding smoking cessation during pandemic processes.

Table 1. Distribution of Healthcare Workers' Disease Status, Smoking Status, and Beck Depression Inventory Scores During and After COVID-19.

	Female (n=467)			Male (n=13				Total (n=59				
	During COVID-19		After COVID-19			During COVID-19		After COVID-19		During COVID-19		After COVID-19	
	n	%	n	%	n	%	n	%	n	%	n	%	
Disease status													
No	350	74.9	271	58.0	106	81.5	77	59.2	456	76.4	348	58.3	
Obesity	31	6.6	88	18.8	15	11.5	39	30.0	46	7.7	127	21.3	
Ulcer - Gastritis	11	2.4	16	3.4	3	2.3	4	3.1	14	2.3	20	3.4	
Type 2 Diabetes	22	4.7	31	6.6	0	0.0	2	1.5	22	3.7	33	5.5	
Hypertension	12	2.6	13	2.8	0	0.0	0	0.0	12	2.0	13	2.2	
Anaemia	10	2.1	11	2.4	6	4.6	7	5.4	16	2.7	18	3.0	
Hyperlipidaemia	24	5.1	30	6.4	0	0.0	1	0.8	24	4.0	31	5.2	
Intestinal Diseases	7	1.5	7	1.5	0	0.0	0	0.0	7	1.2	7	1.2	
McNemar Test; p	0.000*				0.000	*			0.000	*			
Smoking Status													
No, I never smoked	95	20.3	96	20.6	39	30.0	39	30.0	134	22.4	135	22.6	
I used to smoke, I quit	88	18.8	107	22.9	34	26.2	35	26.9	122	20.4	142	23.8	
Yes, I still smoke	284	60.8	264	56.5	57	43.8	56	43.1	341	57.1	320	53.6	
McNemar Test; p	0.000*				0.564				0.000	*			
Beck Depression Invento	ory												
Minimal	0	0.0	33	7.1	0	0.0	8	6.2	0	0.0	41	6.9	
Mild	18	3.9	75	16.1	2	1.5	27	20.8	20	3.4	102	17.1	
Moderate	49	10.5	222	47.5	7	5.4	54	41.5	56	9.4	276	46.2	
Severe	400	85.7	137	29.3	121	93.1	41	31.5	521	87.3	178	29.8	
McNemar Test; p	0.000*				0.000	*			0.000	*			

^{*}p<0.001, p=Significance level

It was reported that there were significant psychological changes (sadness, distress, and irritability) associated with tobacco use and physical activity changes during the first quarantine at the beginning of the COVID-19 pandemic, and tobacco consumers experienced more psychological distress (sadness and stress) than their non-tobacco consuming colleagues (Mounir, et al., 2021). In this study, it was observed that while the depression status

was severe during COVID-19, which decreased to mild-moderate levels after COVID-19. In a study conducted in March-April 2020 at the beginning of the COVID-19 pandemic, it was reported that the health-related anxiety and depression scores of female healthcare workers were higher than those of male healthcare workers (Yıldırım, et al., 2020). In a study conducted by Kolcu and Başer Kolcu (2021) among medical students, it was determined that the participants experienced high levels of anxiety about the transmission of coronavirus to themselves and their family members (Kolcu and Başer Kolcu, 2021). The COVID-19 pandemic has negatively affected both the physical and mental health of healthcare workers and the hypothesis is that it has resulted in a substantial elevation in anxiety levels.

It was observed that there was a statistically significant difference in the water consumption, nutritional supplement use status, reason for using nutritional supplement, and the number of snacks consumed during and after COVID-19 (p<0.05). Accordingly, the mean water consumption of healthcare workers after COVID-19 was higher than that occurred during COVID-19 (p<0.05). It was observed that the use of nutritional supplement by healthcare workers after COVID-19 was lower than that occurred during COVID-19. This rate decreased after COVID-19. Regarding the number of snacks consumed by healthcare workers, while the rate of consuming 3 or more snacks was high during COVID-19, this rate decreased after COVID-19, but healthcare workers did not give up their 3-snack habits (Table 2).

Water consumption is an important element in adequate and balanced nutrition. In the stress management of frontline healthcare workers exposed to COVID-19, it was stated that adequate water consumption (at least 2.5 litres for men and at least 2 litres for women) should be consumed against thirst. Additionally, fruit juices without added sugar, tea, and fruits and vegetables with high water content are recommended as water sources (Maffoni, et al., 2021). In this study, water consumption of healthcare workers after COVID-19 was found to be higher than that occurred during COVID-19 (p<0.05).

It has been reported that the Mediterranean diet may prevent mood disorders and manage stress. In addition, intake of micronutrients, especially folate zinc, magnesium, and selenium, may prevent stress by positively affecting mood and mental health (Maffoni, et al., 2021). According to the United States Food and Drug Administration, a dietary supplement is a non-drug product designed to supplement the diet with one or more of vitamins, minerals, plants, and amino acids. Some dietary supplements may alter the absorption, metabolism, or excretion of a drug, so they should be used with caution (Kolcu and Başer Kolcu, 2021). In this study, it was observed that the use of dietary supplements by healthcare workers after COVID-19 was lower than their use during COVID-19 (Table 2). In a study, it was reported that 55.3% of healthcare workers personally used nutritional supplement during the pandemic, and vitamin C was the most commonly used nutritional supplement with a rate of 81.3% (FDA, 2024). This result suggested that healthcare workers utilised nutritional supplements to protect themselves against COVID-19.

In the case of COVID-19, it has been stated that the purpose of nutrition is to reduce infection and disease progression while improving the disease course; therefore, it is critical for healthcare workers to understand the role of nutrition to protect their health and reduce the risk of disease (Lee, et al., 2021). Skipping meals is not among the nutritional recommendations given for stress management in frontline healthcare workers exposed to COVID-19 (Maffoni, et al., 2021). In this study, it was determined that healthcare workers consumed at least 3 snacks during COVID-19, and it was observed that this number decreased after COVID-19.

It was observed that there was a statistically significant difference in the consumption rates of red meat, chicken and turkey, fish, meat products, eggs, legumes, bread, vegetables, fruits, honey, molasses, jam, chocolate and confectionery, sweets, pastries, cakes, cookies, fast food, coffee, carbonated drinks and energy drinks during and after COVID-19 (p<0.05). Accordingly, while bread and chicken/turkey consumption did not change during COVID-19, it increased after COVID-19. While fish consumption decreased during COVID-19, it decreased after COVID-19. While egg, vegetable, and fruit consumption decreased during COVID-19, this rate increased after COVID-19. Consumption of red meat, honey, molasses, jam, chocolate and confectionery, pastries, cakes, cookies, and fast food increased during COVID-19 and decreased after COVID-19. Dessert consumption increased during and after COVID-19. Consumption of legumes, coffee, carbonated drinks, and energy drinks did not change during COVID-19; however, it decreased after COVID-19 (Table 3).

A previous study indicated that daily nutrition exerts a significant influence on the health of healthcare workers, particularly during the intervention period when workload is elevated and physical and mental fatigue is prevalent. The study also noted the occurrence of certain nutritional concerns, including energy intake imbalances and excessive consumption of fat and salt in the meals provided to healthcare workers during the course of the pandemic (Jaggers, et al., 2020; Zhang, et al., 2020). Macronutrients (protein, carbohydrate, and lipid) and micronutrients (vitamins, minerals, bioactive peptides, and phytochemicals) that the body needs to maintain health and protect against diseases including COVID-19 should be taken through food (Zhang, et al., 2020). In this study, it was determined that there were statistically significant differences in the consumption of red meat, chicken and turkey, fish, meat products, eggs, legumes, bread, vegetables, fruits, honey, molasses, jam, chocolate and confectionery, sweets, pastries, cakes, cookies, fast food, coffee, carbonated drinks and energy drinks by healthcare workers during and after the COVID-19 pandemic (p<0.001). It was determined that there was a statistical decrease

in the consumption of red meat, fish, meat products, honey, molasses, jam, chocolate and candy, pastries, cakes, cookies, fast food, carbonated drinks and energy drinks by healthcare workers after the COVID-19 pandemic (p<0.001) (Table 3). In a study, it was found that there were negative changes in the food consumption of healthcare workers with a high level of burnout, whereas there were positive changes in food consumption frequencies in terms of healthy nutrition in those with moderate and lower level burnout (Chaari, et al., 2020). In another study, it was reported that 40.5% of individuals increased their consumption of cereals and 53% increased their consumption of fruits and vegetables (Erzurum Alim, et al., 2022). Adequate and balanced nutrition, which constitutes an optimally functioning immune system, when supported by healthy dietary choices and informed food choices, can contribute to the development of a better immune response to other pathogenic viruses and microorganisms. This, in turn, can offer benefits in the prevention of infection and complications from COVID-19.

Table 2. Distribution of Water Consumption, Nutritional Supplement Use, Reason for Nutritional Supplement Use, Number of Main Meals Consumed, and Number of Snacks Consumed by Healthcare Workers During and After COVID-19.

	Female				Male				Total				
	(n=467) During		After		(n=130 During	<u> </u>	After		(n=597) During		After		
	COVID-19 Mean Median		COVID-19 Mean Median		COVII Mean	0-19 Median	COVID-19 Mean Median		COVID Mean	0-19 Median	COVID-19 Mean Mediar		
	± SD	(Min- Max)	± SD	(Min- Max)	± SD	(Min- Max)	± SD	(Min- Max)	± SD	(Min- Max)	± SD	(Min- Max)	
Water	1542.	1600	1744.	1800	1476.	1400	1681.	1600	1528.3	1400	1730.	1600	
Consumption	1342. 61	(800-	33	(800-	92	(800-	1061. 54	(1000-	1328.3	(800-	65	(800-	
(ml/day)	±	2600)	±	2800)	# ±	3000)	±	3200)	±	3000)	±	3200)	
(IIII/day)	455.7	2000)	± 457.8	2000)	± 473.4	3000)	± 472.9	3200)	460.03	3000)	± 461.4	3200)	
	2		1		0		5		400.03		8		
T Test; p	0.000*		-		0.000*		<u> </u>		0.000*		0		
	n	%	n	%	n	%	n	%	n	%	n	%	
Use of nutrition	al suppl	ements											
Yes	441	94.4	158	33.8	121	93.1	48	36.9	562	94.1	206	34.5	
No	20	4.3	305	65.3	9	6.9	82	63.1	29	4.9	387	64.8	
Sometimes	6	1.3	4	0.9	0	0.0	0	0.0	6	1.0	4	0.7	
McNemar Test; p	0.000*				0.000*				0.000*				
Reason for using	g nutriti	ional suppl	ements										
To stay healthy	136	30.4	77	47.5	35	28.9	24	50.0	171	30.1	101	48.1	
To not feel tired	2	0.4	5	3.1	2	1.7	8	16.7	4	0.7	5	2.4	
For adequate and balanced nutrition	77	17.2	39	24.1	17	14.0	0	0.0	94	16.5	47	22.4	
To strengthen the immune system	232	51.9	41	25.3	67	55.4	16	33.3	299	52.6	57	27.1	
McNemar Test; p	0.000*				0.030**				0.000*				
Number of main	n meals	consumed											
1	41	8.8	38	8.1	18	13.8	18	13.8	59	9.9	56	9.4	
2	118	25.3	123	26.3	27	20.8	27	20.8	145	24.3	150	25.1	
<u>≥</u> 3	308	66.0	306	65.5	85	65.4	85	65.4	393	65.8	391	65.5	
McNemarTest	0.129				1.000				0.129				
; p													
Number of snac													
1	8	1.7	64	13.7	4	3.1	28	21.5	12	2.0	92	15.4	
2	88	18.8	147	31.5	25	19.2	40	30.8	113	18.9	187	31.3	
≥3	371	79.4	256	54.8	101	77.7	62	47.7	472	79.1	318	53.3	
McNemar Test; p	0.000*				0.000*				0.000*				

^{*}p<0.001, **p<0.05, p=Significance Level

Table 3. Dietary Changes Among Healthcare Workers During and After COVID-19.

	During COVID-19 (n=597))	After COVID-19 (n=597)						McNemar Test; p				
Nutritional changes	I'm eating	I'm eating more than ever		than			I'm eating more than ever		I'm less usual	eating than	No change		
Food Items	n	%	n	%	n	%	n	%	n	%	n	%	
Milk and dairy products	237	39.7	238	39.9	122	20.4	234	39.2	253	42.4	110	18.4	0.608
Red meat	579	97.0	6	1.0	12	2.0	438	73.4	132	22.1	27	4.5	0.000*
Chicken and Turkey	156	26.1	0	0.0	441	73.9	210	35.2	17	2.8	370	62.0	0.000*
Fish	30	5.0	18	3.0	549	92.0	30	5.0	67	11.2	500	83.8	0.000*
Meat products	594	99.5	0	0.0	3	0.5	373	62.5	132	22.1	92	15.4	0.000*
Egg	23	3.9	554	92.8	20	3.4	103	17.3	461	77.2	33	5.5	0.000*
Legumes	63	10.6	27	4.5	507	84.9	88	14.7	91	15.2	418	70.0	0.000^{*}
Bread	285	47.7	0	0.0	312	52.3	317	53.1	34	5.7	246	41.2	0.000^{*}
Cereals and Pasta	276	46.2	35	5.9	286	47.9	276	46.2	35	5.9	286	47.9	0.990
Vegetable	16	2.7	575	96.3	6	1.0	234	39.2	253	42.4	110	18.4	0.000^{*}
Fruit	142	23.8	422	70.7	33	5.5	438	73.4	132	22.1	27	4.5	0.000^{*}
Honey, molasses, jam	298	49.9	6	1.0	293	49.1	210	35.2	23	3.9	364	61.0	0.000*
Chocolate and confectionery	277	46.4	36	6.0	284	47.6	54	9.0	75	12.6	468	78.4	0.000*
Desserts (dairy, pastry)	280	46.9	54	9.0	263	44.1	373	62.5	145	24.3	79	13.2	0.000*
Pastries, cakes, cookies	272	45.6	72	12.1	253	42.4	129	21.6	433	72.5	35	5.9	0.000*
Fast-food	579	97.0	2	0.3	16	2.7	86	14.4	75	12.6	436	73.0	0.000*
Black tea	268	44.9	16	2.7	313	52.4	273	45.7	23	3.9	301	50.4	0.435
Herbal tea	321	53.8	276	46.2	0	0.0	321	53.8	276	46.2	0	0.0	1.000
Coffee	285	47.7	6	1.0	306	51.3	259	43.4	62	10.4	276	46.2	0.000^{*}
Carbonated drinks	164	27.5	69	11.6	364	61.0	145	24.3	133	22.3	319	53.4	0.000^{*}
Energy drinks	155	26.0	24	4.0	418	70.0	116	19.4	261	43.7	220	36.9	0.000^{*}

^{*}p<0.001, p=Significance level

CONCLUSION

The data obtained in this study show that the COVID-19 process affects the nutritional habits of healthcare workers. As a result, although the COVID-19 pandemic has increased the nutritional awareness of healthcare workers and led them to eat healthy, factors such as intense working conditions, feeling of insecurity against COVID-19, and stress have been observed to negatively affect their food preferences. Given the likelihood that perceptions of nutrition will be influenced during and following the post-pandemic period, it is imperative that healthcare workers and institutions implement suitable initiatives and forestall unfavourable alterations in nutritional attitudes by taking into account their requirements and available resources.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors certify that they have no conflicts of interest.

Author contribution

The manuscript is designed in the following manner: HB, BÇ; Acquisition of study data: HB, BÇ; Data analysis: HB, BÇ; Manuscript drafting: HB, BÇ; Content review: HB, BÇ; Version approval: HB, BÇ; Study design: HB, BÇ; Data collection: HB, BÇ; Data analysis: HB, BÇ; Draft preparation: HB, BÇ; Content review: HB, BÇ; Version approval: HB, BÇ.

Ethics committee approval

Ethical approvals and permissions were obtained in writing from Isparta Süleyman Demirel University Ethics Committee (decision 43/5 dated 24/6/2020), Ministry of Health Provincial Health Directorate (16657963-799 number dated 27/08/2020) and Süleyman Demirel University Research and Application Hospital Chief Physician (E-804.01 number dated 04/09/2020).

Acknowledgments

Informed consent and written permission for publication of the data were obtained from all healthcare workers involved in the study.

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International Journal of

Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.20

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 174-189

Effect of Gibberellic acid concentrations on physicochemical attributes and shelf life of different mango (Mangifera indica L.) varieties

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Article History

Received: January 31, 2025 Revised: March 7, 2025 Accepted: March 10, 2025 Published Online: March 12, 2025

Article Info

Article Type: Research Article Article Subject: Post Harvest Horticultural Technologies

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1630584







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Abstract

Mango (Mangifera indica), often referred to as the "king of fruits," is a staple of tropical fruit production, offering high economic and nutritional value. However, mangoes are highly perishable, facing challenges like significant post-harvest losses due to rapid physiological weight loss, reduced fruit firmness, and shortened shelf life. To address these issues, this study evaluated the effects of gibberellic acid (GA3) on the physical, chemical, and storage characteristics of mangoes, aiming to improve post-harvest quality and extend their marketability. The experiment, conducted at Girija Prasad Koirala College of Agriculture and Research Centre (GPCAR), used a Completely Randomized Design (CRD) with six GA3 treatments (0, 50, 100, 200, 300, and 400 ppm). Uniformly sized, newly harvested ripe mangoes were treated with GA3 solutions for 10 minutes, with parameters such as physiological weight loss, total soluble solids, pulp pH, fruit firmness, and titratable acidity assessed after three days. The results revealed that mangoes treated with 400 ppm GA3 had the lowest physiological weight loss (35.75%), highest fruit firmness (1.14), and longest shelf life, with the Maldah variety performing best. Future studies could focus on optimizing GA3 application for diverse mango varieties and explore its integration with advanced storage technologies to further reduce post-harvest losses and improve global mango supply chains.

Keywords: GA₃, Firmness, Ripening delay, Physiochemical traits, Mango shelf life

Cite this article as: Pandit, D.L., Mehata, D.K., Rukhsar, S., Lahutiya, V., Yadav, P.K., Shah, S.K., Timilsina, U. (2025). Effect of Gibberellic acid concentrations on physicochemical attributes and shelf life of different mango (*Mangifera indica* L.) varieties. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 174-189. https://doi.org/10.31015/2025.1.20

INTRODUCTION

Mango (Mangifera indica L.), belonging to the Anacardiaceae family, is one of the most important tropical and subtropical fruit crops grown worldwide (Fitmawati et al., 2017). It is often referred to as the "king of tropical fruits" due to its exceptional flavor, appealing color, pleasant aroma, and rich nutritional content. The fruit is highly valued for its dietary benefits, containing essential vitamins, minerals, and antioxidants. Mango originated in the Indo-Burma region and Southeast Asia, with 69 recognized species (Shankaraswamy et al., 2015). It is cultivated in over 100 countries, with more than 65 nations producing over 1,000 metric tonnes annually (Mitra, 2016). Asia is the dominant producer, contributing approximately 77% of the total global mango production, followed by the USA (13%) and Africa (10%), while Oceania and Europe contribute less than 1% each (FAO, 2012). Major mango-producing countries include India, China, Brazil, Indonesia, Mexico, Pakistan, Egypt, the Philippines, Thailand, and Vietnam. India stands as the largest mango producer, leading in both cultivation area and total production. In Nepal, mango is a high-value fruit crop cultivated in the Terai, inner Terai, and foothills, extending

up to 1100 meters above sea level. It ranks second in terms of total cultivated area and productivity. In the fiscal year 2016/17, Nepal produced 2,98,692 metric tonnes of mangoes across 48,204 hectares (MoAD, 2016/17). The main mango pocket areas in Nepal include Sarlahi (10 mt/ha), Mahottari (11 mt/ha), Dhanusha (11 mt/ha), Kapilvastu (9 mt/ha), Dang (6 mt/ha), Banke (9 mt/ha), and Bardiya (9 mt/ha) (MoAD, 2016/17).

Despite Nepal's potential for high mango production, postharvest losses and poor storage infrastructure lead to significant fruit spoilage annually. One of the major challenges in mango production is postharvest losses due to rapid ripening and short storage life. Mango is a climacteric fruit, meaning it undergoes a sharp rise in ethylene production after harvest, accelerating the ripening process (Singh, 2016). Due to its short shelf life, postharvest losses range from 25–40%, with 25% losses occurring between harvesting and consumption (Evans et al., 2017). The fast deterioration of mangoes reduces their commercial value, making storage and transportation challenging. Although refrigeration slows down the ripening process, mangoes are susceptible to chilling injury, which causes damage to the fruit tissues (Rathore et al., 2007). Mangoes are rich in essential nutrients, providing 64–86 kcal of energy per 100g, along with significant amounts of vitamin C (32–200 mg per 100g of pulp), carotenoids, and minerals like calcium and potassium (Bernardini et al., 2005). Vitamin C plays a crucial role in preventing degenerative diseases such as cancer and cardiovascular disorders. However, due to inadequate postharvest management, a substantial portion of mangoes are lost before reaching consumers. In developing countries, postharvest losses range from 20–50%, while developed nations report losses of up to 5–25% (Kumar et al., 2015).

Effective postharvest treatments, such as controlled atmosphere storage, plant growth regulators, and packaging techniques, are essential to extending shelf life and maintaining mango quality. Plant growth regulators (PGRs) are organic compounds that, even in minute quantities, influence plant growth and development. These include auxins, gibberellins, cytokinins, ethylene, growth retardants, and inhibitors. Gibberellins (GAs), particularly gibberellic acid (GA3), are diterpene acids known for their role in delaying fruit ripening and senescence. GA3 is widely used in agriculture as an endogenous plant growth regulator, although it is rarely produced in significant amounts by plants themselves. The application of GA3 has proven beneficial in preserving mango fruit quality and prolonging its shelf life. GA3 acts as a senescence-delaying agent by slowing the ripening process, reducing ethylene production, and delaying carotenoid synthesis (Lokesh et al., 2013). In papaya, GA3 treatment delays ripening by affecting sucrose metabolism and the breakdown of complex carbohydrates. Similarly, GA3 application in tomatoes has been found to reduce tissue permeability, leading to lower physiological weight loss and decay (Singh et al., 2014). These findings suggest that GA3 can be an effective tool in postharvest mango management. Gibberellic acid application after harvest plays a critical role in maintaining mango quality by reducing metabolic activity and weight loss. GA3 functions as an ethylene antagonist, preserving physiological and enzymatic activity while delaying ripening. It decreases respiration rate, inhibits ethylene synthesis, and slows fruit softening and color development. The reduction in tissue permeability also helps lower physiological losses in weight, contributing to an extended storage life. Several postharvest treatments are employed to improve mango storage, including wax emulsions, fungicides, polythene film, and chemical coatings (Munhuweyi et al., 2020). Among these, GA3 has emerged as a promising alternative due to its ability to modulate ripening and extend fruit shelf life naturally. The use of GA3 in mango storage reduces postharvest losses, maintains nutritional value, and enhances commercial viability.

The goal of this study is to ascertain the ideal concentration of GA3 to improve the shelf life, nutritional value, and postharvest preservation of mangos in storage.

MATERIALS AND METHODS

Experimental site

The study was carried out at G.P. Koirala College of Agriculture and Research Centre (GPCAR) in Gothgaun, Morang, Nepal, from June 16 to June 30, 2023. The experimental site is situated at 26.6806°N latitude and 87.35317°E longitude, as illustrated in the location map (Figure 1). Throughout the study period, environmental conditions were closely observed. The recorded maximum and minimum temperatures were 44.51°C and 24.45°C, respectively, while relative humidity fluctuated between 87.56% and 30.94%. These variations in climatic conditions are visually represented in Figure 2, which depicts the environmental changes recorded in the laboratory during the experiment. Together, Figures 1 and 2 offer a detailed overview of the study location and its climatic conditions, providing essential context for the research.

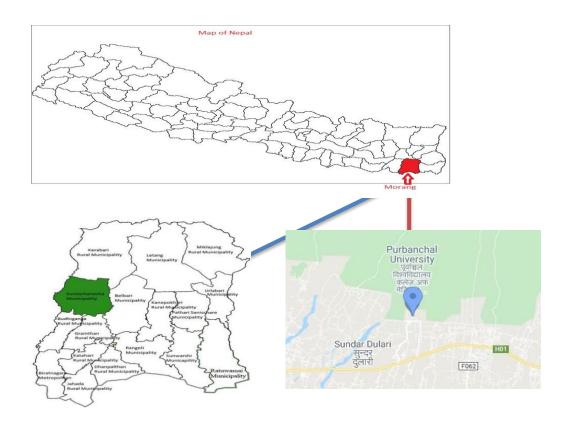


Figure 1. Map of study area

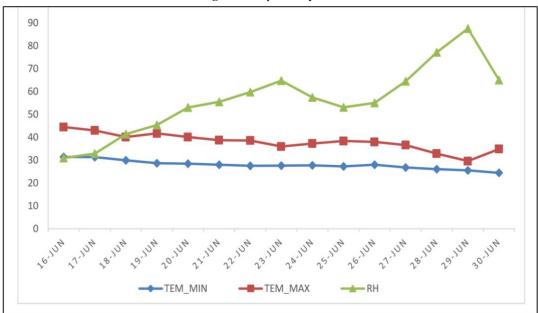


Figure 2. Geographical representation of meteorological data in laboratory.

Experimental design and treatment details

The experiment was conducted using a completely randomized design (CRD) to evaluate the effects of gibberellic acid (GA₃) on three mango varieties: Maldah, Bombay, and Dasheri. A two-factor factorial design was employed, incorporating six GA₃ treatment levels and three replications per treatment combination. Gibberellic acid solutions were prepared at concentrations of 50 ppm, 100 ppm, 200 ppm, 300 ppm, and 400 ppm by dissolving 250 mg, 500 mg, 1000 mg, 1500 mg, and 2000 mg of GA₃ in five liters of distilled water, respectively, while distilled water served as the control treatment. Mango fruits of uniform size and free from defects were first washed thoroughly with tap water, air-dried, and grouped by size before treatment. The fruits were then immersed in the respective GA₃ solutions for 10 minutes to ensure absorption, followed by air-drying on a clean laboratory surface. The mangoes treated were stored under ambient room conditions for further evaluation. The experiment involved three mango varieties and six GA₃ concentrations, arranged in a completely randomized design with three replications, leading to a total of 54 experimental units. Treatments were randomized within each replication to meet the assumptions of CRD, and observations were systematically recorded to analyze the post-treatment effects of GA₃ on mango shelf life and quality. Figure 3 illustrates the details of the mango varieties and treatment combinations used in the study.

Table 1. Experimental design with list of varieties and different concentrations of GA₃.

		Factor B	Factor B					
Factor A	Replication	1	2	3	4	5	6	Total A
	1	T111	T121	T131	T141	T151	T161	
1	2	T112	T122	T132	T142	T152	T162	T-1
1	3	T113	T123	T133	T143	T153	T163	T1
	Total AB	T11	T12	T13	T14	T15	T16	
	1	T211	T221	T231	T241	T251	T261	
2	2	T212	T222	T232	T242	T252	T262	T2
	3	T213	T223	T233	T243	T253	T263	12
	Total AB	T21	T22	T23	T24	T25	T26	
Total B		T1	T2	T3	T4	T5	T6	T

Factor A		Factor B	
S.N.	Varieties	S.N.	Concentration of gibberellic acid
		T1	Control/Distilled water
1	Maldah	T2	50 ppm of GA ₃
2	Bombay	T3	100 ppm of GA ₃
2 3	Dasheri	T4	200 ppm of GA ₃
3	Dashell	T5	300 ppm of GA ₃
		T6	400 ppm of GA ₃

Observations

A total of 54 experimental units were established, considering three replications, six GA₃ treatments, and three mango varieties. Each experimental unit consisted of five mango fruits. Among them, three mangoes were designated as destructive samples for assessing physicochemical parameters, including total soluble solids (TSS), titratable acidity (TA), pH, firmness, and palatability. The remaining two mangoes were maintained as non-destructive samples to evaluate shelf life and physiological loss of weight (PLW) at different observation intervals (6, 9, and 12 days after treatment).

Shelf life and Physiological loss

The shelf life of mango fruits was determined by monitoring the number of days required for them to reach an optimal ripening stage while maintaining their marketable and consumable quality. The calculation was performed based on the duration between the first day of storage after treatment and the last day when the fruits were considered edible (Hasan et al., 2020). The first day of storage after treatment was recorded as Day 0, and the fruits were observed daily for changes in physical appearance, texture, aroma, and overall quality. The percentage of deterioration was recorded for each treatment and replication. The shelf life was considered to have ended when more than 50% of the stored mangoes became unfit for consumption. The number of days from Day 0 to this stage was recorded as the shelf-life duration for that specific treatment. Similarly, at the same time, the physiological loss was calculated using the following formula from (Yadav et al., 2022), PLW, Physiological loss in weight, utilizing non-destructive sampling at three-day intervals.

$$PLW~(\%) = \frac{\text{Initial weight of fruits (IW)-Final weight of fruits (FW)}}{\text{Initial weight of fruits (IW)}} \times 100$$

Potential of Hydrogen (pH)

The pH of mango fruit juice was determined using a digital pH meter, an electronic device that measures hydrogen ion concentration to indicate acidity or alkalinity. Before measurement, the pH meter was calibrated with standard buffer solutions (pH 4.0 and 7.0) to ensure accuracy. It operates based on an electrochemical principle, where a glass electrode detects hydrogen ion activity in the solution and generates a voltage proportional to the H⁺ ion concentration. A reference electrode provides a stable comparison potential, ensuring precise readings. After calibration, the electrode was immersed in mango pulp juice, and the pH value was displayed on the digital screen.

Total Soluble Solids (TSS)

A digital refractometer was used to measure the total soluble solids (TSS) content of mango fruit pulp. A few drops of fresh mango juice were extracted using a clean pipette and placed on the refractometer's prism surface. The reading was recorded at room temperature, ensuring accuracy. After each measurement, the specimen chamber was thoroughly cleaned with distilled water and wiped using a fresh muslin cloth to prevent contamination. The digital refractometer works on the principle of light refraction, where the degree to which light bends as it passes through the sample correlates with the concentration of dissolved solids, primarily sugars. The instrument measures the refractive index and converts it into Brix (% TSS), which is displayed on the screen. Calibration was performed using distilled water (0% TSS) before measurements to ensure precision (Hasan et al., 2020; Yadav et al., 2022).

Titratable Acidity (TA)

By titrating diluted fruit juice (5 ml) against base 0.1 N NaOH solutions using 100 ml of distilled water and 5 drops of phenolphthalein indicator, titrable acidity was ascertained. The following formula (Yadav et al., 2022) was used to calculate the mango juice's TA.

$$TA \ (\%) = \frac{[ml \ of \ NaOH \ used] \times [0.1N \ NaOH] \times [Milliequivalent \ factor]}{Grams \ of \ sample \ used} \times 100$$
 Where,
$$TA, \ Titratable \ Acidity \\ N \ NaOH, \ Normality \ of \ NaOH$$

Milliequivalent factor of predominant acid (malic acid) = 0.0679

Firmness

Fruit firmness is a key quality trait influencing mango shelf life and marketability. In this study, firmness was measured using a handheld penetrometer (Model GY-3, No. 400102024) at 2-day intervals after treatment. The peak puncture force (g) was recorded from three points (top, bottom, and side) on each fruit, with the average considered as the actual firmness. This method helps assess the effect of gibberellic acid (GA_3) on fruit texture, providing insights into its role in delaying softening and extending shelf life.

Palatability

Palatability was evaluated based on color, odor, texture, touch, and taste using a sensory panel of five individuals. Each panelist scored the fruits on a scale from 0 to 5, with 0 indicating very poor and 5 representing excellent palatability. Assessments were conducted at 6, 9, and 12 days after treatment (DAT), and the average score from all panelists was used to determine the overall palatability rating, helping assess the impact of gibberellic acid (GA₃) on fruit quality and acceptability over time.

Statistical analysis

R studio (4.2.2 edition) was used to analyse the acquired data after it was imported into MS Excel. The Duncan Multiple Range Test (DMRT) was used as a statistical technique to compare the means of data for each parameter. Furthermore, the daewr, gvlma, and Agricolae packages in R studio programme (4.2.2 version) were utilised to analyse the interaction impact between the varieties and treatments. Tables and graphs were created using Microsoft Excel.

RESULTS

Effect of GA₃ on physiological weight loss

Variations in gibberellic acid (GA3) concentrations influenced the patterns of physiological weight loss in mangoes. On the twelfth day post-treatment, significant differences were observed in weight loss across different GA3 dosages. Among all treatments, the control group exhibited the highest physiological weight loss for each mango variety. By the end of the study, mangoes treated with 400 ppm GA3 showed the lowest weight loss (35.75%), whereas control fruits experienced the highest loss (61.19%). Tables 2, 3, and 4 present the detailed results. A consistent increase in weight loss was observed across all treatments as storage duration progressed. Fruits treated with 400 ppm GA3 retained the most weight, followed by those treated with 300 ppm, 200 ppm, 100 ppm, and 50 ppm, while control fruits lost the most weight throughout the 12-day storage period. Among the

mango varieties, Dasheri exhibited the highest weight loss (66.06%), followed by Bombay Green (48.08%) and Maldah (38.32%).

Table 2. Effect of different concentrations of gibberellic acids on physiological loss of mango varieties

Varieties		Physiological loss (%)	
	3 DAT (%)	6 DAT (%)	9 DAT (%)	12 DAT (%)
Dasheri	6.22a	15.94 ^a	31.61a	66.06 ^a
Bombay	4.55 ^b	11.60 ^b	22.96 ^b	48.08 ^b
Maldah	3.60°	9.25°	18.35°	38.32°
SEM (±)	0.036	0.093	0.185	0.392
LSD _{0.05}	0.104	0.268	0.531	1.125
F test	***	***	***	***
Treatments				
GA3 ₀	5.37 ^a	14.25 ^a	28.64ª	61.19 ^a
GA3 ₅₀	5.01 ^b	13.41 ^b	26.74 ^b	59.43 ^b
GA3 ₁₀₀	4.86°	12.29°	23.58°	53.71°
GA3 ₂₀₀	4.68 ^d	11.68 ^d	23.42°	52.29°
GA3 ₃₀₀	4.45°	11.13e	22.29 ^d	42.55 ^d
GA3400	4.36e	10.81e	21.17e	35.75°
Grand mean	4.795	12.265	24.310	50.825
CV (%)	3.176	3.209	3.212	3.292
SEM (±)	0.051	0.132	0.262	0.555
LSD _{0.05}	0.147	0.378	0.751	1.591
F test	***	***	***	***

^{***}Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean, CV: Coefficient of variation

Table 3: Interaction of different concentrations of gibberellic acids on the physiological loss of mango varieties

Interactions			Physiological	loss (%)	
Varieties	Treatments	3 DAT	6 DAT	9 DAT	12 DAT
Dasheri	GA3 ₀	6.46a	19.52a	39.25a	83.84ª
	GA350	6.04 ^b	16.12 ^b	32.13 ^b	71.41 ^b
	GA3 ₁₀₀	6.66a	14.82°	28.44°	64.78°
	GA3 ₂₀₀	6.58a	16.41 ^b	32.92 ^b	73.48 ^b
	GA3300	5.70°	14.25 ^{cd}	28.52°	57.07°
	GA3400	5.85 ^{bc}	14.50°	28.40°	45.76 ^g
Bombay	GA3 ₀	5.28 ^d	11.57 ^{fg}	23.27e	49.71 ^f
	GA350	5.63°	13.16e	26.24 ^d	58.33 ^{de}
	GA3 ₁₀₀	3.95 ^g	13.80 ^{de}	26.48 ^d	60.32 ^d
	GA3 ₂₀₀	4.22 ^{ef}	10.52 ^h	21.10 ^f	47.10 ^{fg}
	GA3 ₃₀₀	4.29e	10.73 ^h	21.49 ^f	38.54 ^h
	GA3400	3.95 ^g	9.79 ⁱ	19.17 ^g	34.48 ^{ij}
Maldah	GA3 ₀	4.39e	11.64 ^f	23.41e	50.01 ^f
	GA350	3.36 ^h	10.95gh	21.84 ^f	48.54 ^{fg}
	GA3 ₁₀₀	$3.97^{\rm fg}$	8.24 ^j	15.82 ^h	36.03hi
	GA3 ₂₀₀	3.25 ^h	8.11 ^j	16.26 ^h	36.30hi
	GA3 ₃₀₀	3.36 ^h	8.41 ^j	16.84 ^h	32.04 ^j
	GA3400	3.28 ^h	8.14 ^j	15.94 ^h	27.02 ^k
SEM (±)	<u> </u>	0.089	0.229	0.454	0.961
LSD _{0.05}		0.254	0.655	1.301	2.755
F test		***	***	***	***

GA3: Gibberellic acids, ***Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean

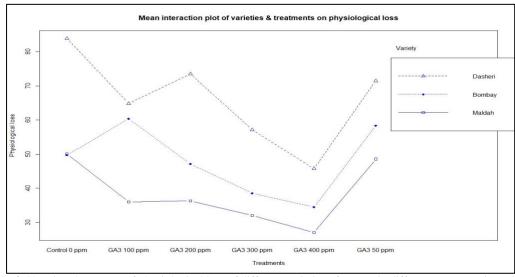


Figure 4. Showing the pattern of physiological loss of different varieties of mango in different concentrations of GA₃.

Effects of GA₃ on Total Soluble Solids and Titratable acidity

A statistically significant variation in total soluble solids (TSS) of mango was observed on the twelfth day after treatment application. Fruits treated with 400 ppm GA3 exhibited the lowest TSS (16.21 °Brix), while the control fruits recorded the highest (20.20 °Brix). Although most treatments showed a continuous increase in TSS throughout the study, fruits treated with 50 ppm GA3 (T2) and the control (T1) followed a distinct pattern, increasing until the ninth day before declining by the twelfth day. The variation in TSS across treatments, as shown in Table 4, Table 5, and Figure 5, reflects the postharvest effects of gibberellic acid application. In control and 50 ppm GA3-treated fruits, TSS initially increased until the ninth day, followed by a decline. Conversely, mangoes treated with 100 ppm, 200 ppm, 300 ppm, and 400 ppm GA3 exhibited a steady increase in TSS throughout the 12-day storage period. A highly significant difference in titratable acidity (TA) was also recorded on the twelfth day. TA consistently declined across all treatments over time, with the lowest TA observed in the control (0.111) and the highest in fruits treated with 400 ppm GA3 (0.162), indicating a gradual increase in TA with higher GA3 concentrations. Details on titratable acidity, variety-treatment interactions, and corresponding graphical representations are provided in Table 4, Table 5, and Figure 6.

Table 4. Effect of different concentrations of gibberellic acids on total soluble solids and titratable acidity of mango varieties

Varieties		TSS (°Br	ix)		TA (%)	
	6 DAT	9 DAT	12 DAT	6 DAT	9 DAT	12 DAT
Dasheri	15.06°	17.84 ^b	17.98°	0.17°	0.15 ^b	0.13 ^b
Bombay	17.17 ^a	18.50a	19.05a	0.18 ^b	0.15 ^b	0.13 ^b
Maldah	16.04 ^b	17.55°	18.40 ^b	0.19 ^a	0.17 ^a	0.16a
SEM (±)	0.0631	0.0601	0.0572	0.000843	0.000957	0.00318
LSD _{0.05}	0.1810	0.1725	0.1641	0.002417	0.002746	0.00911
F test	***	***	***	***	***	***
Treatments						
GA3 ₀	18.80a	20.50a	20.20a	0.14 ^f	0.119e	0.111°
GA3 ₅₀	17.76 ^b	19.27 ^b	19.15 ^b	0.16e	0.151 ^d	0.141 ^b
GA3 ₁₀₀	17.32°	19.01°	19.14 ^b	0.16 ^d	0.159°	0.144 ^b
GA3 ₂₀₀	15.08 ^d	17.46 ^d	19.08 ^b	0.18°	0.172 ^b	0.148 ^{ab}
GA3 ₃₀₀	14.78e	16.04e	17.08°	0.19 ^b	0.174 ^b	0.159a
GA3400	12.81 ^f	15.50 ^f	16.21 ^d	0.23a	0.179a	0.162a
Grand mean	16.095	17.968	18.480	0.182	0.159	0.144
CV (%)	1.655	1.440	1.178	1.965	2.446	9.294
SEM (±)	0.0892	0.0851	0.0809	0.001192	0.001354	0.00449
LSD _{0.05}	0.2560	0.2440	0.2320	0.003418	0.003884	0.01288
F test	***	***	***	***	***	***

^{***}Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean, CV: Coefficient of variation

Table 5. Interaction of different concentrations of gibberellic acids on total soluble solids and titratable acidity of mango varieties

Interactions			TSS (°Brix))		TA (%)		
Varieties	Treatments	6 DAT	9 DAT	12 DAT	6 DAT	9 DAT	12 DAT	
Dasheri	GA3 ₀	18.53 ^b	22.46a	20.43 ^b	0.13 ^k	0.106 ^j	0.115hij	
	GA350	17.36°	18.93 ^d	19.46°	0.17 ^g	0.154 ^f	0.145 ^{defg}	
	GA3 ₁₀₀	17.53°	16.36 ^g	19.60°	0.16 ^h	0.156ef	0.126ghij	
	GA3 ₂₀₀	10.26 ⁱ	17.36 ^f	19.40°	0.17 ^g	0.154 ^f	0.146 ^{defg}	
	GA3 ₃₀₀	14.46 ^f	16.50 ^g	13.43 ⁱ	0.19e	0.173°	0.142 ^{efg}	
	GA3400	12.23 ^h	15.43 ^h	15.56 ^h	0.19 ^e	0.161 ^{de}	0.155 ^{cdef}	
Bombay	GA3 ₀	18.50 ^b	21.56 ^b	19.76°	0.14 ^j	0.118 ⁱ	0.104 ^j	
	GA350	16.70 ^d	19.66°	18.53 ^d	0.15 ⁱ	0.145 ^g	0.109 ^{ij}	
	GA3 ₁₀₀	19.20a	22.50a	20.40 ^b	0.15 ⁱ	0.146 ^g	0.131 ^{fghi}	
	GA3200	19.61a	17.43 ^f	17.43e	0.18 ^f	0.164 ^d	0.136 ^{fgh}	
	GA3300	15.38e	15.20 ^h	21.36a	0.21°	0.196 ^b	0.133 ^{fghi}	
	GA3400	13.63g	14.66 ⁱ	16.80 ^f	0.23 ^b	0.143 ^g	0.185ab	
Maldah	GA3 ₀	19.36a	17.49 ^f	20.40 ^b	0.15 ⁱ	0.131 ^h	0.114 ^{hij}	
	GA350	19.23a	19.23 ^d	19.46 ^c	0.16 ^h	0.154 ^f	0.169 ^{bcd}	
	GA3 ₁₀₀	15.23e	18.16e	17.44e	0.18 ^f	0.175°	0.176 ^{bc}	
	GA3 ₂₀₀	15.38e	17.60 ^f	20.43 ^b	0.20 ^d	0.197 ^b	0.164 ^{bcde}	
	GA3 ₃₀₀	14.50 ^f	16.43 ^g	16.45 ^{fg}	0.19e	0.154 ^f	0.203ª	
	GA3400	12.56 ^h	16.40 ^g	16.26 ^g	0.26a	0.233a	0.146^{defg}	
SEM (±)		0.1546	0.1473	0.1401	0.002064	0.002345	0.00778	
LSD _{0.05}		0.4434	0.4226	0.4018	0.005919	0.006726	0.02231	
F test	•	***	***	***	***	***	***	

GA3: Gibberellic acids, ***Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean

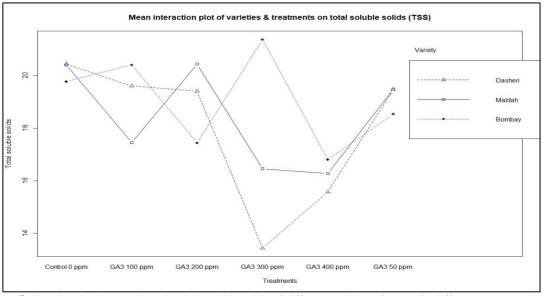


Figure 5. Showing the changes in total soluble solids (TSS) of different varieties of mango in different concentrations of GA₃.

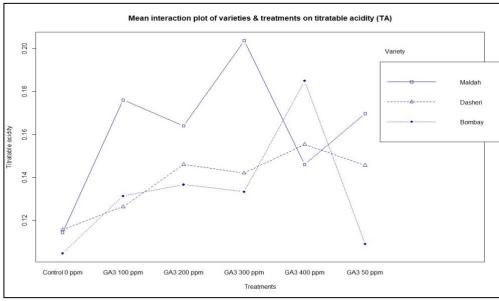


Figure 6. Showing the changes in titratable acidity (TA) of different varieties of mango in different concentrations of GA₃.

Effects of GA₃ on Fruit firmness and Pulp p^H

On the twelfth day after treatment application, a statistically significant variation in fruit firmness was observed across different mango varieties and gibberellic acid (GA3) treatments. The highest fruit firmness (1.14 kg/cm²) was recorded in fruits treated with 400 ppm GA3, while the lowest firmness (0.84 kg/cm²) was observed in the control (0 ppm GA3). Notably, fruit firmness in T4 and T5 was statistically similar on the final day of the study. A lower firmness value indicated a more drastic reduction in fruit firmness, which was most evident in the control (T1). The study results, including interactions between mango varieties and GA3 treatments and the trend of firmness changes over the 12-day storage period, are presented in Tables 6, 7, and Figure 7. Similarly, a highly significant variation in pulp pH was observed throughout the study. The control (T1) exhibited the highest pulp pH, followed in decreasing order by fruits treated with 50 ppm, 100 ppm, 200 ppm, 300 ppm, and 400 ppm GA3. By the end of the trial, mangoes treated with 400 ppm GA3 recorded the lowest pulp pH (5.22), whereas the control group had the highest (6.07). The results, including variety-treatment interactions and the trend of pulp pH changes across different GA3 concentrations, are illustrated in Tables 6, 7, and Figure 8.

Table 6. Effect of different concentrations of gibberellic acids on fruit firmness and pulp pH of mango varieties

Varieties	Fruit firmne	ess (Kg/cm ²)			Pulp pH	
	6 DAT	9 DAT	12 DAT	6 DAT	9 DAT	12 DAT
Dasheri	1.82°	1.33°	0.89 ^b	4.51a	5.07 ^a	5.82a
Bombay	1.90 ^b	1.45 ^b	1.12a	4.27 ^b	4.87 ^b	5.59 ^b
Maldah	2.49a	1.72ª	1.14 ^a	4.03°	4.77°	5.30°
SEM (±)	0.019	0.022	0.023	0.031	0.032	0.025
LSD _{0.05}	0.055	0.064	0.065	0.089	0.093	0.071
F test	***	***	***	***	***	***
Treatments						
GA3 ₀	1.64 ^d	1.26 ^d	0.84°	4.60a	5.13a	6.07a
GA350	1.97°	1.43°	0.98 ^b	4.55a	5.06a	5.71 ^b
GA3 ₁₀₀	2.09 ^b	1.51 ^{bc}	1.11 ^a	4.35 ^b	5.05a	5.55°
GA3 ₂₀₀	2.11 ^b	1.52 ^{bc}	1.12ª	4.12°	5.03a	5.52°
GA3300	2.28a	1.57 ^b	1.12a	4.11°	4.63 ^b	5.35 ^d
GA3400	2.35a	1.72ª	1.14 ^a	3.90 ^d	4.54 ^b	5.22e
Grand mean	2.076	1.503	1.056	4.275	4.909	5.573
CV (%)	3.988	6.374	8.843	3.120	2.465	1.861
SEM (±)	0.027	0.032	0.032	0.044	0.046	0.035
LSD _{0.05}	0.078	0.091	0.092	0.126	0.131	0.100
F test	***	***	***	***	***	***

^{***}Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean, CV: Coefficient of variation

Table 7. Interaction of different concentrations of gibberellic acids on fruit firmness and pulp pH of mango varieties

Interactions	Interactions		nness (Kg/cm ²)			Pulp Ph	
Varieties	Treatments	6 DAT	9 DAT	12 DAT	6 DAT	9 DAT	12 DAT
Dasheri	GA3 ₀	1.21 ^h	1.38 ^{efg}	0.82 ^{de}	4.62abc	5.80 ^a	5.47 ^{de}
	GA350	1.81 ^{ef}	$1.30^{\rm fg}$	0.85 ^{de}	4.63abc	5.14 ^{cd}	6.27 ^b
	GA3 ₁₀₀	1.74 ^{fg}	1.31 ^{fg}	0.76e	4.41 ^{cde}	5.03 ^{de}	6.31 ^b
	GA3 ₂₀₀	2.14 ^c	1.31 ^{fg}	1.08 ^{bc}	4.49 ^{cd}	4.94 ^{def}	5.93°
	GA3 ₃₀₀	1.88 ^{ef}	1.25 ^{fg}	0.88 ^{de}	4.75 ^{ab}	4.86 ^{efgh}	5.51 ^{de}
	GA3400	2.15°	1.43 ^{ef}	0.96 ^{cd}	4.17 ^{ef}	4.67ghi	5.42 ^e
Bombay	GA3 ₀	1.89 ^{ef}	1.02 ^h	0.75 ^e	4.57 ^{abcd}	4.56 ⁱ	6.75a
	GA3 ₅₀	2.15°	1.50 ^{de}	0.88^{de}	4.81a	4.97 ^{de}	5.24 ^{fg}
	GA3 ₁₀₀	1.90 ^{ef}	1.23 ^g	1.38a	4.50 ^{bcd}	5.48 ^b	5.45 ^{de}
	GA3 ₂₀₀	2.07 ^{cd}	1.53 ^{de}	1.13 ^b	3.62g	5.27°	5.57 ^{de}
	GA3 ₃₀₀	1.80 ^{ef}	1.81 ^b	1.25 ^{ab}	4.36 ^{def}	4.72 ^{fghi}	5.38 ^{ef}
	GA3400	1.63 ^g	1.61 ^{cd}	1.34ª	3.76 ^g	4.25 ^j	5.16 ^g
Maldah	GA3 ₀	1.83 ^{ef}	1.38 ^{efg}	0.95 ^{cd}	4.61 ^{abc}	5.03 ^{de}	5.98°
	GA350	1.95 ^{de}	1.50 ^{de}	1.21 ^{ab}	4.22ef	5.06 ^{de}	5.63 ^d
	GA3 ₁₀₀	2.63 ^b	1.98 ^a	1.21 ^{ab}	4.13 ^f	4.65 ^{hi}	4.89 ^h
	GA3 ₂₀₀	2.12°	1.72 ^{bc}	1.13 ^b	4.24 ^{ef}	4.89 ^{efg}	5.06 ^{gh}
	GA3 ₃₀₀	3.17 ^a	1.64 ^{cd}	1.23 ^{ab}	3.24 ^h	4.31 ^j	5.16 ^g
	GA3400	3.27 ^a	2.11a	1.13 ^b	3.77 ^g	4.69ghi	5.08 ^g
SEM (±)		0.047	0.055	0.055	0.076	0.079	0.061
LSD _{0.05}	•	0.135	0.157	0.159	0.217	0.227	0.174
F test		***	***	***	***	***	***

GA3: Gibberellic acids, ***Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean

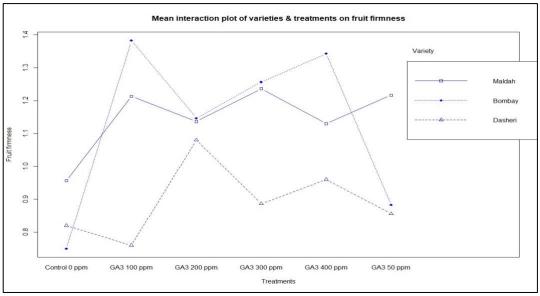


Figure 7. Showing the changes in fruit firmness of different varieties of mango in different concentrations of GA₃.

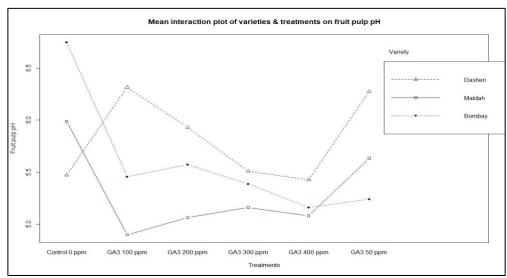


Figure 8. Showing the changes in pulp pH of different varieties of mango in different concentrations of GA3.

Effects of GA₃ on Palatability and Shelf life

Mango palatability varied significantly across different varieties and gibberellic acid (GA3) treatments by the twelfth day after application. The highest palatability score of 4.16 was recorded in T1 (control), T2 (50 ppm GA3), and T4 (200 ppm GA3), whereas T3 (100 ppm GA3) had the lowest score at 3.66. Meanwhile, T5 (300 ppm GA3) and T6 (400 ppm GA3) had scores of 4.00 on the final day. Throughout the study, palatability generally increased across all treatments except for the control, where the trend remained inconsistent. The results, including interactions between varieties and treatments and changes in palatability over 12 days, are presented in Tables 8, 9, and Figure 9. Shelf life also exhibited notable variations based on the gibberellic acid concentration. Defined as the period in which the fruit retains optimal eating and marketing quality without surpassing 50% deterioration, the shelf life ranged from 12.55 to 15.55 days. Fruits treated with 400 ppm GA3 recorded the longest shelf life at 15.55 days, followed by those treated with 300 ppm (14.66 days), 200 ppm (14.11 days), 100 ppm (13.55 days), and 50 ppm (13.11 days). In contrast, untreated mangoes (control) had the shortest shelf life at 12.55 days. These findings indicate that higher GA3 concentrations play a crucial role in prolonging fruit freshness during storage, as detailed in Tables 8, 9, and Figure 10.

Table 8. Effect of different concentrations of gibberellic acids on palatability score and shelf life of mango varieties

Varieties		Palatability score		Shelf life
	6 DAT	9 DAT	12 DAT	
Dasheri	3.25 ^b	3.58°	3.75°	13.72 ^b
Bombay	3.50a	4.08 ^a	4.08 ^b	13.94 ^{ab}
Maldah	3.50a	3.83 ^b	4.25a	14.11 ^a
SEM (±)	0.1734	0.2659	0.1778	0.1242
LSD _{0.05}	0.0207	0.0214	0.0212	0.3563
F test	***	***	***	*
Treatments				
GA3 ₀	3.16 ^d	4.33a	4.16a	12.55e
GA350	3.66a	4.00 ^b	4.16a	13.11 ^d
GA3 ₁₀₀	3.50 ^b	3.33e	3.66°	13.55 ^d
GA3 ₂₀₀	3.66a	3.83°	4.16a	14.11°
GA3 ₃₀₀	3.16 ^d	3.50 ^d	4.00 ^b	14.66 ^b
GA3 ₄₀₀	3.33°	4.00 ^b	4.00 ^b	15.55ª
Grand mean	3.416	3.833	4.027	13.925
CV (%)	0.925	0.824	0.078	3.663
SEM (±)	0.1415	0.2171	0.1452	0.1757
LSD _{0.05}	0.0301	0.0304	0.0324	0.5039
F test	***	***	***	***

^{*}Significant at 5% level of significance, ***Significant at 0.1% level of significance, LSD: Least significant difference, SEM: Standard error of the mean, CV: Coefficient of variation

able 9. Interaction of different concentrations of gibberellic acids on palatability score and shelf life of mango varieties

Interactions			Palatability sco	re	Shelf life
Varieties	Treatments	6 DAT	9 DAT	12 DAT	1
Dasheri	GA3 ₀	3.5 ^b	4.0 ^b	4.0 ^b	12.33 ^h
	GA350	3.0°	3.5°	4.0 ^b	13.00 ^{gh}
	GA3 ₁₀₀	3.5 ^b	3.5°	3.5°	13.33 ^{fg}
	GA3 ₂₀₀	3.5 ^b	3.5°	4.0 ^b	14.00 ^{def}
	GA3 ₃₀₀	3.0°	3.5°	3.5°	14.33 ^{cde}
	GA3 ₄₀₀	3.0°	3.5°	3.5°	15.33 ^{ab}
Bombay	GA3 ₀	3.0°	4.5a	4.0 ^b	12.33 ^h
	GA350	4.0^{a}	4.0 ^b	4.5a	13.33 ^{fg}
	GA3 ₁₀₀	3.5 ^b	4.0 ^b	4.0 ^b	13.66 ^{efg}
	GA3 ₂₀₀	3.5 ^b	4.0 ^b	4.0 ^b	14.00 ^{def}
	GA3 ₃₀₀	3.5 ^b	4.0 ^b	4.0 ^b	15.00 ^{abc}
	GA3400	3.5 ^b	4.0 ^b	4.0 ^b	15.66ª
Maldah	GA3 ₀	3.0°	4.5ª	4.5a	13.00gh
	GA350	4.0^{a}	4.5ª	4.0 ^b	13.33 ^{fg}
	GA3 ₁₀₀	3.5^{b}	2.5 ^e	3.5°	13.66 ^{efg}
	GA3 ₂₀₀	4.0^{a}	4.0 ^b	4.5a	14.33 ^{cde}
·	GA3 ₃₀₀	3.0°	3.0^{d}	4.5ª	14.66 ^{bcd}
·	GA3400	3.5 ^b	4.5a	4.5ª	15.67 ^a
SEM (±)		0.1001	0.1535	0.1027	0.3043
LSD _{0.05}		0.0524	0.0520	0.0517	0.8728
F test		***	***	***	NS

GA3: Gibberellic acids, ***Significant at 0.1% level of significance, NS = Non-significant, LSD: Least significant difference, SEM: Standard error of the mean

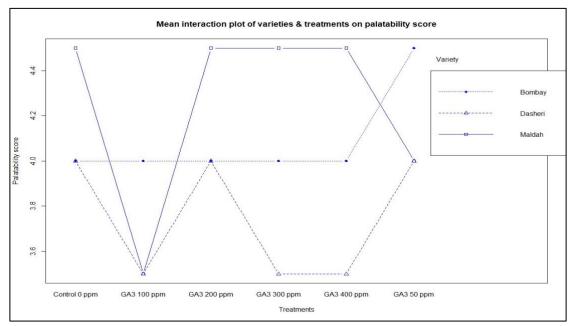


Figure 9. Showing the changes in palatability of different varieties of mangoes in different concentrations of GA_3 .

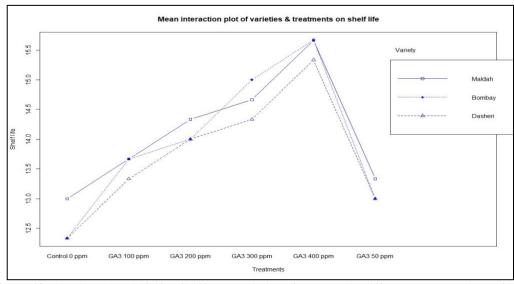


Figure 10. Showing the shelf life of different varieties of mangoes in different concentrations of GA₃

DISCUSSIONS

The findings of this study confirm the efficacy of gibberellic acid (GA3) in extending the shelf life and maintaining the physicochemical qualities of mangoes, aligning with previous research. Yadav et al. (2022) demonstrated that mangoes treated with 400 ppm GA3 exhibited significantly reduced physiological weight loss, which can be attributed to the suppression of respiration and transpiration rates. This effect is crucial in delaying senescence, as excessive water loss leads to shrinkage, textural degradation, and increased susceptibility to microbial spoilage. Similarly, Wahden et al. (2011) emphasized that gibberellic acid functions as a potent ethylene antagonist, delaying ripening and senescence in climacteric fruits. By inhibiting ethylene biosynthesis, GA3 slows down the enzymatic breakdown of cell wall components, including pectin and hemicellulose, thus maintaining fruit integrity for an extended period. Furthermore, the delayed ripening effect of GA3 can be explained by its role in regulating hormonal balance within the fruit. Ethylene triggers the conversion of starch to sugars, leading to rapid softening and degradation of fruit tissue. The anti-senescing properties of GA3 were corroborated by Hu et al. (2018), who demonstrated that GA3 reduces tissue permeability and CO2 production, thereby minimizing water loss during storage. Reduced permeability ensures lower ion leakage, preserving cell structure and delaying the onset of physiological disorders associated with prolonged storage. In the present study, mangoes treated with 400 ppm GA3 exhibited the lowest physiological loss compared to untreated controls. This observation aligns with findings by Chhetri and Ghimire (2023), who reported that GA3 significantly reduced weight loss by slowing down metabolic activities and the enzymatic conversion of starch to sugars. A possible explanation is that GA3 alters the activity of hydrolytic enzymes such as amylases and invertases, thereby regulating the rate at which carbohydrates are stored during respiration. Additionally, the study found that GA3-treated fruits maintained lower pulp pH levels over the storage period. According to Jain and Mukherjee (2000), this effect is due to the delayed oxidation of organic acids, preventing a rapid increase in pH during ripening. Penyimpenan (2013) also noted similar outcomes, highlighting GA3's ability to stabilize pulp acidity by inhibiting metabolic pathways responsible for acid degradation. This is particularly significant since Tosun (2008) established an inverse correlation between pulp pH and titratable acidity, where higher acidity enhances storage quality. The present results suggest that GA3 treatments effectively preserved titratable acidity by minimizing respiration-driven consumption of organic acids, a phenomenon similarly observed by Pal (1998) in other tropical fruits. The reduction in titratable acidity observed in this study aligns with findings by Fatima et al. (2022), who reported that GA3 slows the metabolic conversion of acids into sugars, prolonging the desirable characteristics of fruits during storage. Mango firmness was significantly higher in GA3-treated fruits, consistent with reports by Porat et al. (2001) and Reddy et al. (2014). This outcome is likely due to the inhibition of ethylene synthesis and the subsequent reduction in enzymatic activity responsible for the degradation of cell wall components such as cellulose, hemicellulose, and pectin. Wang et al. (2018) elaborated on this by showing that GA3 modulates ripening-related gene expression, leading to delayed softening. Another possible reason for maintained firmness is the strengthened interactions between cell wall polymers, which prevent rapid textural degradation. Vishwakarma et al. (2022) further provided evidence that GA3-treated mangoes exhibit superior resistance to physiological degradation, likely due to its ability to enhance cell wall reinforcement through calcium retention and lignification processes. Similarly, Panigrahi et al. (2021) explored the application of GA3 coatings and demonstrated their effectiveness in enhancing postharvest quality by reducing surface moisture loss and microbial colonization. These findings are particularly relevant in the present study, where the inhibition of microbial decay in GA3-treated fruits suggests a role in reinforcing fruit cuticle

integrity. Additionally, Siddiqui et al. (2013) reported that GA3, when applied as a pre-harvest spray, not only delayed ripening but also preserved the biochemical composition of fruits by maintaining antioxidant enzyme activity. This aligns with the present findings, where the application of 400 ppm GA3 significantly delayed the accumulation of total soluble solids (TSS) by modulating the enzymatic conversion of starch into sugars. The observed decline in TSS at later storage stages can be attributed to respiratory utilization, as noted by Singh et al. (2019), who explained that during prolonged storage, the respiratory process consumes soluble sugars, leading to a subsequent decline in sweetness. The decline in TSS may also be linked to the delayed hydrolysis of polysaccharides due to GA3's impact on amylolytic enzymes. Finally, the study observed a consistent pattern of reduced metabolic activity in GA3-treated mangoes, which is crucial for extending storage life. This aligns with insights by Reddy and Haripriya (2002), who emphasized that GA3 blocks enzymatic pathways responsible for rapid ripening, thereby mitigating weight loss and quality degradation. Moreover, GA3's role in maintaining chlorophyll stability and delaying anthocyanin synthesis provides an additional explanation for delayed visual ripening and prolonged marketability. These findings collectively underscore the multi-faceted role of GA3 in postharvest management by modulating ethylene synthesis, enzyme activity, organic acid metabolism, and structural integrity of cell walls. The results suggest that GA3 treatments could be an effective postharvest strategy to enhance the storability and quality retention of mangoes, making them more appealing for commercial supply chains.

CONCLUSION

The findings of this study suggest that applying different concentrations of GA3 effectively extends the shelf life of mangoes by delaying ripening, with fruits remaining fresh for an additional 4 to 7 days. Mangoes treated with 400 ppm GA3 exhibited the longest shelf life, lasting nearly 16 days, while untreated mangoes deteriorated within 12 days. Physiochemical traits such as physiological weight loss, total soluble solids (TSS), and pulp pH increased rapidly in untreated mangoes, whereas titratable acidity declined sharply. In contrast, GA3-treated mangoes retained better firmness, lower TSS accumulation, and stable titratable acidity, suggesting reduced metabolic activity and ethylene suppression. The study highlights 400 ppm GA3 as the most effective concentration for maintaining postharvest quality and commercial value. However, certain limitations exist, as the findings are based on a specific cultivar, postharvest handling conditions, and environmental factors that may influence outcomes. Future research should explore varietal responses, economic feasibility, and the integration of GA3 with other postharvest treatments to optimize its practical application. Overall, GA3 treatment, particularly at 400 ppm, proves to be a promising approach for reducing postharvest losses and enhancing the storage potential of mangoes.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed

Declaration of Interests

The authors declare that they have no conflict of interest.

Author Contribution

Daurik Lal Pandit & Dipesh Kumar Mehata: Conceptualization, data curation, investigation, methodology, visualization, original draft writing, review and editing of writing, and validation. Shafat Rukhsar, Pawan Kumar Yadav, Vivek Lahutiya: Data curation, methodology, writing original draft. Sunny Kumar Shah & Umesh Timilsina: Supervision & Validation

Acknowledgements

We would like to give a big thanks to our Girija prasad Koirala College of Agriculture and Research Center (GPCAR) and Prime Minister Agricultural Modernization Project (PMAMP) for providing this platform and opportunity to conduct this research.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.21

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 190-198

Two different methods for rooting blackberry cuttings: comparison of aeroponic and perlite media

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Article History

Received: February 4, 2025 Revised: March 8, 2025 Accepted: March 11, 2025 Published Online: March 12, 2025

Article Info

Article Type: Research Article Article Subject: Horticultural Production

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1633062

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Abstract

This study examines the effects of different IBA concentrations and rooting media on the rooting characteristics of green cuttings from the Jumbo blackberry variety. The cuttings were treated with 0 (control), 500, 1000, and 1500 ppm IBA, then planted in Aeroponic and Perlite rooting systems to compare their performance. Parameters such as rooting rate, root length, development of rootlets, seedling yield, the number of branches per cutting, and disease occurrence were evaluated. In the Perlite medium, the application of 1500 ppm resulted in the most extended root length (7.33 cm), while the highest root number (13.26) was observed at the 500 ppm dose. In the Aeroponic medium, the 1000 ppm application achieved the highest values for root length (10.24 cm) and root number (15.47). However, while the decay rate remained at 0.00% in the Perlite medium, it varied between 16.67% and 20.00% in the Aeroponic medium. The highest rooting rate in the Perlite medium was observed at 500 ppm with 93.33%, whereas in the Aeroponic medium, the rooting rate ranged from 50.00% in the control group to a maximum of 70.00% in the 1500 ppm group. The seedling yield reached 93.33% at 500 ppm in the Perlite medium and peaked at 63.33% in the Aeroponic medium. In conclusion, the Perlite medium provided healthier root development due to its low decay rate, higher callus formation, and higher rooting rates. In contrast, the Aeroponic medium posed a risk of decay even at higher IBA concentrations.

Cite this article as: Dumlu, F., Karadag, H. (2025). Two different methods for rooting blackberry cuttings: comparison of aeroponic and perlite media. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 190-198. https://doi.org/10.31015/2025.1.21

Keywords: Rooting, IBA, Blackberry, Jumbo, Green cuttings

INTRODUCTION

Historically, the relationship between population and agriculture has been a fundamental element shaping economic and social structures. Agriculture has contributed to societies' development through food production and as the basic building block of an environmental, cultural, and economic network. In particular, the increasing prominence of environmental problems poses a problem in terms of the sustainability of traditional agricultural methods (Eryılmaz & Kılıç, 2018). In this context, alternative agricultural methods offer an important option for solving this problem.

The main factors, such as meeting the increasing food demand and the adverse effects of traditional agricultural methods, have necessitated using alternative agricultural methods (Yavuz et al., 2023). In addition, the infertility of the soil due to the unconscious use of water and chemicals, the rapid increase in urbanization and the restriction of production areas, climate change, and environmental factors are other important factors that necessitate the spread of alternative agricultural methods. In a period when traditional agricultural methods face problems such as limited resources, soil erosion, water shortage, and chemical use, the development of more sustainable and efficient alternatives has become inevitable (FAO, 2017). Alternative agricultural methods go beyond traditional agriculture and offer environmentally friendly, sustainable, less resource-consuming approaches. Considering the increasing demands of the population and environmental pressures, alternative methods have become an important factor in shaping the future of agriculture. Developing technology has enabled the diversification and development of alternative agricultural opportunities and has caused Hydroponics, Aeroponics and Aquaponics to gain importance (Bingöl, 2019).

Indoor farming has expanded quickly within the horticultural sector due to yield consistency and environmental control capabilities (Benke & Tomkins, 2017). Aeroponic systems, which stand out among alternative agricultural methods, are among the modern approaches that minimize water use, prioritize sustainability, and allow production in controlled environments. Aeroponic systems allow the producer to precisely control root zone nutrient and water regimes and environmental conditions, as well as have complete access to the roots throughout the life of the crop (Hayden et. al., 2004; Cai et. al., 2023). Aeroponic systems are used in plant production areas for cutting rooting, seedling, and cultivation. Aeroponic rooting is an economical method, especially in terms of rapid reproduction and protection of plants (Mehandru et al., 2014). Aeroponics allows artificial elevation of root zone O₂ to enhance yield (Eldridge et al., 2020).

Perlite is a light and fine-grained rock of volcanic origin and is widely used as a rooting medium, especially in agriculture. In addition to having a high water retention capacity, perlite, which has good air permeability, provides an ideal environment for plant root development. Perlite accelerates the rooting process of cuttings, enabling the growth of strong and healthy new plants (Kalyoncu, 1996). It was noted that perlite can be used alone or mixed with similar products to successfully root wood cuttings (Gregory, 1999; Sengel et. al 2012; Kir, 2025). For this reason, perlite is an effective rooting medium, especially preferred in applications such as propagation by cuttings and plant production (Grillas, 2001).

Propagation by cuttings is widely used in many plant species, especially fruit trees, ornamental plants, shrubs, some vegetables, and field crops (Preece, 2003; Chen & Stamps 2006; Kroin, 2009; Megersa, 2017). One of the most important factors in propagation by cuttings is the selection of a suitable rooting medium (Schimilewski, 2008; Barreett et. al., 2016). These mediums generally consist of materials that can retain water but have good aeration, such as peat, perlite, and cocopeat.

This study aimed to compare the rooting performances of green cuttings of the Jumbo blackberry variety in an aeroponics rooting system and perlite medium. In recent years, the aeroponics rooting system has emerged as a new technique for producing seedling cuttings. It provides advantages such as more efficient use of water and nutrients during the rooting process. This study aims to compare the aeroponics system with traditional rooting media such as perlite and to contribute to the literature on the advantages and disadvantages of the system. It aims to evaluate the potential of aeroponics systems for use in rooting processes.

MATERIALS AND METHODS

Material

This study used green cuttings of the Jumbo Blackberry (*Rubus spp.*) variety as plant material. Jumbo Blackberry is widely grown in Turkiye due to its increasing importance in supplying raw materials to industry and other consumption areas (Akbulut et al., 2003). Cuttings used in the study were obtained from the blackberry parcel belonging to Tokat Gaziosmanpaşa University Agricultural Research and Application Center. Cuttings cut to approximately 20 cm in length were kept in a damp cloth before planting to prevent moisture loss. Plantings were made on the same day.

Method

The study compared the rooting performances of blackberry green cuttings in an aeroponic rooting system and perlite rooting medium. IBA (Indole-3-butyric acid Merck) was applied to the cuttings to promote rooting (Gerçekcioğlu, 2009; Kalyoncu et al., 2016). IBA was applied to blackberry cuttings at doses of 0 (control), 500, 1000 and 1500 ppm. Each cutting was kept for 10 seconds in the recording records and randomly placed in aeroponic and perlite media. The study was set up according to the randomized plot design. 10 cuttings were used for each application. A total of 240 cuttings are used in aeroponic medium (4 applications x 3 replicates x 10 cuttings = 120 cuttings) and perlite medium (4 applications x 3 replicates x 10 cuttings = 120 cuttings). Cuttings were planted in both rooting systems simultaneously on August 17, 2023. The results obtained; Data analysis was performed using the SPSS package program. The data obtained from both media were compared, and the most appropriate IBA dose and rooting media were determined.

Aeroponic Rooting System

The aeroponic system in which the study was conducted was designed in an industrial size, consisting of a three-story structure, and each floor has three boxes with 2 m^2 of growing area. This system has a total planting area of 18 m^2 and is equipped with misting nozzles and circulation fans that provide optimum humidity and air circulation. The system is automated by PLC (Programmable Logic Controller) control, and each cabinet can be operated independently (Figure 1). The nozzles have a hole diameter of 0.30 μ m.

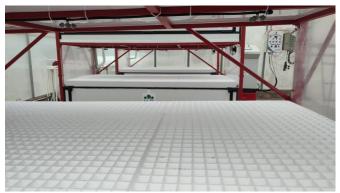


Figure 1. Aeroponic rooting system

Perlite Rooting System

This system used iron-equipped pool-type benches, and the cuttings were planted in a 30 cm deep perlite medium. Irrigation was done with a misting system from above, controlled with a timer (Figure 2). It has been stated that the perlite rooting unit prevents root rot by providing a light, sterile, and air-permeable medium (Gül et al., 2010) and increases rooting success in woody plant cuttings (Mamatha et al., 2024).

The most suitable rooting medium and IBA dose were determined using these methods, and the aim of the study was achieved.

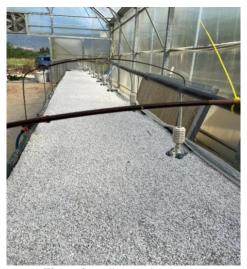


Figure 2. Perlite Rooting System

RESULTS AND DISCUSSION

The trial was started simultaneously on August 17, 2023, using the Aeroponic and Perlite rooting systems. Ten cuttings were used in each replication, and a total of 240 cuttings were used. The placement of the cuttings in these two different environments was carried out with a random distribution. The SPSS statistical package program was used to evaluate the obtained data. Statistical analyses aimed to reveal the significance levels of the effects of doses and different rooting environments on the development of cuttings.

Table 1. Statistical analysis results (Root development)

Media	Application	Root Length (cm)	Root Diameter (mm)	Cutting Diameter (mm)	Root Number (Number)
	Control	6.34 a	0.76 a	8.33 a	11.90 ab
Perlite	500 ppm	5.91 a	0.70 a	7.71 a	13.26 ab
reinte	1000 ppm	5.09 a	0.52 a	8.08 a	10.43 abc
	1500 ppm	7.33 a	0.60 a	7.63 a	12.07 ab
	Control	5.47 a	0.36 a	7.87 a	3.53 с
Aorononio	500 ppm	6.29 a	0.58 a	7.56 a	7.33 abc
Aeroponic	1000 ppm	10.24 a	0.59 a	7.80 a	15.47 a
	1500 ppm	5.44 a	0.88 a	8.27 a	6.74 bc

The statistical analysis results of root development in perlite and aeroponic media are shown in Table 1. Different applications were made in each medium, and their effects on root length, root diameter, cutting diameter, and root number were evaluated. Statistically, no significant difference was found between root length, root diameter and cutting diameter.

However, it is understood from the root lengths that the aeroponic environment promotes root development. 1500 ppm application in perlite medium gave the highest value regarding root length (7.33 cm). However, the 500 ppm application stands out in terms of root number. 1000 ppm application in aeroponic medium provided the highest results in terms of root length (10.24 cm) and root number (15.47 pieces) (Table 1). No significant differences were observed between both medium and application factors regarding root length. This situation reveals that root development varies more in response to genetic and environmental factors. However, it is stated in the literature that root length shows a constant trend regardless of a specific medium and that the composition of the nutrient solution and the oxygen level in the root zone are more effective (Mamatha et al., 2024). In a study, it was reported that the average root storage increase in aeroponically grown crops was more than 20 g dry weight compared to cassava grown under drip irrigation (Selvaraj et al., 2019). It was also reported that burdock grown under aeroponics accumulated 49% more aerial biomass compared to conventional cultivation (Hayden et al., 2004). Root growth in aeroponics has been observed to be relatively high at certain nutrient levels (especially 1000 ppm). However, it should be noted that aeroponics generally results in lower root diameter and number. This indicates that roots grow more densely and rapidly but have smaller diameters.

When we examine the effects of medium applications on root development based on the content of the table, the average root length in the control group in perlite medium is 6.34 cm, which is relatively high compared to the other groups. Aeroponically grown roots may have more capillary roots compared to hydroponically grown roots (Kratsch et al., 2006), which will affect the aerosol capture of the roots. However, at 1500 ppm, the root length increases again, reaching 7.33 cm. Here, it is seen that high ppm levels (1500 ppm) can increase root length. Root diameter and root number also increased in parallel with the high root length at 1500 ppm (Table 1). While the root length was relatively low in the control group in the aeroponics application (5.47 cm), a significant increase was observed with the 1000 ppm application (10.24 cm). While the cutting diameter in the aeroponics medium was generally lower than in the perlite medium, the diameters in the 500 ppm and 1500 ppm applications were at similar levels. A study determined that aeroponics increased potato mini tuber yield by 70% compared to hydroponics, but the average tuber weight was 33% lower (Ritter et al., 2001). In the root number, 15.47 observed in the 1000 ppm group was relatively high compared to the other groups and showed that this medium supported root development (Table 1).

Table 2. Statistical analysis results (rooting status)

Media	Application	Decay rate (%)	Callus rate (%)	Rooting rate (%)	Seedling yield (%)
	Control	0.00 b	100.00 a	86.67 ab	83.33 ab
Da-1:4a	500 ppm	0.00 b	100.00 a	93.33 a	93.33 a
Perlite	1000 ppm	0.00 b	96.67 a	80.00 ab	80.00 ab
•	1500 ppm	0.00 b	96.67 a	90.00 a	76.67 bc
	Control	16.67 a	66.67 b	50.00 d	43.33 e
A amamania	500 ppm	20.00 a	80.00 ab	60.00 cd	53.33 de
Aeroponic	1000 ppm	16.67 a	83.33 ab	70.00 bc	63.33 cd
	1500 ppm	10.00 b	86.67 ab	70.00 bc	63.33 cd

The statistical analysis results of parameters such as decay rate, callus rate, rooting rate, and seedling yield measured in Perlite and Aeroponic media are in Table 2.

The decay rate remained constant at 0.00% in all applications of Perlite media. This shows that Perlite media is not affected by the risk of decay. However, the decay rate in Aeroponic media is at the highest rate of 20.00% at 500 ppm dose. While the rate decreases to 16.67% in control and 1000 ppm applications, it decreases to 10.00% in 1500 ppm. This situation reveals that the risk of decay is higher in Aeroponic media (Table 2). In light of the findings, it can be said that 1000 ppm is the most effective nutrient level for root development in both perlite and aeroponics. The low decay rate and high callus rate in perlite indicate that plant development is healthy in this environment. Even at high nutrient levels, there is no risk of decay, and rooting rates remain pretty satisfactory.

The high decay rate in aeroponics is due to the higher humidity levels and limited air circulation in these systems. It is also reported in the literature that high humidity levels encourage the development of pathogenic organisms, and therefore, the decay rate increases in aeroponics systems (Eldridge et al., 2020). On the other hand, the low decay rate in perlite media can be explained by the physical structure of the perlite, which provides better drainage and prevents water accumulation (Gislerod & Mortenson, 1990). For example, while the decay rate in perlite media was 15%, this rate reached 30% in aeroponics media. The low rate of decay supports healthy root development in this environment. Research indicates that inorganic growth media such as perlite increase the ability to reduce decay (Hendrick & Black, 2002). In aeroponics, it was observed that decay rates were higher

(16.67-20.00%), and seedling yields were low (43.33-63.33%). Although aeroponics systems allow roots to develop freely in the air, theoretically providing healthy root development, the risk of decay increases with high nutrient levels (Lakhiar et al., 2018). The data obtained supports this thesis.

The callus rate in Perlite media has the highest value, with 100% in the control group, and other ppm levels vary between 96.67% and 100%. This shows that Perlite media is effective in healing root wounds. While the callus rate was as low as 66.67% in the control group in the aeroponic system, it increased to 80.00% at 500 ppm, 83.33% at 1000 ppm, and 86.67% at 1500 ppm. High-dose application levels may positively affect callus formation (Figure 3, Figure 4). Perlite medium and 500 ppm application provided high callus and seedling yield by eliminating the risk of decay. This combination is the most effective option.

In aeroponics, it is recommended to prefer high doses (1000 ppm and 1500 ppm) to reduce decay and increase callus/rooting rates. Considering that high doses reduce yield in perlite medium but improve in aeroponics, medium and dose selection should be made carefully in line with the purpose of the experiments.

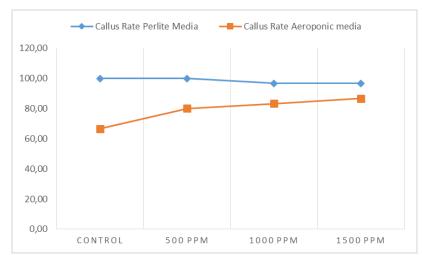


Figure 3. Callus formation rate



Figure 4. Callus formation



Figure 5. Rooting cuttings

While the highest rooting rate in perlite medium was 93.33% with 500 ppm, it decreased to 80.00% and 90.00% in 1000 ppm and 1500 ppm groups. The lowest rooting rate in aeroponic medium was 50.00% in the control group, while this rate varied between 60.00% and 70.00% in other groups. This shows that rooting in aeroponic medium can be increased with better applications (Figure 5, Figure 6). Sharma et al. (2018) in *Tamarix aphylla*, found root development (rooting%, roots/cutting and root length) generally higher in cuttings treated with IBA, they also noted that the highest rooting values (87 %) were performed in cuttings treated with a mixture of IBA and NAA. It was stated in his study that 1 g L^{-1} Indole-3-butyric acid combined with a 10-minute spraying interval increased root biomass yield (Scaltrito et. al 2024).

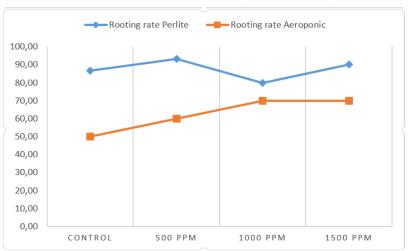


Figure 6. Rooting rate

In perlite medium, seedling yield was the highest, with 93.33% in the 500 ppm group, while it decreased to 76.67% in the 1500 ppm group. In aeroponics, seedling yield varied between 43.33% and 63.33% in all applications. These values indicate that seedling yield in aeroponics was low and could have problems in terms of development. In aeroponics, a low yield (43.33%) was observed in the control group, while this value increased with applications (53.33-63.33%). However, it still lagged behind the perlite medium. 500 ppm application in perlite medium gives the most effective results in seedling yield and rooting, while 1500 ppm in aeroponics medium offers the highest value in callus rate. However, aeroponics applications show lower performance in total. Experiments conducted in perlite medium show that parameters such as root length, diameter, and number give the best results, especially at 500 ppm. Perlite is a frequently used medium for plant development thanks to its lightness, water-holding capacity, and aeration properties (Marschner, 2012). Similarly, there are findings in the literature that perlite media positively affect root development and increase root number and diameter (Baiyin et al., 2021). In addition, the low decay rate (0.00%), high callus rate (100%), and rooting rates (93.33%) observed in the perlite medium show that this medium is quite suitable for healthy plant development.

Rooting experiments were conducted in aeroponics and perlite media using green cuttings in the Jumbo blackberry variety. The analysis results revealed that neither the media nor the application factors showed a significant effect in terms of root length. However, the decay rate varied depending on the media type and was

higher in the aeroponics medium. These findings support some aspects of the existing studies in the literature, while they differ in some aspects.

The fact that no significant difference was observed in terms of root length suggests that root development is mainly driven by genetic factors or other environmental factors other than media and applications. In the literature, it is stated that the effect of different media on root length is generally minimal. Instead, the nutrient solution composition and the root zone's oxygen level are more critical (Pineda et al., 202015). In this context, the results of our study underline this view by showing a constant trend in root length regardless of a particular medium or application.

The fact that the application did not significantly affect root length and decay rate suggests that these applications did not create a critical difference in terms of the parameters examined. Although similar findings are found in the literature, it has been stated that increasing the variety of applications may produce more significant results in some cases (Balliu et al., 2021). For example, a study reported that different application types caused changes of up to 10% in root length. Therefore, it is recommended that the application types be diversified and tested with different plant species in future studies.

Our study observed that different application types did not create a significant difference in root length and decay rate. However, the literature has stated that more significant results can be obtained as the variety of applications increases (Balliu et al., 2021).

As a result, this study emphasizes the critical effects of the environmental factor, especially on the decay rate, while revealing that some parameters, such as root length, are less affected by environmental factors. Future studies should be conducted with a broader range of environments and applications to assess the generalizability of these results. Additionally, strategies to reduce the impact of pathogenic microorganisms may be critical for more effective use of aeroponic systems.

CONCLUSION

This study highlights the critical effects of environmental factors on root length and decay rate. Although the effect of the environment on the decay rate is significant, parameters such as root length are less affected by environmental factors and practices. This shows that optimizing the medium and environmental conditions is important, especially in reducing decay rates. Aeroponic systems may negatively affect productivity when the decay rate is high, while media such as perlite have lower decay rates and can provide healthier root development.

High decay rates in aeroponic systems negatively affect the productivity of such systems. In future studies, various strategies should be developed to reduce decay rates in aeroponic systems. These strategies may include factors such as regulating humidity levels, improving airflow, and optimizing the microclimate of the environment. In addition, using biological or chemical control methods that prevent the proliferation of pathogenic microorganisms may contribute to aeroponic systems being more efficient and sustainable.

It has been stated in the literature that oxygen levels play a critical role in root development (Smith et al., 2015). Therefore, improving oxygen levels in the root zone can be an important strategy to accelerate root development. Increasing oxygen levels, especially in aeroponic systems, can provide healthier root development.

Future studies should be conducted with different media and application types to test the generalizability of these results. Developing strategies to reduce decay rates in rooting studies on green cuttings in aeroponic systems will allow these systems to be used more efficiently and sustainably.

This study was conducted only on green cuttings of the Jumbo blackberry variety. Studies on different plant species can reveal how media types and application diversity differ according to plant species.

In conclusion, this study provides important information by investigating the effects of different rooting media and application types on plant rooting. However, more comprehensive studies in the future can reinforce these results and allow rooting processes to be made more efficient.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no conflict of interest.

Author contribution

All the authors verify that the text, figures, and tables are original and that they have not been published before. This study was produced from the Fatma DUMLU's master thesis.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.22

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 199-209

Comparative study on identification and pathogenicity of fungal pathogens associated with post-harvest rot of tomatoes (solanum lycopersicum 1.) in Umuahia and Okigwe

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Article History

Received: July 8, 2024 Revised: March 8, 2025 Accepted: March 12, 2025 Published Online: March 14, 2025

Article Info

Article Type: Research Article Article Subject: Horticultural Production

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Available at

tps://dergipark.org.tr/jaefs/issue/90253/1505561







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Abstract

Tomato fruits sold in the market and at home present symptoms during storage, but the disease causal agents must be better documented. This study aimed to identify the fungal pathogens associated with tomato rot bought in markets at Umuahia and Okigwe and to evaluate its pathogenicity and disease prevalence. A total of 24 and 16 fungal isolates were recorded, respectively, and were microscopically identified and morphologically to specific fungal isolates. The identified isolates were Alternaria solani, Althelia rolfsii, Colletotrichium phlomoides, Phytophthora nicotinae, Sclerotinia sclerotiorum, and Sclerotium rolfsii. The percentage frequency of isolation of samples from Umuahia ranged from 6.3% - 31%, respectively. Alternaria solani had the highest frequency of 31%, with the lowest percentage of 6.3% recorded in Sclerotium rolfsii from samples obtained from Umuahia. The same trend was also recorded on isolated samples from okigwe with a percentage frequency of isolation of 29% for Alternaria solani and 8.3% for Sclerotium rolfsii. The high percentage frequency of isolation of Alternaria solani indicates a high chance of these tomato fruits being contaminated with mycotoxins since Alternaria solani is a significant mycotoxigenic fungal genus with notable toxicity. The prevalence of disease incidence (PDI) was conducted to ascertain which locations had the highest rate of fungal rot, and there was a higher PDI of 50% in Umuahia against 33% recorded in Okigwe. The highest disease prevalence recorded in Umuahia could result from poor sanitation, poor storage, overcrowding, and unhygienic practices by fruit handlers in this location.

Keywords: Pathogenicity, Fungal rot, Disease prevalence, Percentage incidence, Rot rating

Cite this article as: Nwaru, E.C., Eke, T., Onyeabor-Chinedum, N.P., Ahaiwe, M.C. (2025). Comparative study on identification and pathogenicity of fungal pathogens associated with post-harvest rot of tomatoes (Solanum lycopersicum 1.) in Umuahia and Okigwe. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 199-209. https://doi.org/10.31015/2025.1.22

INTRODUCTION

Tomatoes are an important crop due to their sensory properties and abundant bioactive compounds. Literature reviews show that tomatoes contain essential nutrients that benefit human health. These include vitamins, minerals and trace minerals that are easily absorbed and help the body strengthen immunity and prevent infections (Ali et al., 2021). However, their susceptibility to contamination to rot by fungus and other pests has increased over the years (Furlong and Rodrigues, 2022). Globally, tomatoes are significant vegetables valued for their nutritional benefits and culinary versatility (Bapary et al., 2024). It is extensively consumed in Nigeria and ranked second most valuable vegetable fruit crop (Yusuf et al., 2020). On average, it is consumed daily at a rate of approximately 18%. The crop is highly perishable, resulting in losses of up to 50% from cultivation to consumption. In 2021, FAO reported that Africa imported around 520,000 tons of tomato puree, representing 15% of the global volume worth US\$500 million. However, the susceptibility of tomatoes to postharvest rot and handling processes poses a significant challenge to maintaining their quality and extending their shelf life (Spricigo et al. 2021). This difficulty is particularly pronounced in underdeveloped nations with limited storage and transportation facilities. Postharvest fungal decay can result in significant financial losses and diminish the accessibility of this crucial food resource (Enyiukwu et al., 2014). A study by Gwa and Lum (2023) showed that postharvest losses in tomatoes vary across locations. These damages disrupt the fruit's physicochemical properties, leading to postharvest losses, which account for 25–42 % of losses globally (Qasim et al., 2022; Roy et al., 2024). Nigeria's tropical climate, with its elevated temperatures and humidity, fosters a favourable setting for the proliferation of fungi and the rapid decay of harvested tomatoes. Due to their significant influence on nutrition, functionality, and economy, multiple cultivars have been genetically modified to thrive in semi-tropical and temperate regions. These cultivars exhibit great production and tolerance to the challenges posed by the field environment, distribution, and processing (Siddique et al., 2015).

Fungal contamination of tomatoes, including species such as Penicillium, Aspergillus, Fusarium, and Alternaria, has been identified in screenings throughout several locations, resulting in frequent fruit loss both in the field and during processing; within this group are species that produce toxins, although investigating these poisons in tomatoes has been limited (Rodrigues & Furlong, 2022). Among them are toxigenic species, but their mycotoxins have been little studied in tomatoes. Alternaria sp. synthesizes approximately seventy secondary metabolites, most of which have been identified as having the ability to function as mycotoxins (Sedighi & Mohammadi, 2024). Moreso are *Collectrotrichumtruncatum*, *Phytophthora infestans*, *Pythium aphanidermatum*, *Dipodascus geotrichum*, *Fusarium curvularia spicifera*, *Cladosporium sp.*, *Penicillum chrysogenum*, *Mucor mucedo*, *Botrytis cinerea*, *etc.*, causing different diseases with distinct symptoms (Tolupe & Odebode, 2021). Alternaria, Aspergillus, Fusarium, Mucor species, Penicillium, Rhizopus, and Trichoderma are pathogenic fungi linked to some types of agricultural deterioration. Fungi that cause tomatoes to deteriorate have not been the subject of many investigations in Nigeria. To determine whether fungal diseases cause tomato fruit to rot after harvest, this study was conducted in Nigeria to isolate, identify and check the pathogenicity of these fungal pathogens associated with fungal rot in tomatoes.

Table1. Fungi and mycotoxins in tomatoes and their products.

Product	Fungus	Mycotoxin	Occurrence (µg.kg-1)	Reference
Ketchup	A. alternata	ALT	21.3	Pavon et al., 2012
Dry tomato	A. alternata	ALT	280.0	Pavon et al., 2012
Dry tomato	A. alternata	AOH	376.0	Pavon et al., 2012
Dry tomato	A. alternata	AME	72.0	Pavon et al., 2012
Tomato sauce	A. alternata	ALT	20.0	Pavon et al., 2012
Fresh tomato	P. expansum	PAT	n.d	Cunha et al., 2014

AOH - Alternariol, AME- Alternariol Monomethyl Ether, ALT- Altenuene, PAT - Patulin. n.d - Not detected.

The European Food Safety Authority as reported by Arcella et al. (2016) states that the primary toxins generated by Alternaria sp. include alternariol (AOH), alternariol monomethyl ether (AME), tentoxine (TeA), and tenuazonic acid (TEN). The primary mycotoxin detected in both dried and fresh tomatoes is TeA. *A. alternata* can infiltrate the fruit surface by exploiting micro-cracks or wounds, swiftly establishing itself in the tissue and resulting in substantial harm. Additionally, research demonstrates that this fungus can generate mycotoxins, which could endanger consumers' health. Tomatoes' vulnerability to fungal infection is affected by multiple factors. This study aimed to identify the fungal pathogens associated with tomato rot bought in markets at Umuahia and Okigwe, in the eastern part of Nigeria, and to evaluate its pathogenicity and disease prevalence.

MATERIALS AND METHODS

Collection of Samples

Eight (8) tomato fruits were collected from different locations (Umuahia and Okigwe). They were wrapped in a sterile zip-lock polyethylene bag and sent to the Mycology Laboratory of the Department of Plant Science and Biotechnology, Abia State University.

Sterilization of Glass wares

All the glassware, including beakers, conical flasks, test tubes, measuring cylinders, a spatula, and a test tube rack, was sterilized in a hot air oven for one hour at 121 degrees centigrade and kept in a laminar airflow chamber.

Sample Preparation

The tomato fruits were washed with sterile water and kept at room temperature (22- 25 degrees Centigrade) for five days to allow fungal rot to develop.

Media Preparation

According to the Manufacturer's instructions, 10.53g of Potato dextrose agar (PDA) was dissolved in 270 ml of distilled water, autoclaved at 121 degrees centigrade for 15 minutes, and allowed to cool. It was supplemented

with 0.1 mg of chloramphenicol antibiotics and dispensed into eighteen (18) 9cm diameter Petri dishes, with two plates serving as controls.

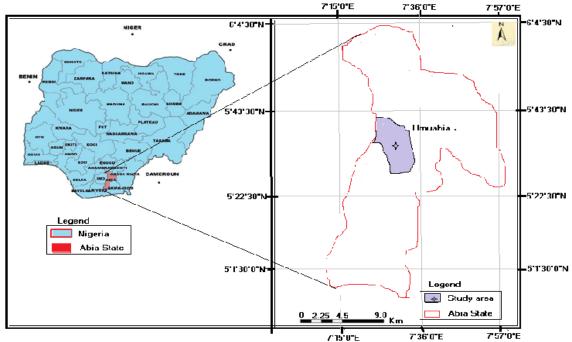


Figure 1. Map of Nigeria showing the study location; Umuahia

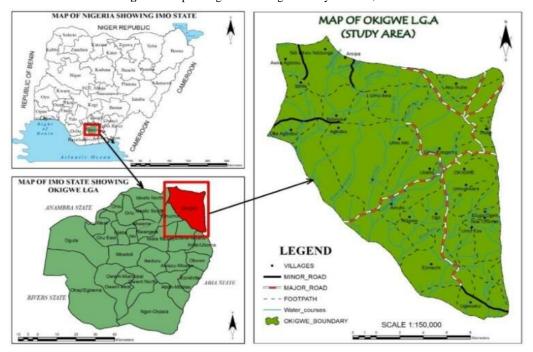


Figure 2. Map of Nigeria showing the study location; Okigwe

Peptone Water Preparation

According to the Manufacturer's instruction, 1.4g of peptone water granules were dissolved in 100 ml of distilled water, autoclaved at 121 degrees Centigrade for 15 minutes, and dispensed into ten (10) beakers (eight for Samples, two for controls). This served as the sample diluent.

Sample Inoculation

A sterile swab stick was used in a randomized control format to swab the tomato fruits with visible fungal rot. Each swab stick was soaked in 10ml aliquots of sterile buffered peptone water for about 120 minutes (2 hours) to dislodge the cells into suspension at ambient temperature. The swab sticks were removed, and the resulting peptone water solution was the test sample. The peptone water was serially diluted (10–1 to 10–6), and each suspension's

10-3 dilution (0.1ml) was inoculated on the already prepared molten potato dextrose agar using the pour plate method. The plates were incubated at 28 degrees Celsius for five days.

Sub-Culturing and Preservation of Isolates

Based on their colonial characteristics, the fungal representative colonies were picked and sub-cultured on potato dextrose agar supplemented with 0.1ml of chloramphenical antibiotics aseptically using the streaking technique and incubated at 28 degrees Celsius. The isolates were preserved in slants of potato dextrose agar in a bottle, which served as a stock for assessing their morphological and biochemical characteristics. The test identification of isolates at the genus level based on cultural morphological and biochemical characteristics was carried out according to Cheesbrough (2000).

Identification of Fungi

After five days, the growth of fungi on potato dextrose agar was examined critically using prepared microscopic slides. The prepared specimens were mounted on KOH Preparation and observed with the microscope's X40 objective lenses.

Pathogenicity Test

The various isolates were subjected to a pathogenicity test to establish Koch's postulates. Healthy tomato fruits were purchased from the market. The fruits were washed with clean tap water to remove any soil debris. The fruits were surface-sterilized in 75% ethanol for about three minutes, rinsed with distilled water, and then air-dried. Each of the isolates was subjected to two methods of inoculation. A sterile cork borer (5mm diameter) was used to wound freshly procured tomato fruits. Mycelial discs of the equivalent diameter obtained from the edge of actively growing pure cultures were placed on the wound concerning the number of isolated pathogens. Two wounded fruits were inoculated with sterile discs and served as controls (Nizamani *et al.*, 2021). The fruits were kept at room temperature of 24 degrees Celsius for five days for possible rot development. The isolates were re-isolated from the new host and compared morphologically to the original isolates. An evaluation was done after five days by cutting the fruits longitudinally and rating the post-harvest fungi rot on a 0-4 rating scale as follows.

0= no visible rot Rot 1 = 1-25%

Rot 2 = 25-50%

Rot 3 = 50-75%

Rot 4 > 75%

RESULTS

Identification and characterization isolates of fungal

A total of five fungal isolates were recorded, namely Alternaria solani, Althelia rolfsii, Colletotrichium phlomoides, Phytophthora nicotinae, Sclerotinia sclerotiorum, and Sclerotium rolfsii. Various parameters, which include the percentage frequency of isolation pathogenicity test, rot ratings, percentage rot range, pathogenicity prevalence, and prevalence of disease incidence, were recorded to ascertain the rot and deterioration effect of the five fungi. Table 2 shows the identification and characterization of the various fungus. The morphology of Alternaria solani showed a straight, flexuous brown mycelium on the PDA. Viewing it under the microscope, oblong conidia were observed. Althelia rolfsii showed smooth, white fruiting bodies with ribbon-like hyphae and clamp connections. Colletotrichium phlomoides showed white to grey pasty colonies with rod-shaped hyphae and single budding cells. The Phytophthora nicotinae showed a dense cottony mycelium that is slightly petaloid in a pattern. Sclerotinia rolfsii showed small tufts of white mycelium that covered the plate in a fan-like pattern. A microscopic view of it showed hyaline thin cell walls. There were sparse, fluffy, creamy white brown to brown colonies observed on Sclerotinia sclerotiorum with spherical to oval hyaline hyphae.

Table 2. Identification and characterization isolates of fungal

Isolates	Morphology	Microscopy	Identified Fungus
A	Straight, flexuous; Brown mycelium on PDA	oblong conidia	Alternaria solani
В	Smooth and white fruiting bodies	ribbon-like hyphae with clamp connection	Athelia rolfsii
С	White to grey pasty colonies	rod-shaped hyphae with single budding cells	Colletotrichium phomides
D	Dense cottony mycelium with slightly petaloid pattern	Papillate ovoid sporangia with oospores	Phytophthora nicotanae
Е	Small tuffs of white mycelium that covers the plate in fan pattern	Hyaline thin cell walls with spare cross walls	Sclerotium rolfsii
F	Sparse fluffy creamy white to brown colonies	Spherical to oval Hyaline hypahe	Sclerotinia sclerotiorum

Percentage frequency of isolation for samples from Umuahia

The result in Table 3 shows the percentage frequency of isolation of samples from Umuahia, which ranged from 6.3% to 31%, respectively. *Alternaria solani* had the highest frequency of 31%, with the least percentage of 6.3% recorded in *Sclerotium rolfsii* from samples obtained at Umuahia.

Table 3. Percentage frequency of isolation for samples from Umuahia

Fungal Isolates	Specific fungal isolates	Total number of fungal isolates	% Isolation Frequency
Alternaria solani	5	16	31%
Athelia rolfsii	3	16	19%
Colletotrichium phomoides	3	16	19%
Phytophthora nicotianae	2	16	13%
Sclerotinia sclerotiorum	2	16	13%
Sclerotium rolfsii	1	16	6.3%

Percentage values were rounded off to the nearest whole numbers.

Percentage frequency of isolation for samples from Okigwe

Table 4 shows the percentage frequency of isolation of Okigwe samples ranging from 8.3% - 29%. The highest percentage frequency of isolation of 29% was recorded on *Alternaria solani*, with the lowest percentage of 8.3% recorded on *Sclerotium rolfsii*

Table 4. Percentage frequency of isolation for samples from Okigwe

Fungal Isolates	Specific fungal isolates	Total number of fungal isolates	% Isolation Frequency	
Alternaria solani	7	24	29%	
Athelia rolfsii	5	24	21%	
Colletotrichium phomoides	4	24	17%	
Phytophthora nicotianae	3	24	13%	
Sclerotinia sclerotiorum	3	24	13%	
Sclerotium rolfsii	2	24	8.3%	

% Isolation =
$$\frac{\text{no. of specific fungal isolate}}{\text{Total no. of isolates fungal isolates}} X = \frac{100}{1}$$

Percentage values were rounded off to the nearest whole numbers

Pathogenicity test, rot ratings and percentage rot range

Results from Table 5 show the pathogenicity test, rot ratings, and percentage rot range of the various fungi. The rot ratings ranged from 1-4. The highest rot rating of 4 was recorded on *Alternaria solani* with a percentage rot range of >75%, followed by *Althelia rolfsii* with a rot rating of 3 and a percentage rot range of 50 -75%. The least rot rating of 1 and percentage rot range of 1-25% was recorded on *Sclerotium rolfsii*

Table 5. Pathogenicity test, rot ratings and percentage rot range

Isolates	Rot Ratings	% Range
Alternaria solani	4	> 75%
Athelia rolfsii	3	50-75%
Colletotrichium phomoides	2	25- 50%
Phytophthora nicotinae	2	25-50%
Sclerotinia sclerotiorum	2	25-50%
Sclerotium rolfsii	1	1-25%.

LEGEND:

Rot 1= 1-25%

Rot 2= 25-50%

Rot 3 = 50 - 75%

Rot 4> 75%

Pathogenicity prevalence

Table 6 shows the pathogenicity prevalence of the isolated fungi on eight samples of the tomato. A pathogenicity prevalence of 13% - 50% was recorded, with the highest prevalence of 50% on *Alternaria solani*, with a rot rating of 4. A pathogenicity prevalence of 25% each was recorded on *Colletotrichum phlomoides*, *Phytophthora nicotinae*, and *Sclerotinia sclerotiorum*. The lowest pathogenicity prevalence, 13%, was recorded in *Sclerotium rolfsii*.

Table 6. Pathogenicity prevalence

Isolates	Rot Ratings	Sample Size	Pathogenicity Prevalence (%)
Alternaria solani	4	8	50%
Athelia rolfsii	3	8	38%
Colletotrichium phomoides	2	8	25%
Phytophthora nicotianae	2	8	25%
Sclerotinia sclerotiorum	2	8	25%
Sclerotium rolfsii	1	8	13%

Pathogenicity prevalence =
$$\frac{\text{Rot ratings}}{\text{Sample size}}$$
 \times $\frac{100}{1}$

Percentage values were rounded off to the nearest whole numbers.

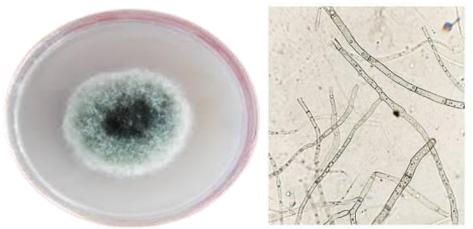


Figure 3. (a) Micrograph of Culture of A. solani (b): Micrograph of Culture of A. Solani



Figure 4. (a): Culture plate of Athelia rolfsii (b): Micrograph of Athelia rolfsii

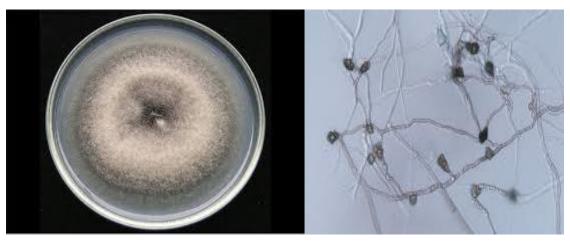


Figure 5. (a): Culture of Colletotrichium phomoides (b): Micrograph of C. phomoides



 $\textbf{Figure 6.} \ \ (a): Culture \ of \ \textit{Phytophthora nicotianae} \ \ \ (b): Micrograph \ of \ \textit{Phytophthora nicotianae}$



Figure 7. (a): Culture of Sclerotinia sclerotiorum (b): Micrograph of Sclerotinia sclerotiorum



Figure 8. (a): Culture of Sclerotium rolfsii (b): Micrograph of Sclerotium rolfsii

Prevalence of disease incidence for the different locations (pdi)

Table 7 shows the disease incidence (PDI) prevalence for the different locations to ascertain which locations had the highest prevalence. There was a higher PDI of 50% in Umuahia against 33% recorded in Okigwe.

Table 7. Prevalence of disease incidence for the different locations

Sample Location	Total no. of Isolates	f Isolates Total no. of samples	
I I	16	0	50
Umuahia	16	ð	50
Okigwe	24	8	33

Prevalence of disease incidence = $\frac{\text{Sample size}}{\text{No. of Isolates}}$ × $\frac{100}{\text{100}}$

DISCUSSION

This study was conducted in two markets: Eke Okigwe, Okigwe Imo state, and Isi gate market, Umuahia North, Abia State, Nigeria. The isolation frequency differed across both towns. The fungus was isolated more frequently in Umuahia than in Okigwe. Comparing our report with that of Kabiru & Yusuf (2023), who reported an isolation frequency of 7.7%—100%, we had an isolation frequency of 8.3%—29% in Okigwe and 6.3%—31% in Umuahia, even though the isolated fungus differed. The differences in the frequency of isolation could be attributed to the tomato variety, locations and the population in the various towns. However, this report shows the susceptibility of tomato fruit to fungal rot and deterioration, which complies with our report. The highest prevalence disease incidence (PDI) of 50% was recorded in Umuahia. This high PDI could result from the high population density in Umuahia compared to Okigwe, which has fewer people. The percentage frequency of isolation for samples from Umuahia showed significant variation from that of Okigwe. Isi gate market located in Umuahia had a percentage frequency (PF) of 6.3% - 31% compared to Eke Okigwe, 8.3% - 29%. *Alternaria solani* from both markets had the highest PF except for *Phytophthora nicotianae and Sclerotinia sclerotiorum*, which had 13% PF in both markets. The high PF frequency, as reported in our work, although higher in *Alternaria solani*, conforms with Osemwegie et al., 2019; Kaur and Banyal, 2019 who carried out a study on fungal pathogens associated with the rot of Tomato fruits and reported *Phytophthora nicotianae and Sclerotium rolfsii* as fungal isolates which causes tomato rot.

In our report, *Alternaria solani* had the highest rot ratings of 4, with a 75% rot range and 50% pathogenicity prevalence in both markets, which conforms to the reports of Schmey *et al.* (2023) and Aminuzzaman *et al.* (2021) on the high disease prevalence of *A. solani* as a blight of tomato. 85.6% and 29% for disease incidence and 29% severity, respectively, were reported by Aminuzzaman *et al.* (2021) for *A. solani, which aligns with* the 29% isolation frequency as reported in our work. One of the primary diseases found in most post-harvest products is caused by fungal pathogens, as reported by Koka *et al.* (2022) on the incidence and severity of tomato rot, which conforms with the identified and isolated fungus in our result.

Of the total of six fungal isolates, Alternaria solani, Athelia rolfsii, Colletotrichium phomoides, Phytophthora nicotianae, Sclerotinia sclerotiorum, and Sclerotium rolfsii, Alternaria solani had the highest pathogenicity prevalence which is however different from what was reported by many researchers who reported Aspergillus

niger as the most frequent in occurrence in their studies (Mailafia et al., 2017). However, reports on *Sclerotinia rot*, caused by *Sclerotinia sclerotiorum*, which affects tomatoes, have also been documented by Laurence *et al.* 2014; McGovern, 2015), which conforms with our report.

Studies on the isolation and identification of fungi associated with spoilt fruits in Gwagwalada market, Abuja, Nigeria, also carried out by Mailafia et al. (2017) in which Aspergillus niger, Fusarium avenaceum, Penicillium digitatum and Rhizopus stolonifer were identified against our report. Reports on Sclerotinia rot, caused by Sclerotinia sclerotiorum, which affects tomatoes, have also been documented (Laurence et al. 2014; McGovern, 2015), which conforms with our report. In storage conditions, Shakya and Aryal (2020) reported that several species of fungi, such as Alternaria alternata and Collectrotrichum truncatum, often infect tomatoes. Similar reports by Schmey et al. (2023) were also reported on the Alternaria diseases in potatoes and tomatoes, which conforms with our report.

CONCLUSION

Six fungal isolates were isolated from the tomato sample collected from the two major cities, Umuahia and Okigwe, in Abia and Imo State, respectively—Alternaria solani, Athelia rolfsii, Colletotrichium phomoides, Phytophthora nicotianae, Sclerotinia sclerotiorum, and Sclerotium rolfsii. The highest pathogenicity prevalence was observed in Alternaria solani and Athelia rolfsii, with the least recorded on Sclerotium rolfsii. Alternaria solani and Athelia rolfsii were also found to be the most pathogenic, with a high percentage rot range. Both fungi also had high percentage isolation frequency, with the highest prevalence of disease incidence recorded in the tomato fruit from Umuahia. Although morphological characteristics, as applied in this report, were used to identify and characterize the fungi isolates, the genus is often tricky due to overlaps in the configuration of morphological features among identical species. Identifying fungi isolates based on morphological characteristics may be deceptive in ascertaining the genus; thus, we recommend combining the morphological characteristics and molecular analysis approach as ideal for accurate identification. There is also the need to develop target fungicides against Alternaria solani, Athelia rolfsii, Colletotrichium phomoides, Phytophthora nicotianae, Sclerotinia sclerotiorum, and Sclerotium rolfsii to reduce post-harvest losses associated with tomatoes in Nigeria.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no known competing conflicting interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Ezeibe Chidi Nwaru: Writing – original draft, Supervision, Methodology, Data curation, Conceptualization; Eke Tobechukwu: Writing – Review and editing; Nkechi P. Onyeabor: Writing – Review and editing; Matthew Chiemerie Ahaiwe: Writing – Review and editing

Acknowledgments

The authors acknowldeges the support of the Head of Department, Plant Science and Biotechnology and the Dean Faculty of Biological Sciences for making available a condusive environment and laboratory to carry the research work.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.23

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 210-220

Impact of high-intensity ultrasound on phenolic content and quality of black tea wine

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Article History

Received: January 11, 2025 Revised: March 8, 2025 Accepted: March 12, 2025 Published Online: March 14, 2025

Article Info

Article Type: Research Article Article Subject: Fermentation Technology

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Available at

://dergipark.org.tr/jaefs/issue/90253/1617958

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Abstract

Tea is the most popular non-alcoholic beverage in Turkiye and worldwide. Black tea, a product of tea leaves fermentation, is the most consumed form of tea in Turkiye. Although a significant amount of tea is produced and consumed globally, there are limited alternative products developed in this field apart from traditional black tea. The objective of this study was to produce tea wine with a high phenolic content from the highly popular and widely consumed black tea in Türkiye. The study aimed to evaluate the influence of ultrasound treatment on the physicochemical properties and overall quality of tea wine, with a particular focus on its impact on phenolic content. The results indicated that ultrasound treatment significantly affected physicochemical properties of tea wine such as total acidity, volatile acidity, total soluble solids, reducing sugar (p<0.05). Ultrasound treatment after brewing increased the total phenolic content (TPC) of tea infusions by 49%. The TPC levels of the samples decreased after fermentation but no significant change occurred in TPC levels duration of two months aging. The color parameters of tea wine were also affected from ultrasound treatment, fermentation process and aging. The L* value of tea wines significantly decreased to 66.41 in samples treated with 50% ultrasound for 8 minutes. Ultrasound treatment was found to influence sensory attributes, with increased amplitude and duration having a negative impact on taste. While there is limited research in the literature on tea-flavored, wine-based beverages, also known as tea wine, our project seeks to produce tea wine using a standardized process. We employed ultrasound, a new food preservation technique, to create an alcoholic drink with high phenolic content from brewed black tea, suitable for year-round production. It is expected that the findings highlighted the potential of tea in the production of diverse products, contributing to the expansion of tea consumption into new areas. Keywords: Black tea, Tea wines, Phenolic compounds, Ultrasound, Functional beverages

Cite this article as: Uzun, H.O., Uzun, S. (2025). Impact of high-intensity ultrasound on phenolic content and quality of black tea wine. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 210-220. https://doi.org/10.31015/2025.1.23

INTRODUCTION

Tea (Camellia sinensis), a plant native to Southeast Asia, is cultivated in 30 countries worldwide, including Türkiye. For the past 80 years, tea has been an economically significant crop in Türkiye, which ranks third globally in per capita tea consumption. Tea, the second most consumed non-alcoholic beverage after water, is also an essential part of Turkish culture.

Turkish tea is renowned globally for its unique flavor, which is attributed to the distinctive climatic conditions of its growing region. Black tea is the most produced and consumed type in Turkey. It contains a range of beneficial compounds, including phenolic compounds, free amino acids, alkaloids, and volatile aroma compounds. Among its polyphenols, epigallocatechin gallate (EGCG), theaflavin, and thearubigin exhibit potent antioxidant properties, with studies highlighting their anti-cancer and strong antiviral activities (Elmas et al., 2019). In black tea production process, catechins are oxidized and polymerized into theaflavins and thearubigins. These compounds

play a crucial role in determining the tea's color, flavor, and brightness. Furthermore, the concentration of specific theaflavins has been strongly associated with tea taster evaluations and quality ratings (Erol et al., 2010).

In Turkiye, black tea is traditionally brewed before consumption. During the brewing process, phenolic compounds from the tea leaves such as theaflavins and thearubigins are released into the water, which shapes the sensory characteristics such as taste, color, and aroma (Erol et al., 2010). To enhance the extraction efficiency of phenolic compounds from foods, modern technologies such as supercritical fluid extraction, pressurized liquid extraction, high hydrostatic pressure, microwave, and ultrasound are increasingly employed (Alara et al., 2021). Ultrasound-assisted extraction, is considered as a promising non-conventional technology for extracting compounds as typically uses less solvent, requires shorter extraction times, and achieves higher yields while preserving thermo-sensitive compounds by minimizing their degradation (Yusoff et al., 2022). Therefore, the application of ultrasound emerges as a promising technique to enhance the release of phenolic compounds during tea brewing. Raghunath & Mallikarjunan (2020) demonstrated that applying ultrasound during the black tea brewing process significantly enhanced the total phenolic and flavonoid content, as well as the antioxidant activity of the tea.

Although Turkiye is a prominent global tea producer, innovation in tea-based products beyond traditional production methods remains limited. While industrial initiatives are underway to expand tea's applications, there is a continued demand for alternative and novel products. One such innovative creation is tea wine, a fermented beverage that blends the flavors of tea and wine into a unique functional drink (Xu et al., 2024). Tea wine is produced by fermenting tea leaf extracts or brewed tea with sugar and yeast, offering a distinctive aroma and a rich profile of polyphenolic compounds, which have made it increasingly popular among consumers (F. Wu et al., 2024).

This study aimed to develop a phenolic-rich tea wine as a new functional product using black Turkish tea. The research focused on optimizing the production process and increasing the phenolic content of the tea wine through ultrasound treatment. The effects of ultrasound at different intensities and durations on the physicochemical properties and phenolic content of the tea wine were explored, with the goal of creating a product that offers both health benefits and sensory appeal.

MATERIALS AND METHODS

Materials

Black tea, specifically 'Çaykur Siftings Tea,' a specialty tea available exclusively in the Rize province, was obtained from Çaykur (Turkiye). The sugar, wine yeast, and lemon needed for the production of tea wine were purchased from a local retail store. Folin–Ciocalteu reagent and all other chemicals used in the analysis were purchased from Merck (St. Louis, MO, USA).

Production of tea wine with ultrasound-assisted method

5 g of black tea leaves were weighed and placed into a stainless-steel teapot. Boiling water (0.5 L at 100 °C) was poured over the tea leaves, and the mixture was allowed to brew for 10 min. After the infusion time, the tea infusion was transferred to a 500-mL beaker, and the sonicator probe was immersed to ensure maximum exposure to sonic waves. Ultrasound treatment was applied to the tea infusions at amplitudes of 50% and 70% for durations of 4, 6, and 8 min. All sonication treatments were performed using an ultrasonicator (Bandelin Sonoplus, Germany) equipped with a titanium alloy flat-tip probe with a diameter of 13 mm. The samples were left at room temperature for 30 minutes to facilitate mass transfer. Subsequently, the ultrasonicated black tea infusions were filtered to separate the tea leaves from the infusion. Sugar (80 g) and yeast (1 g) were then added to the black tea infusions, and the mixture was stirred at 600 rpm for 10 minutes to ensure complete dissolution. Finally, the prepared tea infusion was transferred to a fermentation container and incubated at 25 °C for three months. After fermentation, the broth was extracted, clarified, filtered, and pasteurized at 65 °C for 10 minutes to produce the final tea wine. The samples were coded based on the ultrasound amplitude and treatment duration. Samples treated at 50% amplitude were labeled as U50-4, U50-6, and U50-8 for 4, 6, and 8 minutes, respectively, while those treated at 70% amplitude were designated as U70-4, U70-6, and U70-8.

Physicochemical analysis

Tea wine density was determined using pycnometer according to Turkish Standards 522. The following formula was used to calculate tea wine density:

density of tea wine =
$$\frac{\text{mass of pycnometer full of tea wine} - \text{mass of pycnometer}}{\text{volume of pycnometer}}$$

Total soluble solids, alcohol, and ash content of tea wine was measured according to OIV Compendium of International Methods of Analysis of Wine and Musts (OIV, 2023). pH of tea wine samples was measured with a pH meter (HI2002, Hanna Instruments, USA) at room temperature.

Total acidity was determined by mixing 25 mL of tea wine with a few drops of 2% phenolphthalein solution and titrated with N/3 NaOH (Y. Y. Huang et al., 2021). The titration was stopped when the sample turned a violet

color, and the amount of NaOH consumed was used to calculate the total acidity of the tea wine. The amount of NaOH used indicates the total acidity in terms of tartaric acid, expressed as g/L.

To determine the volatile acidity of the tea wines, 20 mL of the sample was mixed with 35 mL of distilled water and distilled for 30 minutes until a sufficient amount of liquid was collected. Subsequently, 1–2 drops of phenolphthalein were added to the distillate, and it was titrated with 0.1N NaOH. The amount of alkali consumed was multiplied by 0.375 and 0.306 to calculate the volatile acidity in terms of acetic acid and sulfuric acid, expressed as g/L (Aktan and Yıldırım, 2012).

The reducing sugar content was determined according to Neto et al. (2015) in this method, 25 mL tea wine sample was diluted with 250 mL of distilled water. The sample was then filtered, and titration was performed. 5 mL of Fehling A and 5 mL of Fehling B solutions were added into 25 mL of distilled water and then the mixture was heated to boiling. After boiling for 2 minutes, 2–3 drops of methylene blue were added, and titration was carried out by adding tea wine dropwise to the mixture over a flame, until the color changed from blue to copper red. The volume of titrant used was then used to calculate the reducing sugar content.

$$reducing \ sugar \ (\frac{g}{100} \, ml) = \frac{Factor \times dilution \ factor}{volume \ of \ titrant} \times 100$$

Total phenolic analysis

The total phenolic content of tea wine samples was evaluated as expressed by Stevanato et al. (2004). 1 mL of tea wine was added to 75 mL of distilled water. Then, 5 mL of Folin-Ciocalteu reagent was introduced, and the mixture was shaken thoroughly to ensure proper mixing. After a 3-minute incubation, 10 mL of saturated Na₂CO₃ solution was added, and the volume was adjusted to 100 mL with distilled water. The mixture was vortexed to achieve homogeneity and left to stand for 1 hour. Absorbance was measured at 720 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). The obtained absorbance values were evaluated using a standard calibration curve (Başoğlu and Uylaşer, 2016). For the calibration curve, gallic acid solutions with concentrations of 50, 100, 200, 300, and 400 mg/L were prepared, and absorbance readings were recorded. The calibration curve was constructed based on these absorbance values (Cemeroglu, 2013).

Color analysis

The color analysis of the tea wines was performed by direct reading on a Konica Minolta CR-5 colorimeter (Konica Minolta Sensing, Netherlands). The color values (L, a, b) of the product were measured, and the total color difference (ΔE) was calculated using the following formula:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}$$

where, ΔE represents color difference; L_0 , a_0 , and b_0 represent lightness, redness and yellowness values of tea infusions before fermentation and L, a, and b represent lightness, redness and yellowness values of tea wine after fermentation.

Sensory analysis

The sensory characteristics of the tea wine were evaluated using a hedonic scale. Sensory analysis was conducted by 11 panelists' ages at 18 or older. The product's taste, aroma, fragrance, color, acidity, turbidity, bitterness, and overall liking were assessed. A scoring system ranging from 1 to 5 was used for the sensory characteristics of the product. In this scoring system, 1 indicates very poor, 2 indicates poor, 3 indicates average, 4 indicates acceptable, and 5 indicates very good.

Statistical analysis

The effect of ultrasound application on the physicochemical properties and phenolic compound content of tea wines during production was analyzed using a one-way ANOVA test at a 95% confidence level, performed with OriginLab (Origin Pro 8.5, MA, USA) software. The significance of differences between groups was determined using the Tukey test.

RESULTS AND DISCUSSIONS

Influence of ultrasound process parameters on the physico-chemical properties of tea wine Density and alcohol concentration

The density and alcohol concentration results of tea wine are shown in Table 1. After fermentation, the density of these beverages was found to range between 987.3 and 991.7 g/mL. Among the samples, the control (brewed tea before fermentation) had the highest density, while the sample treated with ultrasound at 50% amplitude for 6 min. after fermentation exhibited the lowest density. This difference is attributed to the alcohol content generated during fermentation, as alcohol's lower density compared to water reduces the overall density of beverages with higher alcohol levels. The highest alcohol content was observed in samples treated with ultrasound at 50% amplitude for 6 and 8 minutes, while the lowest was found in samples without ultrasound treatment. Table 1 indicates that the alcohol content of the tea wines formed through fermentation ranged from 5.87% to 9.52%.

Ultrasound application was found to influence alcohol production during fermentation. Previous studies on wine production from black tea have reported achieving alcohol levels of up to 14% by volume (Chen et al., 2023). The lower alcohol content in the current samples may result from the low alcohol tolerance of the yeast used or the absence of bioreactors providing optimal conditions for controlled fermentation. Enhancing the alcohol content in tea wine could involve using a yeast strain with higher alcohol tolerance and adjusting sugar levels.

Acidity

The total acidity values, expressed as tartaric acid, for tea wine are presented in Table 1. Acidity is a key indicator of wine quality, as it enhances flavor and inhibits the growth of harmful microorganisms during fermentation. The acidity of both ultrasound treated and untreated tea wines ranged from 7.87 to 9.41 g/L. The lowest acidity was observed in the control sample without ultrasound treatment, while the highest was found in the sample treated with ultrasound at 50% amplitude for 8 min. Among the samples, only the U50-8 sample showed a statistically significant difference in acidity compared to the others (p<0.05) following ultrasound treatment. An increasing trend in wine titratable acidity was also observed in a previous study when ultrasound treatment time was extended (Ifrim et al., 2024). Patel et al., (2010) reported that the total acidity of Sauvignon Blanc wine, expressed as tartaric acid, ranges from 7.86 to 8.85 g/L. Similarly, the titratable acidity of Cabernet Sauvignon wines was found to vary between 6.10 and 7.33 g/L in another study (Berbegal et al., 2023). In contrast, studies on tea wines have shown a wide range of results. Xu et al., (2023) reported that the total acidity of wines produced from Ziyan tea fermentation ranged from 0.99 to 2.76 g/L, depending on the fermentation duration. For rice tea wine, a traditional Asian beverage, Wang et al. (2023) reported a total acidity of 6.08 g/L. Kumar et al. (2020) noted that in tea-flavored wines prepared by brewing tea in apple juice, total acidity ranged from 0.86% to 1.02% malic acid during a six-month fermentation period. Huang et al. (2024) investigated the effects of low-temperature plasma, high-temperature high-pressure, and UV treatments on the physicochemical properties of tea wine. They observed significant variations in the titratable acidity of tea wines depending on the treatment, with acidity ranging from 4.0 to 4.8 g/L.

Volatile acidity

During alcoholic fermentation, volatile acids are produced, with acetic acid being a major component. The quantity of volatile acids formed depends on the composition of the must (acids, sugars, and nitrogenous compounds), the yeast strain used, and the fermentation conditions (Burin et al., 2015; Mangas et al., 2023). The results of volatile acid analysis after three months of fermentation for tea wine are summarized in Table 1.

In ultrasound-treated and untreated tea wines, volatile acid concentrations ranged from 0.20 to 0.50 g/L (expressed as acetic acid). Overall, fermentation and ultrasound treatment had no significant effect on volatile acid levels (p > 0.05). However, the U70-6 sample, among the ultrasound-treated beverages, showed significantly higher levels of volatile acids (p < 0.05). Due to their high sensory threshold, volatile acids generally have a limited impact on wine aroma. However, excessive levels of volatile acidity, generated during alcoholic fermentation, is particularly harmful to the quality of wine. In the tea wine, volatile acid levels remained below the acceptable limit of 0.72 g/L of acetic acid (Ribéreau-Gayon et al., 2006). For comparison, volatile acid levels were reported to be 0.03% (as acetic acid) in wines made by fermenting tea brewed in apple juice (Kumar et al., 2020). Furthermore, research showed that increasing the power and duration of ultrasound treatment during the tea-based aromatization of Chardonnay wines resulted in elevated volatile acid levels (Liang et al., 2023). Similarly, Merlot red wines pretreated with higher ultrasound power exhibited increased levels of volatile acidity which is consistent with our findings (Xie et al., 2023).

pН

Grapes, one of the most commonly used fruits in winemaking, possess naturally acidic properties, with acidity levels varying by variety. Puertas et al. (2008) reported a pH of 3.84 for free-run juice derived from Tempranillo grapes. Similarly, Jiang & Zhang (2010) found that juice from Cabernet Sauvignon grapes grown in China's Loess Plateau had a pH of 3.1, while Chardonnay grape juice had a pH of 3.1 (Szövényi et al., 2024). However, tea does not naturally exhibit acidic properties, making it more susceptible to spoilage during fermentation compared to grapes. To reduce this risk, lemon juice was added to the tea-based wine before fermentation, adjusting the pH to 3.86. Fermentation was then carried out under these conditions. The pH values of the resulting wine, after a threemonth fermentation period, are presented in Table 1. The pH of the tea wines was found to range from 3.90 to 4.14. While the U50-8 sample exhibited a slightly higher pH, the ultrasound treatment overall did not have a significant effect on the pH of the tea wine. Gómez Gallego et al. (2013) observed that the pH of Moravia Dulce wine ranged from 3.67 to 3.83, Rojal wine from 3.69 to 3.85, and Tortosi wine from 3.80 to 3.87 (Tetik and Selli, 2018). While the composition of tea-based wine differs from that of traditional grape wine, it was notable that the control sample without ultrasound treatment and the U70-6 group had pH values similar to those of Rojal wine. The tea wine demonstrated acidic characteristics, with pH levels close to those of standard grape wines. The results of this study are consistent with findings in the literature. For instance, in a study on Ziyan tea wine, Lin et al. (2024) reported that the pH decreased during aerobic fermentation but showed no significant change once anaerobic fermentation began.

Total soluble solids

The impact of ultrasound application on the total soluble solids (TSS) content of tea wine was investigated, and the resulting TSS values are presented in Table 1. The TSS content of the wines ranged from 0.35% to 0.76%. The lowest TSS levels were observed in the U70-8 and U50-8 samples, while the highest levels were found in the control group and in the U70-6 wines treated with ultrasound at 70% amplitude for 6 minutes. The observed variations in TSS content are believed to be linked to yeast activity during fermentation. Lin et al. (2024) reported a significant decrease in TSS content, from 15% to 8%, in Ziyan tea wines after 12 days of fermentation due to yeast activity. Similarly, Zou et al. (2023) found that TSS content in tea wines decreased significantly over a 20-day fermentation period, attributing the decrease to the role of tea in supporting yeast sugar consumption. These results suggest that ultrasound treatment influences the production and consumption of compounds, such as sugars, during fermentation, likely due to the activity of yeast, which leads to changes in total soluble content.

Ash

The ash content of tea wines is presented in Table 1. Ultrasound treatment was found to increase the ash content, with the highest values observed in the samples treated with ultrasound at 70% amplitude. Among these, the U70-6 samples exhibited the highest ash content, while the control samples, which were obtained without ultrasound treatment, had the lowest. The elevated ash content in the samples treated with 70% ultrasound amplitude may be attributed to the fragmentation of tea leaves due to the high ultrasound power, leading to the formation of small particles that were not fully separated during the filtration process. Additionally, it was observed that these small tea leaf particles contributed to increased turbidity.

Reducing sugar

Table 1 presents the reducing sugar content of the tea wines. In tea wines, the reducing sugar content ranged from 4.6 to 8.3 g/L. Since tea does not contain the necessary nutrients for yeast fermentation, sucrose was added to facilitate the process. At the beginning of fermentation, the brewed tea contained no reducing sugar; however, the added sucrose was converted into reducing sugar by the Saccharomyces cerevisiae yeast during fermentation, resulting in an increase in reducing sugar levels. Upon examining the reducing sugar content of the tea wine, it was found that ultrasound treatment significantly (p < 0.05) impacted the reducing sugar levels after three months of fermentation. In the control samples without ultrasound treatment, the reducing sugar level was 4.6 ± 0.3 g/L, whereas the level increased to 8.3 ± 0.1 g/L in the ultrasound-treated brewed teas. Ultrasound treatment was shown to increase the reducing sugar content in the tea wine. The highest reducing sugar levels were observed in the samples treated with ultrasound at 70% amplitude. The reducing sugar content was 8.3 ± 0.1 g/L in the samples treated for 6 minutes with ultrasound at 70% amplitude, and 6.6 ± 0.5 g/L in the samples treated for 8 minutes. Previous studies have also highlighted that ultrasound treatment increases the reducing sugar content in alcoholic beverages like wine (Martínez-Pérez et al., 2020). For example, in wines produced from Monastrell red grapes, ultrasound treatment resulted in an increase in reducing sugar content from 1.8 g/L to 2.1 g/L (Martínez-Pérez et al., 2020). Additionally, Joshi & Kumar (2017) found that in tea wines made with different sugar sources, such as sucrose (3.14 g/L), apple concentrate (6.09 g/L), and honey (6.91 g/L), the reducing sugar content varied depending on the sugar source.

Total Phenolic Content

The amount of phenolic compounds in tea wines is presented in mg GAE/L in Figure 1. The total phenolic content (TPC) was measured both before fermentation, after brewing the tea, and in the ultrasound-treated brewed teas. The phenolic content before fermentation is expressed as "initial" in both the control (untreated) and ultrasound-treated samples in Figure 1. The TPC of the brewed black tea (315.71 ± 6.06 mg GAE/L) is consistent with the study conducted by Bagheri et al. (2021). It was observed that ultrasound treatment increased the transfer of phenolic compounds from the tea leaves into the water. The amplitude and duration of ultrasound significantly (p<0.05) affected the transfer of phenolic compounds (Figure 1). After ultrasound treatment, the phenolic content significantly (p<0.05) increased in all samples. The highest phenolic content was found in the samples treated with ultrasound at 50% amplitude for 4 min (U50-4). Ultrasound treatment enhances the extraction process by causing cavitation bubbles to collapse, which breaks cell walls and releases active compounds. This process reduces particle size, accelerates mass transfer through diffusion, and increases extraction efficiency (Ozsefil & Ziylan-Yavas, 2023). Borah et al. (2024) reported that ultrasound duration increased the extraction efficiency of polyphenolic compounds from green tea leaves in the aqueous phase with increasing temperature. Similarly, Both et al. (2014) found a 15% increase in polyphenol extraction using ultrasound-assisted methods compared to conventional methods for black tea, while a 49% increase in TPC was noted in the U50-4 tea wine.

Although ultrasound treatment increased TPC, no linear correlation was established between the amplitude and the transfer of phenolic compounds. The highest TPC was obtained at 50% amplitude, while a decrease in TPC was observed at 70% amplitude. Generally, there was no statistically significant difference between the total phenolic content at 50% and 70% amplitudes. However, in the samples treated for 8 minutes, the phenolic content was significantly lower at 70% amplitude. Similarly, increasing ultrasound treatment duration did not result in a linear increase in phenolic transfer. This may be due to the formation of larger cavitation bubbles during ultrasound treatment, the effects of decomposition, or the increased formation of hydroxyl radicals under high ultrasonic power, which may react with phenolic compounds and lead to their degradation (Afroz Bakht et al., 2019; Ozsefil

& Ziylan-Yavas, 2023; Z. L. Wu et al., 2008). Qiao et al. (2013) investigated the effect of ultrasound treatment on the stability of phenolic acids in different solvents, finding that ultrasound power and frequency, as well as the liquid volume, significantly affected the degradation of phenolic compounds. Based on the obtained data, the optimal amplitude and duration for producing tea wines with high phenolic content were determined to be 50% and 4 minutes, respectively.

The phenolic compound content of tea wines was analyzed after three months of fermentation, and the results are presented in Figure 1. A significant reduction (p<0.05) in phenolic content was observed across all samples following fermentation. The lowest phenolic content was recorded in the control sample at 106.43 mg GAE/L, while the highest was found in the U50-8 sample, which contained 204.29 mg GAE/L. The U50-4 sample showed the second-highest phenolic content. The degradation of phenolic compounds over time is a well-documented phenomenon. Šilarová et al. (2017) studied the degradation of catechins and other green tea phenolic compounds during a six-month storage period and found that the most significant degradation occurred within the first three weeks. During fermentation, the activity of yeasts likely contributes to the observed reduction in phenolic content. The degradation or biotransformation of phenolic compounds due to microbial activity is a common occurrence during the fermentation of phenolic-rich foods. For instance, Valero-Cases et al. (2017) reported that fermentation of pomegranate juice with various lactic acid bacteria led to the complete degradation of epicatechin and catechin. However, bacterial activity also resulted in the formation of new catechin-derived phenolic compounds and a decrease in ellagic acid content. Similarly, Chen et al. (2023) found that increasing the yeast concentration during fermentation significantly reduced the total phenolic content in tea wines. To evaluate changes in phenolic compounds during storage, an analysis was conducted after two months of storage following fermentation. The results revealed no significant changes in the total phenolic content during this storage period.

Color parameters in tea wine

Color is one of the most critical quality parameters in wine production and a key factor influencing consumer preferences (Fairchild, 2018). Grapes, pears, apples, strawberries, and raspberries are commonly used in winemaking, with phenolic compounds and anthocyanins from these fruits playing a significant role in determining the color of the wine. The development of wine color begins during fermentation and continues throughout storage, as the phenolic compounds in the fruit interact to produce a stable color profile.

Tea is a beverage naturally rich in phenolic compounds, which also contribute to the color development of tea wines. The results of color analysis conducted after a 3-month fermentation period for tea wine are shown in Table 2. The analysis revealed that ultrasound treatment significantly influenced the color properties of the beverages.

The brightness (L*) values decreased significantly in samples treated with ultrasound at 50% amplitude, indicating a darker product. The brightness value of the beverage treated with ultrasound at 50% amplitude for 8 minutes (U50-8) was measured at 66.41 ± 1.14 . This reduction in brightness is attributed to the increased extraction of phenolic compounds from tea leaves facilitated by ultrasound. The enhanced phenolic content in ultrasound-treated tea samples (Figure 1) likely contributed to the observed decrease in brightness. However, no significant statistical difference (p>0.05) in brightness was found in the U70-8 sample.

Ultrasound treatment also caused a significant increase (p<0.05) in the redness (a^*) values of the beverages. The highest redness value, 14.8, was observed in the U50-8 sample, while the untreated control group exhibited the lowest redness. Similarly, the yellowness (b^*) values were significantly higher in the ultrasound-treated samples, with the U50-8 sample showing the highest yellowness value. Among all the samples, the U50-8 beverages exhibited the most significant overall color change (ΔE) due to ultrasound treatment.

After a 2-month storage period following fermentation, the color properties of the tea wines are presented in Table 3. During storage, the brightness (L*) values decreased further, indicating a continued darkening of the beverages. Additionally, the redness (a*) values declined, while the yellowness (b*) values increased over time. The most pronounced color changes during storage were observed in the ultrasound-treated U50-8 samples, whereas the untreated control group exhibited the least changes.

These findings highlight the substantial impact of ultrasound treatment and storage duration on the color attributes of tea wines, emphasizing the role of phenolic compounds in defining and maintaining color stability.

Sensory analysis

Phenolic compounds not only play a crucial role in determining the color of tea but also significantly contribute to its sensory characteristics. During brewing, these compounds transfer from the tea leaves to the water, imparting attributes such as bitterness and astringency, or enhancing aroma and flavor, depending on their concentration and composition.

The sensory evaluation of black tea wine was conducted by 11 panelists. The wines were assessed for taste, aroma, clarity, color, acidity, aroma, astringency, and overall acceptability. The results, displayed in a radar chart (Figure 2), revealed that tea wine samples without ultrasound treatment received the highest overall acceptability scores (>4). Ultrasound treatment was found to influence sensory attributes, with increased amplitude and duration having a negative impact on taste. Among the samples, U50-4 was the most favored in terms of taste, followed by the control sample, while U70-8 was the least preferred.

Astringency levels were highest in the U70-8 sample, followed by U70-6, U50-8, U50-6, U50-4, and the control group. The main contributors to taste and astringency in the beverages include phenolic compounds, free amino acids, sugars, organic acids, and caffeine (Alasalvar et al., 2012). The highest phenolic content was observed in the U50-4 sample, highlighting the significant role of black tea's phenolic compounds in shaping the sensory profile of tea-flavored aromatized wine. The specific type of phenolic compound also affects the resulting taste and aroma. For instance, oligomeric or polymeric flavan-3-ols exhibit less bitterness and astringency compared to their monomeric counterparts (Kraujalyte et al., 2016). Conversely, tannic acid, theaflavin, and thearubigin are known to be responsible for the astringent taste in black tea (Alasalvar et al., 2012). Additionally, caffeine has been reported to contribute to astringency (Xu et al., 2018).

The pronounced astringency observed in the U70-8 sample may not solely be due to its phenolic content but rather to the ultrasound treatment at 70% amplitude, which caused increased fragmentation of tea leaves. This likely facilitated the release of astringent compounds such as tannic acid, theaflavin, thearubigin, or caffeine into the liquid phase. Supporting this, Kowalski et al. (2019) demonstrated that ultrasound-assisted brewing of black tea increased flavonoid content by 29%, polyphenol content by 34%, and caffeine content by 51%.

In terms of clarity, the control sample was rated the highest, followed by the U50-4 sample. Clarity was influenced by the amplitude and duration of ultrasound application. The reduction in clarity and the appearance of turbidity were attributed to the fragmentation of tea leaves during ultrasound treatment, which resulted in the formation of small particles suspended in the liquid.

Tablo 1. Physico-chemical parameters of tea wine treated with ultrasound before fermentation

	Density	Total soluble solids (%)	Ash (%)	Reducing sugar	Alcohol concentrati	pН	Total acidity (g/L)	Volatile acid (g/L)
		solids (70)		(g/L)	on (%)		(g/L)	aciu (g/L)
Control	1018.2 ± 0.4^{a}	0.73 ± 0.21^{a}	0.08 ± 0.01^a	4.6 ± 0.3^{a}	5.87 ± 0.27^{a}	3.90 ± 0.01^{a}	7.87 ± 0.07^{a}	0.31 ± 0.01^{a}
U50-4	990.4 ± 0.3^{b}	0.56 ± 0.13^{b}	0.18 ± 0.02^{b}	5.5 ± 0.8^a	6.88 ± 0.28^{b}	4.02 ± 0.06^a	8.61 ± 0.71^{a}	0.26 ± 0.01^a
U50-6	987.3 ± 5.7^{b}	0.64 ± 0.25^{a}	0.12 ± 0.01^a	5.1 ± 1.1^{a}	9.52 ± 1.84^{b}	4.03 ± 0.10^{a}	8.42 ± 0.87^{a}	0.30 ± 0.03^a
U50-8	988.3 ± 0.6^{b}	0.36 ± 0.08^b	0.14 ± 0.01^{b}	5.6 ± 0.6^a	8.82 ± 0.52^{b}	4.14 ± 0.08^a	9.41 ± 0.63^{b}	0.20 ± 0.02^a
U70-4	990.9 ± 0.8^{b}	0.52 ± 0.13^{b}	0.18 ± 0.01^{b}	7.2 ± 0.4^{b}	6.72 ± 0.21^{b}	3.99 ± 0.03^{a}	8.61 ± 0.41^{a}	0.36 ± 0.03^a
U70-6	990.4 ± 1.2^{b}	0.76 ± 0.16^{a}	0.21 ± 0.02^{b}	8.3 ± 0.1^{b}	6.68 ± 0.08^a	3.94 ± 0.04^{a}	8.42 ± 0.32^{a}	0.50 ± 0.01^{b}
U70-8	990.3 ± 1.1^{b}	0.35 ± 0.12^{b}	0.19 ± 0.02^{b}	6.6 ± 0.5^a	6.95 ± 0.87^{b}	4.01 ± 0.09^{a}	7.92 ± 0.87^{a}	0.31 ± 0.03^{a}

Different letters within the same column indicate a statistically significant difference at the p < 0.05 level.

Tablo 2. Color values of tea wines after fermentation

	L	a	b	ΔE	
Control	81.76 ± 3.57^{a}	5.65 ± 2.06^{a}	35.78 ± 5.17^{a}	8.02	
U50-4	73.14 ± 1.25^{b}	10.37 ± 0.64^{b}	47.48 ± 0.51^{b}	15.28	
U50-6	78.79 ± 0.92^{a}	7.96 ± 2.10^{a}	42.59 ± 3.95^{b}	7.85	
U50-8	66.41 ± 1.14^{b}	14.80 ± 1.72^{b}	52.89 ± 1.57^{b}	17.49	
U70-4	76.09 ± 1.82^{a}	7.13 ± 1.12^{a}	43.03 ± 1.81^{b}	9.14	
U70-6	74.14 ± 1.82^{b}	9.50 ± 1.06^{a}	44.97 ± 2.04^{b}	12.27	
U70-8	80.03 ± 2.18^{a}	6.36 ± 1.38^{a}	41.53 ± 5.01^{a}	7.51	

Different letters within the same column indicate a statistically significant difference at the p < 0.05 level.

Tablo 3. Color values of tea wines after two months aging

	L	a	b	ΔE
Control	72.32 ± 0.64^{a}	4.31 ± 1.31^{a}	38.22 ± 3.14^{a}	9.87
U50-4	64.71 ± 1.09^{b}	9.41 ± 0.04^{b}	49.52 ± 0.08^{b}	22.25
U50-6	69.36 ± 0.07^{a}	6.53 ± 1.52^{a}	45.31 ± 0.92^{b}	15.69
U50-8	57.81 ± 0.80^{b}	13.64 ± 1.19^{b}	54.78 ± 1.01^{b}	31.63
U70-4	63.67 ± 0.09^{b}	6.12 ± 0.67^{a}	44.54 ± 0.78^a	20.62
U70-6	62.49 ± 1.02^{b}	8.03 ± 0.81^{b}	46.84 ± 0.84^{b}	22.37
U70-8	71.63 ± 0.03^{a}	5.73 ± 0.54^{a}	43.37 ± 1.59^{a}	12.69

Different letters within the same column indicate a statistically significant difference at the p < 0.05 level.

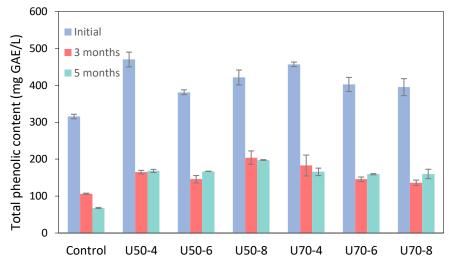


Figure 1. The effect of ultrasound amplitude and duration on the Total Phenolic Content of tea wines before and after fermentation and 2 months aging.

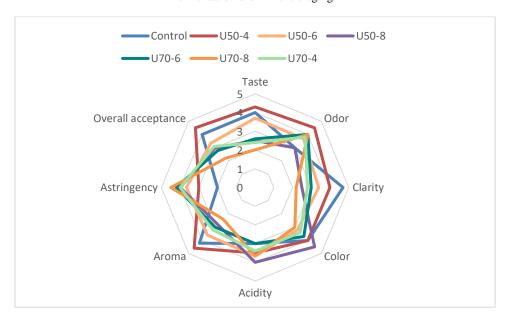


Figure 2. Radiation map of sensory profiles for tea wines. Taste, odor, clarity, color, acidity, aroma, astringency, and overall acceptance ranged from very unattractive (1) to very attractive (5).

Conclusion

This study successfully developed black tea wines through a 3-month fermentation process, incorporating ultrasound treatment at varying amplitudes and durations. The ultrasound application not only avoided any negative impact on the fermentation process but also enhanced the phenolic content of brewed black tea and influenced its color properties positively. The physicochemical analyses demonstrated significant variations in parameters such as total solids, ash, reducing sugar, alcohol, and total acidity. While a reduction in total phenolic content was observed during fermentation, ultrasound-treated samples consistently retained higher phenolic levels compared to the control. Notably, the highest phenolic content was achieved in samples treated with ultrasound at 50% amplitude for 4 minutes. Moreover, although the total phenolic content declined during fermentation, it remained stable during a 2-month storage period, underscoring the durability of these beverages. Sensory evaluations revealed changes in clarity, astringency, aroma, and color, influenced by the amplitude and duration of ultrasound treatment. Among all tested samples, the U50-4 beverage, characterized by its high phenolic content, emerged as the most preferred choice by the sensory panel. The findings of this research demonstrate the potential of tea—a widely consumed and culturally significant product—as a base for creating innovative beverages with high consumer acceptance. This work highlights the versatility of tea and opens new avenues for its application in diverse beverage categories, contributing to the development of value-added products with unique sensory and functional properties.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors have no conflict of interest to declare.

Author contribution

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

This study was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) (Application No. 1919B012307455).

Acknowledgments

The authors express thanks to TUBITAK (The Scientific and Technological Research Council of Turkey) for providing funding.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.24

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 221-232

Estimation of energy production of solar panels installed in agricultural areas with machine learning algorithms

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Article History

Received: February 3, 2025 Revised: March 9, 2025 Accepted: March 13, 2025 Published Online: March 14, 2025

Article Info

Article Type: Research Article Article Subject: Agricultural Energy Systems

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1632319







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Abstract

Predicting solar power generation is used to ensure that solar power plants operate with optimum efficiency, meet the demands of the energy grid and stabilize energy prices. This study aims to predict the medium-term electricity generation of photovoltaic panels with machine learning algorithms. Boosting Regression, Decision Tree Regression, K-Nearest Neighbors Regression, Neural Network Regression, Random Forest Regression, Regularized Linear Regression, and Support Vector Machine Regression algorithms were evaluated. Mean Squared Error (MSE), Root Mean Squared Error (RMSE), Mean Absolute Error (MAE), Mean Absolute Percentage Error (MAPE), and R-Squared (R²) were calculated. It was found that the Random Forest algorithm has the best prediction metrics. A hypothesis was formulated to evaluate the difference between the actual energy generation of the photovoltaic panels and the predicted energy by the Random Forest algorithm. The hypothesis was evaluated by the Mann-Whitney U hypothesis test and the p-value was calculated as greater than 0.05. It was concluded that there is no significant difference between the predicted energy by the Random Forest (RF) algorithm and the actual energy generated by photovoltaic panels. Based on the results of this study, we recommend using the Random Forest algorithm for medium-term energy generation prediction for photovoltaic solar panels.

Keywords: Photovoltaic, Energy, Prediction, Machine Learning

Cite this article as: Tez, T., Akyol, E. (2025). Estimation of energy production of solar panels installed in agricultural areas with machine learning algorithms. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 221-232. https://doi.org/10.31015/2025.1.24

INTRODUCTION

The energy demand has been increasing recently with the increase in the world population. There has been a significant increase in the electricity consumption of industrial facilities and individual users in a large number of countries (IEA,2022). It has become inevitable to turn to alternative renewable resources for electricity generation due to the increase in energy demand, the decrease in fossil fuels, and the increase in fossil fuel prices. In this context, solar electricity generation has recently become one of the most favored among renewable energy sources (Rabaia et al., 2021). Electricity generation from solar energy is an irregular and intermittent process (Li et al., 2020). Factors such as day and night cycles, seasonal characteristics, meteorological events, geographical location, and impurities of photovoltaic (PV) panels reduce energy conversion efficiency. Therefore, it is necessary to accurately predict the amount of electricity that solar power plants connected to the electricity grid can generate. A true and accurate electricity generation prediction is crucial for the planning, operation, and control of electricity grids and for stabilizing electricity prices (Akman et al., 2018). In addition, solar power generation prediction contributes to increasing the competitiveness of solar power plants in the electricity market and reducing the dependence on fossil fuels for economic and social development (Jebli et al., 2021).

A review of the literature reveals that various methods have been used to make predictions about the use of PV panels for solar electricity generation. These studies include the following:

Sridharan et al. (2018) presented a model using fuzzy logic techniques to predict the power output of photovoltaic/thermal hybrid (PV/T) collectors. In experimental tests, the predicted results were compared with the developed fuzzy model and it was shown that there is a good agreement between this model and the experimental results

Li et al. (2019) proposed a new prediction model using the Recurrent Neural Networks (RNN) method with only previous PV power data as input without weather data. This method showed a high prediction quality in very short-term predictions, demonstrating the feasibility and effectiveness of the prediction model.

Arce et al. (2019) trained and applied different regression techniques and machine learning algorithms such as linear and polynomial regression, support vector regression, and Random Forest to predict the total electricity consumption of a residential house in a residential neighborhood. Random Forest regression predicted the next day's electricity consumption with an error rate of 0.58% [8].

Shapsough et al. (2019) used linear regression models and artificial neural networks to predict the power output of photovoltaic systems under soiling conditions. Only PV Maximum Power Point (MPP) characteristic variables obtained from online current-voltage tracers such as solar irradiance and ambient temperature were used in this study. The results predicted the maximum power output of soiled PV modules with an accuracy of about 97%.

Liu et al. (2019) used the Differential Evolution Gray Wolf Optimizer and Random Forest Regression algorithm optimized by Principal Component Analysis and K-means clustering algorithm to model photovoltaic power generation in three different regions. Comparative experiments showed that the proposed model has higher prediction accuracy.

Sadorsky (2021) used the random forest machine learning method to predict the stock price direction of clean energy exchange-traded funds. For a 20-day prediction horizon, the researchers achieved accuracy rates between 85% and 90% with random forest methods.

Qu et al. (2021) introduced a new Gated Recurrent Unit-based hybrid model for predicting distributed PV power generation. They achieved a 62.7% increase in the prediction accuracy of this model compared to the single-gate recurrent unit model.

Didavi et al. (2021) compared the performance of Decision Tree, Random Forest, and XGBoost algorithms to predict the power generation of solar energy systems. The researchers concluded that all three models perform the prediction task effectively, but XGBoost is the best-performing model in terms of Mean Square Error and Regression Value.

Visser et al. (2022) investigated the performance of 12 different models that can predict electricity generation for the next day in accordance with market conditions. These models were evaluated in terms of their technical and economic performance.

Anupong et al. (2023) intended to evaluate the effectiveness of WANN, WSVM, and ANFIS methods in solar energy prediction at Wasit and Dhi Qar stations. The results showed that WANN and WSVM methods yield similar results in solar energy modeling. ANFIS results were acceptable but showed lower performance.

The unique aspect of this study is that many studies aim to predict PV panel power generation, but these studies do not have a hypothesis test to test the difference between the predicted values and the actual values. Unlike the literature, firstly, we conducted prediction studies on all algorithms. Then, we evaluated the performance metrics of these algorithms to determine the most appropriate algorithm. We hypothesized whether the algorithm with the best performance metric shows a significant difference between the predicted values of the regression equation and the actual values.

MATERIALS AND METHODS

Material

A Jinko brand JKM260P-60 PV module with 260 Wp power, shown in Figure 1, was used for the prediction of PV panel energy production with machine learning regression algorithms. The installation type was a land installation, the placement direction was 180° and the tilt angle was 30°. The PV panels were installed at the Faculty of Engineering, Trakya University, Edirne, Turkey at 41°37′58.38" N latitude and 26°37′26.97" E longitudes. Table 1 shows the technical specifications of the panel.



Figure 1. Photovoltaic System Installation.

Table 1. Photovoltaic Panel Features.

Parameter	Value
Maximum Power (Pmax)	260W
Power Tolerance	0 ~ +3%
Maximum Voltage (Vmp)	31.1V
Maximum Current (Imp)	8.37A
Open Circuit Voltage (Voc)	38.1V
Maximum System Voltage	1000VDC
Panel Type	Jinko JKM260P-60

Jasp 0.16.4.0 was used to create the regression models of the learning algorithms and to generate performance metrics in this study (Jasp, 2023). Jamovi 2.3.28 was used for statistical analysis of the results (Jamovi, 2023).

Research Hypothesis

This study investigates the electrical energy generation prediction of a PV panel using traditional machine learning regression algorithms. In addition, we sought to answer the question "Is there a difference between the values predicted by the regression algorithm that finds the best prediction result among the predictions made by traditional machine learning regression algorithms and the actual values generated by the PV panel?".

For this purpose, we created the following hypothesis test by utilizing Mann-Whitney U-Test on the prediction data of the model generated by using machine learning regression algorithms and the actual values generated from the PV panel:

 H_0 : There is no difference between the values predicted by the regression model and the actual values generated by the PV panel.

 H_1 : There is a difference between the values predicted by the regression model and the actual values generated by the PV panel.

The study aims to evaluate the performance of traditional machine learning regression algorithms for PV panel electrical energy generation prediction and to evaluate the performance of the algorithm that gives the best prediction result. Mann-Whitney U-Test is used as a statistical test to compare the values predicted by the regression algorithm with the actual values.

Data Collection

1705 data units from PV panels recorded by sensors in a data logger were used in this study. The data were obtained in time periods of 10 minutes each for 30 days. Four independent variables were used in the study. The variables include Air Temperature (°C), Air Humidity (%), Wind Speed (m/s), and Solar Radiation (W/m²). There is also a dependent target variable, which is Produced Energy (Wh). All dependent and independent variables are numerical data.

Prediction

Predictions of PV panel energy generation were calculated with machine learning regression algorithms. The following machine learning regression algorithms and sub-functions were used for the predictions:

- Boosting Regression
- Decision Tree Regression
- K-Nearest Neighbors Regression
- Neural Network Regression
- Random Forest Regression
- Regularized Linear Regression
- Support Vector Machine

Random Forest Regression Algorithm

The Random Forest Regression algorithm is a supervised learning algorithm. A random forest is first created in this algorithm. By using more than one decision tree in this random forest, a regression model is achieved to make accurate predictions by generating more adaptive models. Since decision trees are used in this algorithm, it is intermittent. In other words, it yields the same results for the desired predictions in a certain interval. One of the biggest problems with decision trees is that they overlearn the data. The mathematical syntax of the Random Forest regression algorithm includes the following steps (Mahmud et al., 2021):

- 1. Data Set:
- a. Training data set: X_train (size: m x n), y_train (size: m x 1)
- b. Test data set: X test (size: m' x n)
 - 2. Decision Trees:
- a. Number of decision trees: N
- b. Maximum depth for each tree: D
 - 3. Algorithm Steps:
- a. For each tree:

Step 1: Randomly select a training data set sample and train a decision tree using this sample.

Step 2: Use a random subset of features to select the split point at each node when generating the decision tree.

Step 3: Check if the tree has reached the maximum depth. Stop if the depth limit has been

Step 4: Complete the tree, which finally gives a decision tree.

b. To make predictions:

reached.

Step 1: Apply decision trees to each sample in the test data set and get the prediction result of each tree.

Step 2: Calculate the final prediction by combining the prediction results of all trees.

Performance Evaluation Methods for Machine Learning Regression Models

Performance evaluation methods are used to objectively measure and evaluate the predictive capabilities and accuracy of machine learning regression models. These methods are also used to determine which model performs better using different regression algorithms or different hyperparameters of the same algorithm. This study employs the machine learning regression algorithms shown below to calculate performance evaluation criteria to measure the performance of the predicted values for the energy generation of the PV panel.

Mean Squared Error (MSE): This is calculated by averaging the squares of the differences between the actual values and the predicted values. MSE emphasizes the magnitude of errors more and can be more sensitive to outliers. Lower MSE values indicate better performance. In Equation (1), n is the number of observations, y_j is the actual values and \hat{y}_i is the predicted values.

$$MSE = \frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$$
 (1)

Root Mean Squared Error (RMSE): This is calculated by taking the square root of the MSE. RMSE also shows the magnitude of errors and is interpreted in a similar way to MSE. Lower RMSE values indicate better performance. RMSE can take values from 0 to ∞ . It is sometimes better to use the RMSE when the MSE value is too large to be compared. The RMSE is calculated by taking the square root of Equation (1), i.e. the MSE, to find the equation in Equation (2).

$$RMSE = \sqrt{MSE} = \sqrt{\frac{1}{n} \sum_{j=1}^{n} (y_j - \hat{y}_j)^2}$$
 (2)

Mean Absolute Error (MAE): This is calculated as the average of the absolute differences between the actual and predicted values. Lower MAE values indicate better performance. MAE can take values from 0 to ∞ . Lower MAE values indicate that the model performs well. In Equation (3), n is the number of observations, y_j is the actual values and \hat{y}_i is the predicted values.

$$MAE = \frac{1}{n} \sum_{j=1}^{n} |y_j - \hat{y}_j|$$
 (3)

Mean Absolute Percentage Error (MAPE): This is calculated by averaging the absolute percentage differences between the actual values and the predicted values. MAPE is a way to compare errors without depending on the scales of the measured values. MAPE < 10% is considered good, $10\% \le \text{MAPE} \le 20\%$ is considered fair, MAPE > 20% is considered poor performance. In Equation (4), n is the number of observations, y_j is the actual values and \hat{y}_i is the predicted values.

$$MAPE = \frac{100}{n} \sum_{j=1}^{n} \frac{|y_j - \hat{y}_j|}{y_j}$$
 (4)

R-Squared (R^2): This is calculated as the ratio of the variance of the actual values that can be explained by the regression model to the total variance. The coefficient of determination R^2 is found by the mathematical equation given in Equation (5). In Equation (2), y_j is the actual values, \hat{y}_j is the predicted values and \bar{y}_j is the average of the actual values. It takes values in the range of $0 < R^2 < 1$. When $R^2 = 0$, it is concluded that the change in the dependent variable is not related to the independent variable, and when $R^2 = 1$, it is concluded that the change in the dependent variable is 100% caused by the independent variable (Chicco et al., 2021).

$$R^{2} = 1 - \frac{\sum_{j=1}^{n} (y_{j} - \hat{y}_{j})^{2}}{\sum_{j=1}^{n} (y_{j} - \bar{y}_{j})^{2}}$$
 (5)

Normality Distribution

Real-world data sets often do not exactly follow the normality distribution. In this case, if the normality assumption is not valid, alternative statistical methods such as nonparametric tests or data transformations can be used. In the present study, it was decided to check whether the data were normally distributed using the Anderson-Darling test. A² and p-value were found for the Anderson-Darling test. When the p-value was greater than 0.01, it was accepted that the data were normally distributed, and when the p-value was less than 0.01, it was accepted that the data were not normally distributed (Das and Imon, 2016).

Mann-Whitney U Hypothesis Testing

The Mann-Whitney U hypothesis test is a nonparametric test used to test the null hypothesis in samples that are not normally distributed. For this test, the dependent variable must be categorical and the independent variable must be continuous or ordinal. The test statistic is a value referred to as U. U in Equation (6) is the minimum value of U and U_1 (Heumann et al., 2022).

$$U = min(U_1, U_2) \tag{6}$$

$$U_1 = n_1 \cdot n_2 + \frac{n_1(n_1 + 1)}{2} - R_{1+} \tag{7}$$

$$U_2 = n_1 \cdot n_2 + \frac{n_2(n_2 + 1)}{2} - R_{2+}$$
 (8)

Each observation in Equation (7) and Equation (8) has an order between 1 and $(n_1 + n_2)$. R_{1+} is the sum of the orders of sample x and R_{2+} is the sum of the orders of sample y. The U value found as a result of the test determines whether the null hypothesis is accepted or rejected.

RESULTS AND DISCUSSION

The independent variables Temperature of the Air (°C), Humidity of the Air (%), Wind speed (m/s), Solar Radiation (W/m²), and the dependent variable Produced Energy (Wh) were analyzed, the Descriptive Statistics are shown in Table 2 and the performance metrics obtained using machine learning regression algorithms are shown in Table 3.

Table 2. Descriptive Statistics.

Tubic 2. Descriptive Statistics.							
	Valid	Mode	Median	Mean	Std. Deviati	on Minimun	n Maximum
Solar Radiation (W/m)	1705	946.000	745.000	698.058	238.022	70.000	1164.000
Humidity of the Air (%)	1705	36.000	35.000	37.263	10.410	19.000	69.000
Temperature of the Air (C)	1705	32.800	31.700	31.353	3.662	20.100	40.000
Wind speed (m/s)	1705	1.800	1.800	1.674	0.537	0.400	3.600
Produced Energy(Wh)	1705	31.430	25.210	21.895	10.757	0.140	38.260

Table 3. Results of machine learning regression algorithms tested in the prediction of PV panel energy generation.

Algorithm Function		Evaluation	n Metrics			
		MSE	RMSE	MAE	MAPE	\mathbb{R}^2
Boosting Regression	Gaussian	0.086	0.293	0.183	134.59%	0.914
Boosting Regression	Laplace	0.084	0.29	0.172	136.18%	0.915
Decision Tree Regression		0.104	0.322	0.22	234.00%	0.895
K-Nearest Neighbors Regression	Rectangular	0.125	0.354	0.218	49.78%	0.883
K-Nearest Neighbors Regression	Triangular	0.144	0.379	0.207	36.11%	0.854
K-Nearest Neighbors Regression	Epanechnikov	0.102	0.319	0.194	101.94%	0.893
K-Nearest Neighbors Regression	Biweight	0.117	0.342	0.206	228.10%	0.88
K-Nearest Neighbors Regression	Triweight	0.129	0.359	0.211	212.14%	0.868
K-Nearest Neighbors Regression	Cosine	0.11	0.332	0.205	231.60%	0.887
K-Nearest Neighbors Regression	Inverse	0.1	0.316	0.195	128.16%	0.898
K-Nearest Neighbors Regression	Gaussian	0.102	0.319	0.2	170.15%	0.897
K-Nearest Neighbors Regression	Rank	0.106	0.326	0.204	193.01%	0.892
K-Nearest Neighbors Regression	Optimal	0.108	0.329	0.204	175.55%	0.89
Neural Network Regression	Linear	0.099	0.315	0.211	202.67%	0.901
Neural Network Regression	Binary	1.821	1.349	0.972	966.89%	0.02
Neural Network Regression	Logistic sigmoid	0.574	0.758	0.485	96.02%	0.685
Neural Network Regression	Sine	0.464	0.681	0.484	652.17%	0.524
Neural Network Regression	Cosine	0.48	0.693	0.492	591.41%	0.514
Neural Network Regression	Inverse tangent	0.098	0.313	0.201	138.05%	0.902
Neural Network Regression	Hyperbolic tangent	0.157	0.396	0.264	150.44%	0.853

N. 1N. 1 D.	D III	0.077	0.000	0.000	100.000/	
Neural Network Regression	ReLU	0.977	0.988	0.868	100.00%	
Neural Network Regression	Softplus	0.57	0.755	0.481	88.20%	0.694
Neural Network Regression	Softsign	0.161	0.401	0.271	363.49%	0.86
Neural Network Regression	ELU	0.142	0.377	0.25	101.85%	0.87
Neural Network Regression	SiLU	0.977	0.988	0.869	100.77%	0
Neural Network Regression	Mish	0.78	0.883	0.772	134.60%	0.762
Neural Network Regression	GeLU	0.977	0.988	0.868	100.00%	0
Random Forest Regression		0.083	0.288	0.175	257.54%	0.917
Regularized Linear Regression	Lasso	0.099	0.315	0.211	198.43%	0.902
Regularized Linear Regression	Ridge	0.104	0.322	0.231	176.95%	0.9
Regularized Linear Regression	Elastic net	0.099	0.315	0.211	198.21%	0.902
Support Vector Machine Regression	Linear	0.096	0.31	0.204	177.48%	0.902
Support Vector Machine Regression	Radial	0.088	0.297	0.153	67.77%	0.91
Support Vector Machine Regression	Polynomial	0.312	0.559	0.397	221.11%	0.715
Support Vector Machine Regression	Sigmoid	11677.815	108.064	90.211	107372.21%	0.008

Table shows that the algorithm with the best performance metrics is Random Forest Regression. Therefore, we decided to analyze the PV solar panel energy generation prediction with the Random Forest Regression algorithm. Table 4 shows the data obtained by analyzing the PV panel energy generation prediction using Random Forest Regression algorithm.

Table 4. Results of the Random Forest Regression algorithm for PV panel energy generation predictions.

Trees	Features per split	n(Train)	n(Validation)	n(Test)	Validation MSE	Test MSE	OOB Error
100	2	1091	273	341	0.116	0.083	0.106

For Random Forest Regression analysis with PV panel energy generation data, 1091 of the total 1705 data units were used for training, 341 for testing, and 273 for validation. 100 tree were generated in the analysis. Figure 2 shows the data split amounts.



Table shows the PV panel Random Forest Regression Evaluation Metrics.

Table 5. Evaluation Metrics

Metrics	Value
MSE	0.083
RMSE	0.288
MAE	0.175
MAPE	257.54%
R ²	0.917

Table shows the PV panel Random Forest Regression Feature Importance.

Table 6. Feature Importance

Independent Variables	Mean decrease in accuracy	Total increase in node purity
Solar Radiation (W/m.)	1.685	456.889
Humidity of the Air (%)	0.082	40.704
Temperature of the Air (.C)	0.045	29.461
Wind Speed (m/s)	8.156×10^{-4}	6.062

Figure 3 shows the PV panel Random Forest Regression Out-of-bag Mean Squared Error Plot.

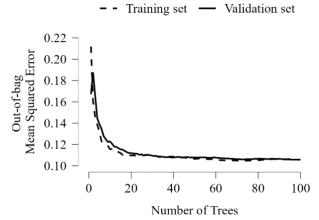


Figure 3. Out-of-bag Mean Squared Error Plot

Figure 4 shows the PV panel Random Forest Regression Predictive Performance Plot.

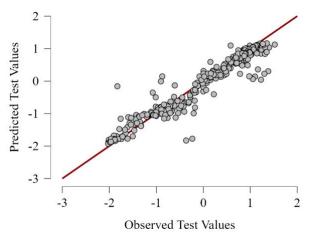


Figure 4. Random Forest Regression Predictive Performance Plot

Figure 5 shows the PV panel Random Forest Regression Mean Decrease in Accuracy.

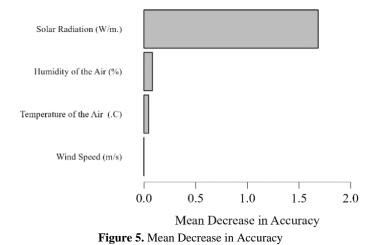


Figure 6 shows the PV panel Random Forest Regression Total Increase in Node Purity

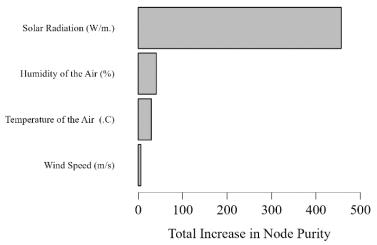


Figure 6. Total Increase in Node Purity

Normality Testing

Before the hypothesis test, which checks whether there is a difference between the predicted energy values and the actual produced energy values, the normality test was performed with the JMP Pro 17 statistical analysis software. Table 2 shows the results of the Anderson-Darling normality test and Figure 7 and Figure 8 show the distribution plots.

Table 2. Anderson-Darling normality test values for produced and predicted energy.

	A ²	Simulated p-Value
Produced energy	66.696148	< 0.0001
Predicted energy	66.956766	< 0.0001

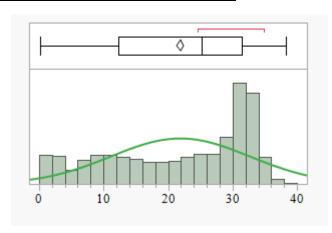


Figure 7. Distribution of produced energy values.

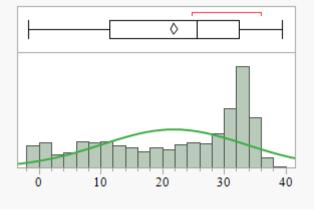


Figure 8. Distribution of predicted energy values.

Since p<0.0001 in the Anderson-Darling normality test for the produced and predicted energy values, it was assumed that the data were not normally distributed. Since the data were not normally distributed, the Mann-Whitney U hypothesis test was used to test whether there was a difference between the predicted energy values and the actual produced energy values. Table shows the descriptive statistics of the Mann-Whitney U hypothesis test and Table shows the results of the Mann-Whitney U hypothesis test.

Table 8. Descriptive statistics of the Mann–Whitney U hypothesis test

Group	N	Mean	Median	SD	SE
Produced Energy(Wh)	1705	21.9	25.2	10.8	0.261
Predicted Energy(Wh)	1705	21.9	25.6	11.7	0.283

Table 9. Mann–Whitney U hypothesis test results

Statistic	n	Mean difference	95% Confidence Interval		
	P	Mean difference	Lower	Upper	Effect Size
1.40e+6	0.091	0.441	-0.0705	0.935	0.0334

Discussion

The purpose of this paper was to identify which of the traditional machine learning algorithms provides the best prediction of the electrical energy generated by PV panels installed on the campus of Trakya University in Edirne, Turkey. According to the performance metrics obtained as a result of the study, the Random Forest algorithm provided the best prediction values. The values of MSE = 0.083, RMSE = 0.288, and MAE = 0.175 are extremely low and close to zero, which shows how well the regression curve fits the data. In addition, the fact that $R^2 = 0.917$ is very close to 1 and also supports this fit. However, the value of MAPE = 257.54% is above the 20% limit, indicating poor performance.

In the Mann-Whitney U hypothesis test conducted to determine whether there is a difference between the prediction results achieved by the Random Forest algorithm and the actual values recorded on the PV panel, p>0.05 was observed as shown in Table . Therefore, hypothesis H_1 is rejected and hypothesis H_0 is accepted. Thus, there is no significant difference between the values predicted by the Random Forest algorithm and the actual electrical energy generated by PV panels.

Many scientific studies have been conducted on the prediction of electrical energy generation by PV panels using the Random Forest algorithm. Table 3 shows some of these studies. Indeed, it has been reported in previous studies that there are differences between the location where PV solar panels are installed and the prediction interval. However, when the studies shown in Table 3 are reviewed, we see that the studies usually focus on making a prediction specific to the location and time of the study. What differentiates this paper from the studies shown in Table 3 is that this paper aims to identify the algorithm that finds the best result among traditional machine learning algorithms. Comparing the performance metrics in Table 3 with the values found in this study, we observe that Jebli's MSE is quite good, but the Time Step used in Jebli's study is larger than the data in this study. The MSE values in other studies are higher than the MSE values in this study. In terms of RMSE values, the values found by Jebli, AlSkaif, and Cattani are better, but the RMSE values in other studies are higher. A comparison of MAPE values reveals that the results of Zhang et al. were better. When R^2 values are compared, the results of Munawar et al. and Jebli are better than the result found in this study, but close to the result of Munawar et al.

Table 3. Scientific studies on the prediction of electrical energy generation by PV panels using the Random Forest algorithm.

Reference	Data Set Location	Time Step	Error Metrics				
			MSE	RMSE	MAE	MAPE	R^2
Liu et al.	Region 1 - (h+1),	1 April 2012 -	-	9.24	4.94	-	-
(2019)	(GEFCom2014)	29 June 2012					
Liu et al.	Region 2 - (h+1),	1 April 2012 -	-	8.32	5.35	-	-
(2019)	(GEFCom2014)	29 June 2012					
Liu et al.	Region $3 - (h+1)$,	1 April 2012 -	-	10.02	5.69	-	-
(2019)	(GEFCom2014)	29 June 2012					
Munawar et	Hawaii, US	September -	-	6299.84	-	-	0.9389
al. (2020)		December					
		2016					
AlSkaif et al.	Austin, US	3 years	-	0.16	0.11	-	-
(2020)							
AlSkaif et al.	Utrecht, Holland	3 years	-	0.12	0.085	-	-
(2020)							
Didavi et al.	Naitingou, Benin	1 January	3.0583	0.9999	-	-	-
(2021)		2005 - 31					

		December 2016					
Jebli, Imane, et al. (2021)	Errachidia, Morocco	2016 - 2018	0.000000993	0.0009	0.0000264	-	0.9999
Zhang et al. (2022)	Dataset-A Results, Australian	1 January 2018 - 10 September 2021	0.6982	0.8356	0.4787	12.7396	-
Zhang et al. (2022)	Dataset-B Results, China	1 January 2018 - 10 September 2021	2367.1626	48.6535	431.2076	29.5431	-
Cattani (2023)	Australia	2010–2020	-	0.076	-	-	-

The hypothesis that there is no significant difference between the performance metrics achieved by the Random Forest algorithm and the values of electrical energy generated by PV panels was made using one month of data. Therefore, this assumption should be taken into account when conducting similar research for this type of prediction. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences. International Journal of Agriculture, Environment and Food Sciences.

This study identified the best-performing traditional machine learning algorithm for predicting PV panel energy generation, demonstrating the superior performance of the Random Forest algorithm in this field, while also guiding future research to investigate the reasons behind the high MAPE value.

CONCLUSION

In this study, mid-term energy production forecasts for photovoltaic (PV) panels integrated into agricultural areas were compared using traditional machine learning algorithms, and it was demonstrated that the Random Forest algorithm achieved the highest performance. The algorithm outperformed other models with low MSE (0.083), RMSE (0.288), MAE (0.175), and high R² (0.917) values. However, the high MAPE (257.54%) value indicates that the model's relative error rates increase in low energy production scenarios (e.g., cloudy weather or nighttime hours). This suggests that while the predictions maintain accuracy in absolute terms, they need to be supported by additional parameters (cloud cover, panel cleanliness, etc.) in low production ranges.

The Mann-Whitney U test results (p=0.091) proved that there is no statistically significant difference between the predicted values and the actual energy production, supporting the reliability of the model. These findings demonstrate that the Random Forest algorithm can be used as a practical tool for energy planning and grid management in agrivoltaic integration projects. Particularly, with improvements in demand-response mechanisms, the model is expected to play a critical role in reducing energy costs and supporting sustainable agricultural practices.

The main limitations of the study include the collection of data over only a 30-day period and the limited geographical scope. In future studies, collecting long-term datasets from different seasons and geographical regions (high-altitude areas, desert climates) will enhance the model's generalizability. Additionally, incorporating variables such as wind direction, panel age, and dust accumulation could help improve the MAPE value.

In conclusion, while this study proves the effectiveness of traditional machine learning algorithms in PV energy forecasting, the development of hybrid models that simultaneously optimize energy and agricultural productivity in agrivoltaic systems will provide significant contributions to the renewable energy field. The widespread adoption of such models will enable both the reduction of carbon footprints and the synergistic management of agricultural production and energy generation.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors state there is no competing interest.

Author contribution

Erhan Akyol contributed to the literature search, writing and editing. Taşkın Tez contributed to the experimental setup and theoretical analysis.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.25

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 233-238

Seedling growth response of sesame (Sesamum indicum L.) to PEG-induced drought stress and different boron levels

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Article History

Received: September 17, 2024 Revised: March 9, 2025 Accepted: March 14, 2025 Published Online: March 16, 2025

Article Info

Article Type: Research Article Article Subject: Industrial Crops

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1551396

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Abstract

Sesame (Sesamum indicum L.) is an important oilseed crop; however, its productivity is often adversely affected by drought stress, particularly during the seedling stage. Some micronutrients, such as boron (B), can significantly enhance plants' drought resistance; nevertheless, excessive levels may be toxic. The development of drought-resistant sesame varieties is essential for sustainable cultivation. The purpose of this study was to investigate the effects of drought stress, boron, and the combination of both on sesame seedling traits. Different doses of polyethylene glycol solution (PEG 6000) (PEG) as a drought stress (0; control, -2; P1, and -4; P2 MPa) and boric acid (H₃BO₃) (B) as a boron source (0; control, 5; B1, and 10; B2 mM) were used to apply to seeds. Drought stress adversely affected sesame seedling growth trait. The increase in PEG levels from 0 to -4 MPa significantly reduced root and shoot length, whereas they did not generate a significant difference in fresh root and fresh shoot weight. Furthermore, the findings indicated that increased B treatments reduced all seedling characteristics in sesame. The overall results indicate that the growth parameters of sesame seedlings were significantly reduced at -4 MPa of PEG and 10 mM concentrations of boron.

Keywords: Fresh root, Fresh shoot, Root lenght, Shoot lenght

Cite this article as: Guden, B. (2025). Seedling growth effect of sesame (Sesamum indicum L.) to PEG-induced drought stress and different boron levels. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 233-238. https://doi.org/10.31015/2025.1.25

INTRODUCTION

Sesame (Sesamum indicum L.) is a prominent oilseed crop cultivated mostly in tropical and subtropical regions of Asia, Africa, and South America (Mehmood et al., 2021). Sesame seeds contain a significant quantity of oil, ranging from 50% to 60%, which might vary based on the specific cultivar and environmental factors (Wei et al., 2015). This oil has a high concentration of exclusive antioxidants called lignans, sesamin, and sesaminol, which contribute to its capacity for oxidative stability (Erbas et al., 2009). Sesame oil mostly comprises oleic and linoleic acids, which constitute around 80% of the total fatty acids (Yol et al., 2015). Sesame seeds are rich in minerals such as calcium, iron, zinc, and iodine, as well as vitamins including E, B6, thiamin, riboflavin, niacin, and folic acid (Pickersgill and Bedigian, 2011; Tripathy et al., 2019). These nutrients are particularly beneficial for a healthy human diet.

The rapid increase in the global population and the drastic changes in the climate are posing a serious threat, such as the occurrence of drought. This abiotic stress factor can affect plants, potentially hindering their optimal function and risking their survival (Fahad et al., 2017). Severe droughts result in significant reductions in agricultural yields due to adverse effects on plant growth, physiology, and reproduction (Barnabas et al., 2008). Sesame is mostly cultivated in arid and semi-arid regions, where its productivity is constrained by drought. Therefore, it is crucial to develop tolerances to these abiotic stress factors in order to ensure sustainable sesame productivity (Islam et al., 2016).

The significance of several micronutrients in plant metabolism has been well established. Boron (B) is a vital micronutrient necessary for the proper development of the majority of plants (Shireen et al., 2018). This micronutrient is involved in plant processes such as the movement of carbohydrates inside the plant, aiding in the transportation of sugar, and the production of DNA in meristems (Seervi et al., 2018). Boron is crucial for maintaining the integrity of plant cell walls and membranes (Bassil et al., 2004). It also promotes plant development and productivity by enhancing leaf expansion and yield components (Qamar et al., 2016). Shireen et al. (2018) reported that B improved growth and yield. Dey et al. (2023) found that B had a significant influence on the oil content, seed yield, and other components of the sesame yield. Khuong et al. (2022) found that foliar B application was beneficial in increasing the sesame growth in terms of plant height, number of leaves, chlorophyll content, and yield component. Similar results were obtained by Hamideldin and Hussein (2014), who reported that the application of boron B solutions enhances both the growth and yield of the sesame. However, the range of boron availability between insufficiency and toxicity is quite limited (Brdar-Jokanović, 2020). For example, Mertens et al. (2011) identified that increased boron concentrations adversely affect barley. Torun et al. (2006) documented same findings on wheat. In sunflowers, where a concentration of 0.5 ppm promotes healthy growth, an increase to 1 ppm results in toxicity (Eaton, 1940).

Abiotic stresses may have a negative impact on all stages of life, including the growth and development of plants (Khaeim et al., 2022). Seed germination and the establishment of seedlings are crucial phases in the life cycle of plants. However, drought stress during these stages is a significant limiting factor that restricts the effective establishment of crops (Pushpavalli et al., 2020). Sesame is also more susceptible during the germination and seedling stages, similar to other important crops (Orruno and Morgan, 2007). Dissanayake et al. (2020) and Ahmed et al. (2021) reported that high concentrations of PEG negatively affected seed germination and the seedlings. Micronutrient growth regulators, such as B, improve its capacity to tolerate abiotic stress conditions and improve parameters for growth (Abdel-Motagally and El-Zohri, 2018). Dehnavi et al. (2017) confirmed that boron has positive effects by leading to osmotic adjustment, reduction of oxidative damage, and maintenance of cell turgor on sesame in drought stress conditions. However, the optimal concentration range for boron is notably restricted (Hilal et al., 2011).

The impact of drought on the yield and quality of sesame seeds is significantly observed, particularly during the germination and seedling that are vital phases in the life cycle of plants (Bahrami et al., 2012; Dissanayake et al., 2020). Some micronutrients, such as boron, can help germination by combating the adverse effects of drought. However, it becomes toxic to plants when the amount of boron is slightly greater than required (Hilal et al., 2011). Therefore, this study aimed to understand the response of sesame seedling growth traits to different concentrations and different combinations of these concentrations of PEG, which causes drought stress, and boron, which is used as a micronutrient.

MATERIALS AND METHODS

The research used a sesame cultivar called Muganli-57, which had been previously released, as the genetic material. Seed sterilization was performed with 2% sodium hypochloride for 10–20 min, after which it was washed using double-distilled water. Each 10 cm diameter Petri dish contained a layer of Whatman No. 1 filter paper and ten healthy sesame seeds. We performed the experiment with nine treatments: two levels of boric acid (H3BO3) (5 and 10 mM; B1 and B2), two levels of PEG 6000 (Polyethylene glycol 6000) solution (-2 and -4 MPa; P1 and P2), four combinations of B and PEG (B1P1, B1P2, B2P1, and B2P2), and a control group (0) with tree replicates. The petri dishes were put in a growth chamber for a duration of 10 days at a temperature of 20 °C, following a photoperiodic condition of 14 hours of light and 10 hours of darkness each day for both treatments. The relative humidity was established at 70% throughout both the day and night. The Petri dishes were moistened, utilizing either deionized water for use as a control or 10 ml of treatment solution for each application. After a period of 10 days following seed germination, measurements were taken for root length, shoot length, root fresh weight, and shoot fresh weight.

These measurements were then analyzed using ANOVA and the least significant difference (LSD) test for comparisons. SAS version 9.3 (Anonymous, 2011) conducted the statistical analysis.

RESULTS AND DISCUSSION

One of the most significant agricultural and environmental challenges in cultivated regions is scarcity of water resources (Ceccarelli et al., 2010). According to climate forecasts, the availability of water resources is anticipated to decline. On the other hand, the growing global population and the rising demand for food require improvements in agricultural production to enhance food security. Identifying genotypes that exhibit drought resistance is critically important for crop breeding programs. Although sesame exhibits a greater tolerance to water scarcity than many other oilseed crops, its productivity and quality are adversely affected by severe drought conditions (Hassanzadeh et al., 2009; Bahrami et al., 2012). Some micronutrients such as boron can serve as beneficial elements for plant growth, particularly in conditions of drought stress. While a minimal quantity of boron is essential for the growth and development of plants, it can become toxic if present in slightly excessive amounts (Hilal et al., 2011). Therefore, in this study investigated the influence of boron on the characteristics of sesame seedlings subjected to drought stress.

Utilizing osmotic materials to generate drought potential is an important method for investigating the impact of drought stress for plants. Therefore, we used various dosages of PEG to investigate the effects of drought on sesame at the seedling stage. In this study, the statistical analysis revealed significant differences in root length

and shoot length across the varied doses of PEG applications (Table 1). In comparison to the control condition, the root length showed a significant decrease as PEG levels increased; the control and -4 MPa had the highest and lowest values with 4.30 cm and 1.67 cm, respectively (Table 2). The reduction in root length may be attributed to diminished cellular reproduction during the germination phase (Frazer et al. 1990). The result aligned with the reports of Kızıl and Yol (2018), who reported that increasing concentrations of PEG from -2 to -6 MPa drastically reduced root length. The ability of advanced root systems to withstand drought conditions and are the first to be impacted under drought stress conditions make root traits important selection factors (Saxena et al., 1993). Reduced root lengths due to drought conditions have been documented in maize (Khodarahmpour, 2011), rapeseed (Bouchyoua et al., 2024), and oats (Mut and Akay, 2010). We identified the same negative effect on shoot lengths, recording 4.13 cm at 0 (control), 3.36 cm at -2, and 2.77 cm at -4 MPa. Previously, Ahmed et al. (2021) reported that shoot length decreased with the increase in drought stress in sesame. Although it was not statistically significant, fresh shoot and fresh root weight drastically reduced from -2 to -4 MPa, which was a result of restricted water conditions inhibiting plant development. These stress levels probably represent crucial values for sesame production (Kızıl and Yol, 2018).

Table 1. ANOVA on mean of squares of measured traits in Muganli-57 under control and different levels of PEG, Boron, and PEG x Boron

Source of Variation	df	Root	Shoot Length	Fresh	Root	Fresh	Shoot
		Length		Weight		Weight	
Boron (B)	2	12.500**	10.509**	0.018**		0.059**	
PEG (P)	1	12.339**	18.157**	0.005		0.133	
B x P	2	8.204**	5.966**	0.014		0.053**	

^{*} and **; significant at 5% and 1% probability levels, respectively. df, represents degree of freedom

Boron has a limited range between deficiency and toxicity, and it is a vital plant micronutrient absorbed almost entirely in the form of boric acid via the roots (Brdar-Jokanović, 2020). Consequently, the soil boron that is insufficient for one crop may demonstrate toxic effects on another. In this study, different concentrations of boron had a statistically significant effect on all measured traits (Table 2). All traits were significantly reduced by increasing doses of boron. The control group exhibited the maximum values for all traits, while the lowest values were reached at 10 mM, measuring 0.23 cm for root length and 0.45 cm for shoot length. Excessive boron hinders cell division and impairs the thylakoid structure via influencing photosynthesis, therefore decreasing CO₂ uptake and leading to decreased root and shoot development (Reguera et al., 2009). Our findings supported this theory as we observed a significant reduction in the length of root and shoot at 10 mM. Culpan and Gürsoy (2023), and Eroğlu and Topal (2022) reported similar results, observing a decline in linseed and barley seedling characteristics as the boron dose increased, respectively. Moreover, the highest values were identified at control, while there was no statistical difference increase from B1 (5 mM) to B2 (10 mM) in fresh root and fresh shoot weight.

Table 2. Mean comparison of different doses of Bor (B), PEG (P), and Bor x PEG on sesame cultivar, Muganlı-57

Applications	Root Length	Shoot Length	Fresh Root Weight	Fresh Shoot Weight
	(cm)	(cm)	(g)	(g)
Boron				
Control	4.30 ^a	4.13 ^a	0.16 ^a	0.47 ^a
B1	1.66 ^b	1.71 ^b	0.04^{b}	0.24^{b}
B2	0.23^{c}	0.45^{c}	0.01^{b}	0.22^{b}
PEG				
Control	4.30^{a}	4.13 ^a	0.16	0.47
P1	2.54 ^b	3.36^{b}	0.06	0.36
P2	1.67 ^c	2.77°	0.03	0.25
Boron x PEG				
Control	4.30 ^a	4.13 ^a	0.16	0.47 ^a
B1P1	1.51 ^b	1.48 ^b	0.01	0.16^{b}
B1P2	0.32^{c}	0.65^{b}	0.35	0.27^{b}
B2P1	1.81 ^b	1.43 ^b	0.02	0.20^{b}
B2P2	0.32^{c}	0.64^{b}	0.05	0.21 ^b

^{*:} Mean with different letter(s) in each trait is significantly different according to LSD multiple range test.

Generally, boron application is more effective when the absorption of nutrients in the roots is insufficient due to water scarcity (White et al., 2013). However, in this study, it was determined that increasing the PEG dose, especially with the addition of boron, had a more negative effect on the root length (Table 2). The highest and lowest values for root length were found in the control group and both at B1P2 and B2P2, respectively. These same effects were also observed in shoot length. There were no significant differences in fresh shoot weight for different combinations of boron and PEG and the control group had the highest value. This is thought to be due to two reasons. Firstly, although boron is important for plants, an excessive amount of it might result in certain

adverse consequences in plant growth and development (Goldbach et al., 2001). Plants subjected to elevated levels of boron typically exhibit diminishing growth in both shoots and roots (Nable et al., 1990). Secondly, under conditions of drought stress, plants have a reduction in water absorption (Hussain et al., 2018). Consequently, there is a decrease in the rate of B influx into the root and its subsequent transportation from the root to the aerial organs (Liu et al., 2018).

CONCLUSION

Drought represents a reduction in water availability due to inadequate precipitation, which constrains plant growth, development, and productivity across various crops globally. A greater focus on developing drought-tolerant cultivars in breeding programs is gaining significance. This study examined the impact of drought on the seedling growth traits of sesame subjected to varying boron treatments. Drought stress negatively impacted the seedling growth traits of sesame (Muganli 57). When combined with drought stress and boron, the reduction of seedling traits may be the result of drought stress as well as B toxicity. This cultivar should be evaluated under different ecological conditions in the field, focusing on its agro-morphological characteristics.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing interests in this study.

Author contribution

The author contributed in the full study.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.26

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 239-251

Prevalence, antibiotic resistance, and biofilm formation of coagulase-positive staphylococci in Izmir Tulum Cheese

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Article History

Received: December 7, 2024 Revised: March 9, 2025 Accepted: March 14, 2025 Published Online: March 17, 2025

Article Info

Article Type: Research Article Article Subject: Food Microbiology, Veterinary Food Hygiene and Technology

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Available at

gipark.org.tr/jaefs/issue/90253/1597915

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Abstract

Coagulase-positive staphylococci (CPS) are the main causative bacterial agents of staphylococcal food intoxication, posing a significant public health risk and causing infections in humans and animals. In this study, a hundred Izmir Tulum Cheese samples were collected from various retail outlets in the Izmir province. CPS isolates from cheese samples were identified using standard cultural methods. The phenotypic antibiotic resistance of CPS isolates was determined using the agar disk diffusion test method, while their biofilm formation capacity was assessed using the colorimetric method. In the study, CPS was isolated from 30 out of 100 analyzed Izmir Tulum Cheese samples (30%), and it was determined that 27 of these samples (27%) had CPS levels exceeding the maximum acceptable limit of 103 CFU/g set by the Turkish Food Codex Microbiological Criteria Regulation. Antimicrobial resistance analysis revealed that among the 30 CPS isolates, 90% were resistant to penicillin, while resistance rates to other commonly used antibiotics were 83.3% for clindamycin, 56.7% for ciprofloxacin, and 53.3% for tetracycline. Additionally, 76.7% of the isolates were multidrugresistant, meaning they were not easily killed by different antibiotics, which limits treatment options. Furthermore, 83.3% of the CPS isolates had the capacity for biofilm formation, highlighting its impact on food safety. These findings emphasize the need for stricter hygiene protocols, controlled antibiotic use, and innovative strategies to combat biofilms in dairy production.

Keywords: Coagulase-positive Staphylococci, Antimicrobial Resistance, Biofilm Formation, Izmir Tulum Cheese, Food Safety

Cite this article as: Col, B.G., Yalcin, S., Cakmak Sanca, B., Akhan, M., Saglam, K., Yikmis, S. (2025). Prevalence, antibiotic resistance, and biofilm formation of coagulase-positive staphylococci in Izmir Tulum Cheese. International Journal of Agriculture, Environment and Food Sciences, 9 (1): 239-251. https://doi.org/10.31015/2025.1.26

INTRODUCTION

Cheeses are dairy products that are produced and consumed on a global scale (Tilocca et al., 2020). In Türkiye, tulum cheese is among the most widely consumed varieties. Its name is derived from the Turkish word 'tulum,' meaning 'goat or sheep skin,' which refers to the material traditionally used for packaging and maturation (Hayaloglu et al., 2007). Izmir Tulum Cheese, a geographically indicated product, is among Turkey's leading traditional cheese varieties. The cheese is produced in the Aegean region using milk derived from sheep, goats, and cows. In the traditional production process, starter cultures are not used, and the cheese is aged in brine. (Yerlikaya and Akbulut, 2019). Since there are no starter cultures in the cheese, it can age with the help of the natural milk microbiota. The quality of cheese depends on the composition of its microbiota (Coelho et al., 2022). Although the production process is similar to that of Erzincan Tulum Cheese, the ripening of Izmir Tulum Cheese in brine, filled tins differentiates it from other tulum cheese varieties. In producing Izmir Tulum Cheese, raw milk is either used directly or pasteurized at 60–68°C for 30 minutes before being cooled to the fermentation temperature

(27–37°C) and allowed to coagulate for 45–60 minutes. After curd cutting and whey drainage, the curd is shaped and salted. The cheese is then ripened in tins filled with 12–14% brine at 4–6°C for 90–180 days, with periodic turning, until it is ready for consumption (Koca, 1996; Yerlikaya and Akbulut, 2019).

Raw milk is the primary source of contamination in cheese production (André et al., 2008). Pasteurization eliminates harmful and spoilage bacteria in raw milk, but heat-resistant toxins made by some microorganisms can still change the microbiological quality (Penna et al., 2021). Mainly, staphylococcal enterotoxins produced by CPS, such as *Staphylococcus aureus* (*S. aureus*), are generally heat-resistant and can remain in food even after thermal processes like pasteurization (Balaban and Rasooly, 2000; Angulo et al., 2009; Larkin et al., 2009). These heat-resistant enterotoxins pose a significant risk of food poisoning for consumers (Ferreira et al., 2016; Calahorrano-Moreno et al., 2022).

Bacteria belonging to the *Staphylococcus* genus are Gram-positive, non-motile, and non-spore, forming facultative anaerobic cocci that typically form grape-like clusters (Götz et al., 2006). At least nine *Staphylococcus* species, including *S. aureus*, *S. intermedius*, *S. pseudointermedius*, *S. delphini*, *S. lutrae S. schleiferi* subsp. *coagulans*, *S. hyicus*, *S. argenteus*, and *S. schweitzeri*, have been identified. Among these CPS *S. aureus* is the most common cause of foodborne illnesses (Esemu et al., 2023). In addition to foodborne illnesses, *S. aureus*, a member of the CPS group, can cause purulent skin and soft tissue infections by leading to wound infections, pneumonia, meningitis, and bacteremia (Pereira et al., 2022; Ryan and Ray, 2004).

According to the Turkish Food Codex Microbiological Criteria Regulation, the maximum acceptable limit for CPS in cheese is 10³ CFU/g (Anonymous, 2025). However, studies conducted in Türkiye indicate that CPS contamination in cheese often exceeds this threshold. Unal Turhan, (2019) detected CPS in all 20 traditional cheese samples analyzed, with 45% exceeding the regulatory limits. Similarly, Gökmen et al. (2017) found CPS in 10% of 100 cheese samples they examined. The presence of *S. aureus* in various cheese samples has been reported at different rates: 48% (Gundogan and Avci, 2014), 12.5% (Can et al., 2017), 22% (Kayili and Sanlibaba, 2020), 37.5% (Güngören et al., 2022) and a study conducted in Aydın province reported that 18 out of 100 tulum and white cheese samples (18%) contained *S. aureus* (Taşcıoğlu, 2022).

Staphylococcal food poisoning caused by cheese is a significant global issue. Several factors contribute to this problem, including the pathogen's high salt tolerance (capable of growing in salt concentrations of 10% and even 20%), the use of raw milk contaminated with *S. aureus* without pasteurization, insufficient activity of starter cultures (Yıldırım et al., 2016), improper cooling and storage conditions, and contamination of processed foods by food handlers who are infected or natural carriers of *S. aureus*, either through direct hand contact or respiratory secretions. (Fernandes et al., 2022). Figure 1. shows the primary contamination sources of CPS in dairy processing.

Besides foodborne microbial diseases, another significant concern in food safety is the development of antimicrobial resistance resulting from the misuse and abuse of antimicrobial agents in humans and animals (de Souza Paiva et al., 2021). The unregulated access to veterinary antibiotics used for therapeutic and prophylactic purposes in animal husbandry, insufficient knowledge about antibiotic dosage and resistance development, excessive use of antibiotics, and the addition of antibiotics to animal feed play a key role in the development of antibiotic resistance (Bacanlı and Başaran, 2019; Tiseo et al., 2020).

CPS is commonly detected in milk samples and is associated with mastitis, a widespread disease in dairy cattle. Excessive use of intramammary antibiotics in mastitis treatment has led to the emergence of antibiotic-resistant bacteria and the horizontal transfer of resistance genes to other bacteria (Capurro et al., 2010; Vanderhaeghen et al., 2010).

The use of antimicrobials in animal husbandry significantly contributes to the development of antibiotic resistance in both human and animal pathogens, posing a considerable threat to public health, especially regarding infections caused by multidrug-resistant bacteria (Kupczyński et al., 2024). Antibiotic-resistant bacteria, including methicillin-resistant *S. aureus* (MRSA), are widespread in both communities and healthcare settings and have developed resistance to various antibiotics like tetracycline, tobramycin, and gentamicin (Silva et al., 2014). Increased antibiotic-resistant infections remain a global issue, resulting in treatment failures, higher morbidity and mortality rates, and increased healthcare costs (Spellberg et al., 2008; Arefi et al., 2014; Uddin et al., 2021). Furthermore, resistance genes that can spread through food via environmental factors directly risk human health (Endres et al., 2023). Morar et al. (2021) recommend establishing an integrated surveillance system throughout the food chain to address this.

Biofilms can persist despite cleaning and disinfection efforts, making their removal even more difficult (Yang et al., 2012). Moreover, the biofilm matrix enhances bacterial antibiotic resistance, increasing food contamination risk (Kroning et al., 2016). Bacterial biofilms that form on food processing surfaces and equipment can spread to other areas or directly contaminate food, acting as a persistent source of contamination (Kasnowski et al., 2010). Given these risks, effective biofilm prevention and control strategies are essential to ensuring food safety in processing environments.

In conclusion, the presence of CPS in dairy products is significant due to its potential to produce enterotoxins, which poses a major concern for product and consumer safety. Literature on the microbiological quality parameters of Izmir Tulum Cheese is limited (Büyükyörük and Soyutemiz, 2010; Şen et al., 2023). This study aims to examine

the prevalence, antimicrobial resistance profiles, and biofilm formation capacities of CPS isolated from Izmir Tulum Cheese in the city center and surrounding districts and assess their impact on food safety. Furthermore, the hypothesis of a statistically significant correlation between antimicrobial resistance and biofilm production ability will be tested.

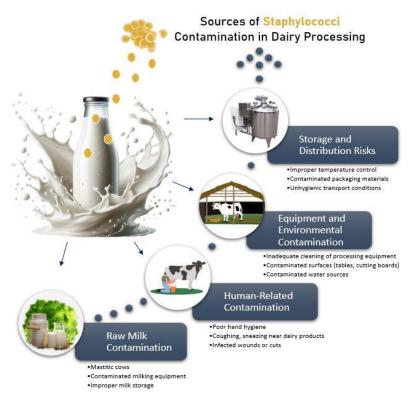


Figure 1. Primary contamination sources of CPS in dairy processing

MATERIALS AND METHODS Material

A total of 100 Izmir Tulum Cheese samples were obtained from several local markets, commercial establishments, and retail outlets in the Izmir province center and surrounding districts (Dikili, Bergama, Aliağa) between August and September 2024. The sample size was determined based on representativeness, considering Izmir's significance in cheese production and consumption, feasibility due to resource and time constraints, and alignment with previous studies. However, some limitations exist, including the restricted geographical scope, as samples were collected only from specific districts, the non, random sampling method, which may affect generalizability, and the sample size, which, while sufficient for analysis, could be expanded for broader insights. Cheese samples made from a mixture of goat, cow, and sheep milk and matured for at least 90 days were transported to the laboratory under cold chain conditions (2–8 °C). To minimize potential food safety and contamination risks, sterile sample collection containers and monitoring forms were used during sampling. The work plan is given as a graphical abstract in Figure 2.

Coagulase-positive Staphylococci Analysis

Under aseptic procedures, 25 grams of cheese samples were placed in a sterile stomacher bag, to which 225 milliliters of Maximum Recovery Diluent (MRD, Merck, 112535) were added and homogenized for two minutes. Appropriate dilutions of 1/10, 1/100, and 1/1000 from the homogenized sample were prepared by mixing one milliliter of the sample with nine milliliters of sterile dilution fluid. 0.3,0.3,0.3,0.4 mL of each dilution (1 mL in total) was transferred to three separate Petri dishes containing Baird Parker Agar (BPA, Merck, 105406) supplemented with 5% Egg Yolk Tellurite Emulsion (Merck, 103785) and spread quickly with a sterile Drigalski spatula without touching the sides of the petri dish. The Petri dishes were maintained at laboratory temperature for 15 minutes to permit the inoculum to be absorbed by the medium. Subsequently, the BPA medium was inverted and incubated at 35,37°C for 48±2 hours. Following the incubation period, typical and suspicious colonies with a diameter of 2,3 mm, black in color, shiny, and forming a transparent zone around them were evaluated for a coagulase test using a commercial latex test kit (OXOID Staphylase Test Kit, DR0595). All samples were studied at a biosafety level of 2 cabinets. Coagulase-positive colonies were counted, and results were calculated in CFU/g (ISO 6888,1:2003).

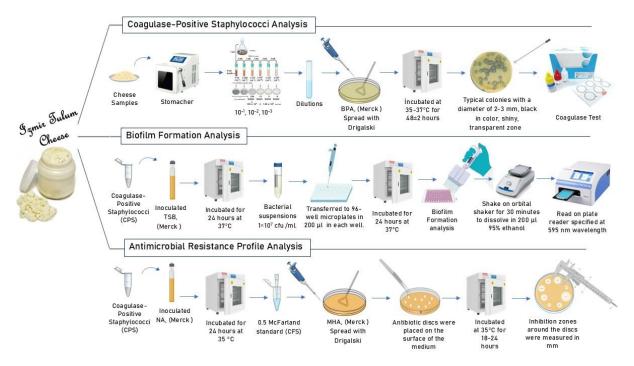


Figure 2. Graphical abstract

Antimicrobial Resistance Profile Analysis

Antibiotic selections were made by choosing one representative antibiotic from each group used to treat staphylococcal diseases in dairy animals, with phenotypic zone diameters available as specified by the Clinical and Laboratory Standards Institute (CLSI, 2020) documents. The antimicrobial susceptibility of CPS isolates was determined using the Kirby- Bauer disc diffusion method (Yao et al., 2021). In the tests, discs selected from seven different antibiotic groups were used: cefixime (5 μg), sulfamethoxazole/trimethoprim (25 μg), tetracycline (30 μg), penicillin G (10 μg), gentamicin (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg) and clindamycin (2 μg) (Bioanalyse). For antimicrobial susceptibility testing, bacterial suspensions were prepared from fresh and pure cultures grown on nutrient agar (NA, Merck, 105450) under aerobic conditions at 35 °C by 0.5 McFarland standard (Biomerieux, 70900). 0.1 mL of these suspensions were taken and spread inoculated on Mueller, Hinton agar (MHA, Merck, 103872) plates. Subsequently, antibiotic discs were placed on the surface of the medium. The plates were incubated at 35 °C for 18,24 hours, after which the inhibition zones around the discs were measured in millimeters. The *S. aureus* ATCC 29213 strain was employed as the positive control. The results were classified according to the CLSI (2020) criteria as susceptible (S), intermediate susceptible (I), or resistant (R).

Biofilm Formation Analysis

In this experiment, CPS bacteria obtained from cheese samples were incubated with 5 mL Tryptic Soy Broth (TSB, Merck, 105459) containing 1% glucose for 24 hours at 37°C to obtain fresh cultures. Then, bacterial suspensions at approximately 1×10⁷ CFU/mL density were prepared and transferred to 96 well microplates in 3 replicates with 200 µL in each well. The microplates were incubated at 37°C for 24 hours. A TSB solution containing 1% glucose was the negative control, while the positive control was the S. aureus ATCC 29213 strain. After the incubation period, the wells were washed three times with 250 µL of physiological saline to remove any unbound bacteria, then dried and proceeded to the next step. To facilitate fixation, 200 µL of 99% methanol was added to each well and allowed to stand for 15 minutes. Then, the methanol was aspirated, the microplates were dried, and the staining stage was started. The dried wells were stained with 200 µL of a 0.1% crystal violet solution for five minutes. The excess stain was removed by rinsing with tap water, and the microplates were then allowed to air dry. The stained biofilms were shaken on an orbital shaker for 30 min to dissolve in 200 µL of 95% ethanol and transferred to the measurement stage. The optical density of the resulting solution was determined at a wavelength of 595 nm (OD595) (Li and Tang, 2004). According to the measurement results, biofilm formation ability was evaluated as weak, medium, and strong. ODc = Mean OD of negative control + (3X SD value of the standard deviation of negative control); OD: Optical Density; ODc: Optical Density cut, off value; OD MB: Microorganism Biofilm Optical Density; SD: Standard Deviation. As a result of the measurements, biofilm formations are scored as 0, +, ++, +++. Measurement result; OD MB ≤ ODc: Nonbiofilm formation and score value 0, ODc < OD MB < 2 X ODc: Weak biofilm, score value +1, 2 X ODc < OD MB < 4 X ODc: Moderate biofilm, score value +2, 4 X ODc < OD MB: Strong biofilm, score value +3 (Kim et al., 2019; Yılmaz, 2020).

Statistical Analysis

The distributions between CPS levels, antimicrobial resistance rates, and biofilm formation abilities were presented with frequency and percentage analyses. Frequency and 95% Confidence Interval (CI) were calculated for categorical variables. The relationship between antibiotics and biofilm formation capacity was evaluated by Fisher's exact test. All analyses were performed SPSS Statistics v29.0.1 package program (SPSS Inc., Chicago, Ill., USA).

RESULTS AND DISCUSSION

Coagulase- positive Staphylococci Analysis Results

In this study, CPS was isolated from 30 out of 100 analyzed Izmir Tulum Cheese samples (30%). It was determined that 27 of these samples (27%) had CPS levels above the maximum CPS limit (10^3 CFU/g) accepted in cheese according to the Turkish Food Codex Microbiological Criteria Regulation (Anonymous, 2025). The CPS results (ranging from 2.0×10^1 to 6×10^5 CFU) are presented in Table 1, while the contamination levels and distribution of these 30 CPS-containing İzmir Tulum Cheese samples are shown in Table 2.

Table 1. CPS results in Izmir Tulum Cheese

Sample no	Coagulase-positive staphylococci (CFU/g)	Sample no	Coagulase-positive staphylococci (CFU/g)	Sample no	Coagulase-positive staphylococci (CFU/g)
1	2.0×10¹	11	$7,0\times10^{3}$	21	3.6×10 ⁴
2	1.8×10 ²	12	1.4×10 ⁴	22	3.7×10 ⁴
3	7.1×10 ²	13	1.5×10 ⁴	23	4.9×10 ⁴
4	4.0×10³	14	1.6×10 ⁴	24	5.1×10 ⁴
5	4.5×10³	15	1.6×10 ⁴	25	6.4×10 ⁴
6	4.6×10³	16	2.0×10 ⁴	26	6.6×10 ⁴
7	5.0×10³	17	2.1×10 ⁴	27	6.8×10 ⁴
8	5,0×10³	18	2,6×10 ⁴	28	1.0×10 ⁵
9	5,0×10³	19	2,8×10 ⁴	29	4.0×10 ⁵
10	6,0×10³	20	2.9×10 ⁴	30	6.0×10 ⁵

Table 2. Prevalence of CPS in Izmir Tulum Cheese

Total complex	Number with CPS (%)	Number of samples with CPS (%) at different levels		
Total samples		<10 ³ CFU/g	$\geq 10^3 \text{CFU/g}$	
100	30 (30)	3(3)	27 (27)	

Gökmen et al. (2017), in a study on milk and dairy products, reported that they detected CPS in 10% of 100 cheese samples examined. Similarly, Sylejmani et al. (2015) detected 22% CPS in artisan cheese samples and reported that these values ranged between 2.0x10² and 6.5x10⁵ CFU/g. Normanno et al. (2005) detected 23.7% CPS in cheeses produced from heat-treated milk sold in markets in Italy, and this rate was determined as 24.2% in ricotta cheese. Morar et al. (2013) found CPS in 34.8% of 51 cheese samples produced from fresh and matured raw milk, and more than 50% of these cheeses had CPS levels above 10⁵ CFU/g. In a study conducted in Egypt, CPS was detected in 76% of 50 cheese samples (Endres et al., 2023). The current study revealed that 27% of Izmir Tulum Cheese exceeded the maximum level (10³ CFU/g) specified in the Microbiological Criteria Regulation terms of CPS. These include contaminated raw milk, recontamination after pasteurization, poor storage conditions, and staff not following proper hygiene practices. Inadequate personnel adherence to hygiene protocols can markedly contribute to contamination in food production (Kousta et al., 2010; Sospedra et al., 2012). Moreover, while standard pasteurization can inactivate CPS, it cannot eliminate pre-formed enterotoxins. These toxins are recognized as the causative agents of foodborne illnesses, even at exceedingly low levels (Medeiros et al., 2019). Unal Turhan (2019) detected CPS in all 20 traditional cheese samples analyzed and reported that 45% exceeded the Turkish Food Codex limits. In a study conducted in Romania, CPS was found in 35.5% of traditional cheeses obtained from raw milk, and 68.1% of these samples had CPS levels above 10^s CFU/g (Rosengren et al., 2010). Radoslava et al. (2010) detected CPS in 20.48% of 415 cheese samples collected from local markets, and Normanno et al. (2005) reported that 20.7% of 3,097 milk and milk product samples were contaminated with CPS. In a study conducted in Brazil, CPS was detected in 43% of samples taken from raw milk, curd, matured cheese, and colonial cheeses (Grecelle et al., 2020). The prevalence of CPS can vary across regions and studies, with factors such as hygiene conditions, contamination levels, and the procedures applied during food processing influencing this variability.

Antimicrobial Resistance Profile Analysis Results

Antibiotics used for treatment or protection in animals promote the development of antimicrobial resistance in pathogens and normal flora bacteria. Bacteria carrying resistance genes can pass into the human flora through food and cause transmission of this problem to humans (Barton, 2000). This situation represents a substantial risk to

both food safety and public health. Our findings are consistent with those observed in other studies (Ferreira et al., 2016; Kürekçi, 2016; Da Silva, 2021; Kizanlik and Goksoy, 2024), with penicillin exhibiting the most significant level of resistance (90%). In addition, phenotypic resistance to antibiotics from three or more different groups was observed in 23 (76.7%) of the thirty CPS isolates for which antibiogram analyses were performed. Similarly, Endres et al. (2023) observed that all *S. aureus* strains obtained from cheese samples exhibited multidrug resistance. In this study, antibiotic resistance rates were found to be high for clindamycin (83.3%), ciprofloxacin (56.7%), and tetracycline (53.3%) antibiotics, respectively. Table 3 presents the antibiotic susceptibility and multidrug resistance profiles of the 30 CPS isolates, while Table 4 shows the phenotypic susceptibility percentages of the isolates to antibiotics.

Table 3. Antibiotic susceptibility and multidrug resistance profiles of CPS isolates

Sample No.	Penicillins (P)	$Sulfon a mides \ (SXT)$	Tetracyclines (TE)	Aminoglycosides (CN)	Quinolones (CIP)	Lincosamides (DA)	Macrolides (E)	M D R
1	R	S	R	S	R	R	I	MDR
2	R	S	R	S	S	R	S	MDR
3	R	S	R	S	R	R	I	MDR
4	R	I	R	R	R	R	R	MDR
5	R	R	I	S	I	R	R	MDR
6	R	R	R	R	R	R	R	MDR
7	R	S	R	R	I	R	I	MDR
8	R	R	R	S	R	R	R	MDR
9	R	R	R	S	R	R	I	MDR
10	R	S	R	R	R	R	R	MRD
11	R	S	S	S	I	S	S	
12	R	R	I	I	R	R	I	MDR
13	R	R	I	R	I	R	I	MDR
14	R	R	R	R	R	R	R	MDR
15	R	S	I	I	I	R	I	
16	S	S	S	S	S	S	S	
17	R	I	I	I	R	R	I	MDR
18	R	I	R	I	I	I	I	
19	R	S	I	S	I	R	I	
20	R	R	I	R	R	R	I	MDR
21	R	R	R	I	R	R	I	MDR
22	R	R	I	S	R	R	I	MDR
23	R	I	R	S	R	I	I	MDR
24	S	S	S	S	S	I	S	
25	S	R	S	R	S	R	I	MDR
26	R	S	R	S	I	R	I	MDR
27	R	I	I	I	I	R	I	
28	R	R	R	I	R	R	R	MDR
29	R	R	R	R	R	R	I	MDR
30	R	I	I	I	R	R	R	MDR
Total								(23/30)
R	27	13	16	9	17	25	8	·
I	-	6	10	8	9	3	18	
S	3	11	4	13	4	2	4	

*Resistant (**R**; Red); Intermediate Susceptible (**I**; Orange); Susceptible (**S**; Green), Multidrug resistance (**MDR**; Blue), MDR Negative (**Grey**), penicillin G (**P**); trimethoprim/sulfamethoxazole (**SXT**); tetracycline (**TE**); gentamicin (**CN**); ciprofloxacin (**CIP**); clindamycin (**DA**); erythromycin (**E**)

Table 4. Phenotypic susceptibility percentages of CPS isolates to antibiotics

Antibiotic Class	Antibiotic	R	R	I	I	S	S
	Name	[%]	95% CI	[%]	95% CI	[%]	95% CI
Penicillins	P	90	[79.2- 100]	0	[0-0]	10	[0-20.8]
Sulfonamides	SXT	43.3	[25.7-60.9]	20	[5.9- 34.1]	36.7	[19.6- 53.8]
Tetracyclines	TE	53.3	[35.5-71.1]	33.3	[16.5- 50.1]	13.3	[1.2- 25.4]
Aminoglycosides	CN	30	[13.6-46.4]	26.7	[10.9- 42.5]	43.3	[25.7-60.9]
Quinolones	CIP	56.7	[39.0-74.4]	30	[13.6-46.4]	13.3	[1.2- 25.4]
Lincosamides	DA	83.3	[70.0- 96.6]	10	[0-20.8]	6.7	[0- 15.8]
Macrolides	Е	26.7	[10.9- 42.5]	60	[42.6- 77.4]	13.3	[1.2- 25.4]

*penicillin G (P); trimethoprim/sulfamethoxazole (SXT); tetracycline (TE); gentamicin (CN); ciprofloxacin (CIP); clindamycin (DA); erythromycin (E); R: Resistant; I: Intermediate Susceptible; S: Susceptible; 95% Confidence Interval: 95% CI

In parallel with our findings, Endres et al. (2023) reported 50% resistance to tetracycline group antibiotics. Tetracycline, widely used in human and animal health due to its broad, spectrum effect, causes an increase in resistance rates due to its use as a growth factor (Aydin et al., 2011). The tetracycline resistance rate obtained was higher than the previously reported resistance rates between 6.9% and 30% in animal foods in Turkiye (Yücel and Anıl, 2011; Can et al., 2017; Hızlısoy et al., 2018). Antibiotic-resistant foodborne isolates pose serious health

threats to consumers, indicating inadequacies in hygiene practices. Uncontrolled and widespread use of antimicrobials, especially in developing countries, is one of the leading causes of this problem (Aslim, 2008). The extensive use of penicillin, particularly for animal treatment and prevention, plays a significant role in developing resistance. In the early years of penicillin treatment, all staphylococcal strains were susceptible to this antibiotic. However, in time, due to the production of β - lactamase, these bacteria acquired resistance to penicillin to a significant extent (Barton, 2000; Enright, 2003). The presence of residues of penicillins, which are included in the β , lactam antibiotic group, in dairy products can lead to serious health issues such as allergic reactions and anaphylactic shock (da Silva Abreu, 2021). Yücel and Anıl (2011) reported penicillin (13.9%), methicillin (11.9%), gentamicin (5%), and erythromycin (3.7%) resistance rates in cheese isolates. Morar et al. (2021) determined resistance rates of 53.1% for penicillin, 30.6% for clindamycin, and 22.4% for erythromycin. Samaržija et al. (2007) found that 54% of *S. aureus* isolates in cheese samples were resistant to penicillin. William and Withers (2010) reported 33% penicillin resistance, while Rola et al. (2016) found a resistance rate of 69.2%. Borelli et al. (2006) detected penicillin resistance in 70% of cheese isolates.

Mahdavi and Isazadeh (2019) reported that 21 *S. aureus* isolates from 100 cheese samples showed resistance to several antibiotics, with the highest resistance to penicillin. Similarly, Demirsikan and Tuncer (2021) detected penicillin resistance in 60% of Tulum cheeses sold in Isparta province. To conclude, the antibiotic resistance profiles of *S. aureus* isolates from foods of animal origin, such as cheese, are critical for food safety. Differences in resistance profiles are influenced by factors such as changes in hygiene levels, production technologies, and sensitivity of detection methods used (Papadopoulos et al., 2019). Increasing molecular characterization studies is important regarding the similarity of virulence properties of strains isolated from foods with clinical strains (Kürekçi, 2016; Rodrigues et al., 2017). One of the most urgent issues of contemporary concern is antimicrobial resistance (AMR), which poses a significant threat to the well, being of humans and livestock. Various factors are believed to contribute to the emergence of antibiotic, resistant bacteria, including the inappropriate use of pharmaceuticals in the food industry, particularly within the dairy sector, and insufficient biosecurity protocols on farms. Consequently, to ensure the sustainability of dairy farming in the future and safeguard consumer health, it is imperative to implement strategies that effectively prevent the proliferation of AMR on agricultural premises. These strategies should emphasize reducing antimicrobial usage while upholding animal welfare and productivity standards (Neculai-Valeanu et al., 2024).

Biofilm Formation Results

The ability of *Staphylococcus* species to form biofilms provides resistance to antimicrobial and disinfectant agents and adverse environmental conditions such as desiccation, UV radiation, pH changes, osmotic shocks, and thermal stress. This characteristic can contribute to food contamination within industrial facilities, causing economic losses and facilitating the transmission of foodborne diseases (Chavant et al., 2007; Cha et al., 2013). Biofilms that adhere to surfaces and cannot be cleaned increase the risk of cross, contamination by acting as a reservoir for pathogenic microorganisms. This adversely affects the microbial quality of foods, shortens shelf life and can cause serious diseases when consumed (Flemming and Wingender, 2010). Extracellular DNA, polysaccharide components, teichoic acid, protein adhesives, minerals, and vitamins make up staphylococcal biofilms (Heilmann, 2011). Although biofilm formation has been widely studied in clinical conditions, especially on implants and medical materials, studies on biofilm formation in foods have been limited (dos Santos et al., 2014; Di Ciccio et al., 2015; Cruzado-Bravo et al., 2019). Figure 3 shows the biofilm-forming ability of CPS isolates obtained from cheese samples of the present study.

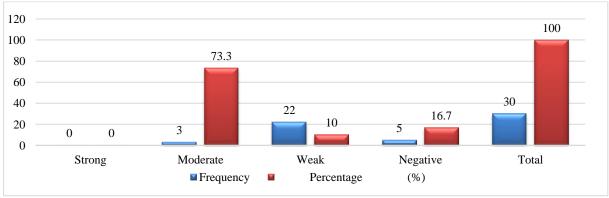


Figure 3. Frequency and percentage distribution of biofilm-forming capacity of CPS isolates

In the study, 25 out of 30 CPS had biofilm-producing capacity (83.3%) in total, which was negative (16.7%), moderate (10%), and weak (73.3%). In a study conducted in Turkey, 83.3% of 30 *Staphylococcus* isolates were capable of biofilm formation which resulted aligning the current study and the resistance rates of biofilm-forming isolates to various antibiotics were; penicillin (P) 73.3%, trimethoprim/sulfamethoxazole (SXT) 40%, tetracycline

(TE) 40%, gentamicin (CN) 26.6%, ciprofloxacin (CIP) 46.6%, clindamycin (DA) 66.6% and erythromycin (E) 20% (Gundoğan and Ataol, 2013). In another study conducted in Erzincan Tulum Cheeses, 37 (60.65%) of 61 S. aureus positive isolates were found to be capable of biofilm formation at a decent rate than the current study. Also, in the study, penicillin (P) exhibited the highest antibiotic resistance rate among biofilm-forming isolates, recorded at 45.90%. In addition, the highest rate of penicillin resistance in biofilm-forming isolates was found to be 25% (Özpınar and Gümüşsoy, 2013). In a study by Castro et al. (2020), 69.73% of Staphylococcus species isolated from raw milk, Minas artisanal cheese, and food workers' hands were found to form biofilms; while no strong biofilmforming isolates were found in the study, the distribution according to biofilm capacity was reported as negative (18.42%), moderate (7.90%) and weak (73.68%) aligning the current study results. Unlike the present study, in which no isolates with strong biofilm-forming capacity were found, Gajewska et al. (2020) reported that 22.2% of 54 staphylococcal bacteria isolated from 30 milk samples were CPS, and all isolates exhibited biofilm-forming capacity, with 79.6% demonstrating strong biofilm formation. Another study in the Kayseri province found that 26% of S. aureus strains from 23 tulum cheese samples could form biofilms. Of these isolates, 2 had strong, 2 had moderate, and 2 had weak biofilm-forming ability (Akyol et al., 2023), which is lower rates than the current study. Table 5 presents the antibiotic resistance profiles of CPS isolates based on their biofilm production capacity, and the correlation between antibiotic resistance and biofilm formation was analyzed using Fisher's exact test.

Table 5. Antibiotic resistance profiles based on biofilm production capacity in CPS isolates.

	Moderate Biofilm Producer			Weal	Weak Biofilm Producer			Negative		
		(n=3)			(n=22)			(n=5)		
Antibiotic	R	I	S	R	I	S	R	I	S	
	%	%	%	%	%	%	%	%	%	
	[95% CI]	[95% CI]	[95% CI]	[95% CI]	[95% CI]	[95% CI]	[95% CI]	[95% CI]	[95% CI]	
P	66.7	0.0	33.3	90.9	0.0	9.1	100.0	0.0	0.0	
	[0.0-100.0]	[0.0-0.0]	[0.0-100.0]	[70.8-98.9]	[0.0-15.4]	[1.1-29.2]	[56.6-100.0]	[0.0-43.4]	[0.0-43.4]	
SXT	33.3	0.0	66.7	50.0	36.4	13.6	20.0	20.0	60.0	
	[0.0-100.0]	[0.0-0.0]	[0.0-100.0]	[29.9-70.1]	[18.0-57.5]	[3.9-31.7]	[1.1-70.1]	[1.1-70.1]	[14.7-94.7]	
TE	33.3	33.3	33.3	50.0	36.4	13.6	80.0	20.0	0.0	
	[0.0-100.0]	[0.0-100.0]	[0.0-100.0]	[29.9-70.1]	[18.0-57.5]	[3.9-31.7]	[29.9-98.9]	[1.1-70.1]	[0.0 - 43.4]	
CN	33.3	0.0	66.7	31.8	31.8	36.4	20.0	20.0	60.0	
	[0.0-100.0]	[0.0-0.0]	[0.0-100.0]	[14.7-53.0]	[14.7-53.0]	[18.0-57.5]	[1.1-70.1]	[1.1-70.1]	[14.7-94.7]	
CIP	66.7	0.0	33.3	54.5	36.4	9.1	60.0	20.0	20.0	
	[0.0-100.0]	[0.0-0.0]	[0.0-100.0]	[33.7-74.2]	[18.0-57.5]	[1.1-29.2]	[14.7-94.7]	[1.1-70.1]	[1.1-70.1]	
DA	66.7	0.0	33.3	81.8	13.6	4.5	100.0	0.0	0.0	
	[0.0-100.0]	[0.0-0.0]	[0.0-100.0]	[59.7-94.8]	[3.9-31.7]	[0.1-22.8]	[56.6-100.0]	[0.0-43.4]	[0.0-43.4]	
E	33.3	33.3	33.3	22.7	68.2	9.1	40.0	40.0	20.0	
	[0.0-100.0]	[0.0-100.0]	[0.0-100.0]	[8.6-42.8]	[45.1-86.1]	[1.1-29.2]	[5.3-85.3]	[5.3-85.3]	[1.1-70.1]	

*R: Resistant; I: Intermediate Susceptible; S: Susceptible; 95% Confidence Interval: 95% CI; penicillin G (P); trimethoprim/sulfamethoxazole (SXT); tetracycline (TE); gentamicin (CN); ciprofloxacin (CIP); clindamycin (DA); erythromycin (E)

Based on Fisher's Exact test, significant correlations were found between biofilm formation and resistance to penicillin (p = 2.82×10^{-10}), gentamicin (p = 0.004), clindamycin (p = 3.56×10^{-7}), and erythromycin (p = 0.00066). However, no statistically significant differences were observed for trimethoprim/sulfamethoxazole, tetracycline, and ciprofloxacin (p> 0.05). Compared to the study by Pajohesh et al. (2022), which found significant correlations between strong biofilm formation and resistance to penicillin G, ampicillin, oxacillin, and gentamicin, the current study similarly identified significant associations with penicillin and gentamicin. However, unlike their findings, significant correlations with clindamycin and erythromycin were not observed. Despite these differences, both studies found no statistically significant relationship between biofilm formation and certain antibiotics (p> 0.05).

The results show that CPS species are often found in milk and dairy products. Their ability to form biofilms makes them more resistant to various conditions, increasing the likelihood of cross-contamination and foodborne illnesses. Additionally, the study demonstrates a correlation between biofilm formation and resistance to multiple antibiotics, further highlighting the challenges of controlling these bacteria in food environments.

CONCLUSION

This study's results indicate that CPS contamination was present in 30% of the samples, with 27% exceeding the microbiological limits set by Turkish food regulations. High levels of MDR (80%) and biofilm formation potential (73.3%) were identified, highlighting significant food safety risks. The development of antibiotic resistance and biofilm-forming capacity in coagulase-positive staphylococci isolated from cheeses was phenotypically examined. However, the genetic resistance profile was not examined, which is the study's limitations. To reveal the spread of antimicrobial resistance among bacteria via genetic means in food sources, further studies should be conducted, including molecular analyses such as *mecA* and *ica* gene detection, to better understand resistance and biofilm formation mechanisms. It is thought that increasing such studies is important in emphasizing the importance of animal-based food-borne infections resistant to antimicrobial treatment in terms of human health and increasing awareness and precautions in ensuring food safety from farm to table. The presence of CPS, such as *S. aureus*, in the human microbiota suggests that food handlers play a crucial role in contamination

during cheese production. The high antibiotic resistance observed, particularly against penicillin, clindamycin, ciprofloxacin, and tetracycline, indicates the potential misuse of antibiotics in dairy farming. Furthermore, the high percentage of CPS's biofilm-formation ability increases its resistance to cleaning agents, posing a risk for cross-contamination throughout the dairy production chain. To reduce these risks, stricter hygiene controls should be enforced in dairy processing environments, emphasizing the need for improved sanitation protocols targeting biofilms. Using new cleaning technologies that focus on breaking down biofilms and studying the genetic factors that help biofilms form is crucial for creating better antimicrobial treatments and managing these harmful bacteria. Also, the findings support the necessity for antibiotic stewardship programs in dairy farms, which align with Türkiye's Antimicrobial Resistance Action Plan. By addressing these challenges through evidence-based interventions, regulatory adjustments, and consumer education, food safety can be significantly enhanced, reducing the public health risks associated with CPS contamination in dairy products.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declared no actual, potential, or perceived conflict of interest in this research article.

Author contribution

The authors contribute equally to the present study. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

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International Journal of Agriculture, Environment and Food Sciences

e-ISSN: 2618-5946 https://dergipark.org.tr/jaefs

DOI: https://doi.org/10.31015/2025.1.27

Int. J. Agric. Environ. Food Sci. 2025; 9 (1): 252-260

Effects of different doses of cadmium on physiological, biochemical, and phytoextraction potential of mustard (Brassica juncea L.)

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Article History

Received: October 10, 2024 Revised: March 10, 2025 Accepted: March 16, 2025 Published Online: March 17, 2025

Article Info

Article Type: Research Article Article Subject: Agricultural Biotechnology Diagnostics, Soil Sciences and Plant Nutrition

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Available at

https://dergipark.org.tr/jaefs/issue/90253/1564689







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Abstract

This study investigated the physiological and biochemical tolerance and response, cadmium (Cd) accumulation capacity of the mustard plant (Brassica juncea L.) to different doses of Cd (0.0 (control)-, 25-, 50-, 100-, 200-, and 300 ppm) under greenhouse conditions. After harvesting the mustard plant, physiological parameters (plant length, plant fresh and dry weight, roots fresh weight and dry weight), and biochemical parameters such as chlorophyll a (Chl a), and chlorophyll b (Chl b), carotenoids, proline, malondialdehyde (MDA), antioxidant enzymes such as peroxidase (POX), and catalase (CAT) were examined. Cd content was measured in leaves and roots to determine phytoextraction capacity. Cd stress decreases plant and root fresh weight (Fwt) and dry weight (Dwt). Chl a-Chl b, and carotenoid contents 100 ppm of Cd decrease Cd doses increase p≤0.05. The osmolyte molecule proline increased to 100 ppm Cd dose and then declined to 300 ppm. Accumulation of MDA (2.9 to 33.8 nmol g⁻¹ Fwt), H₂O₂ (2.9 to 30.4 µmol g⁻¹ Fwt), and antioxidant enzymes (POX and CAT) showed an increasing trend with increasing Cd doses, p≤0.05. Cd accumulation in leaves (0.0 to 53.8. mg kg⁻¹) and roots (0.0 to 67.7. mg kg⁻¹) increased depending on the applied Cd concentration. The highest Cd accumulation was determined at 300 ppm Cd level. These findings suggest that mustard plants can accumulate high levels of Cd in both leaves and roots, indicating that they are hyperaccumulators. As a result, mustard plants can be utilized as phytoremediation plants in Cdcontaminated soils.

Keywords: Phytoremediation, Phytoextraction, Mustard, Heavy metal

Cite this article as: Altintas, R., Karakas, S., Dikilitas, M., Ugurlar, F. (2025). Effects of different doses of cadmium on physiological, biochemical, and phytoextraction potential of mustard (Brassica juncea L.). International Journal of Agriculture, Environment and Food Sciences, 9 (1): 252-260. https://doi.org/10.31015/2025.1.27

INTRODUCTION

Rapid population growth has reduced agricultural area and increased environmental pollution (Ozyürek, 2016; Maja et al., 2021). Soil pollution resulting from heavy metal contamination has risen significantly in recent years. Heavy metals are one of the natural components of soils in trace amounts (Rahimzadeh et al., 2017; Priya et al., 2023). There are heavy metals known as essential nutritional elements including iron (Fe), copper (Cu), cobalt (Co), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn), which are necessary for plant growth and physiological functions (Farooq et al., 2016; Daulta et al., 2022). In comparison, non-essential heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd), and lead (Pb) are not required by plants for their physiological functions (Bortoloti and Baron, 2022). Several heavy metals have a long history of accumulating in soil through industrial waste and wastewater disposal, including Fe, Mn, Cu, Ni, Co, Cd, Zn, and Hg. Excessing some of these metals can affect plant growth, metabolism, physiology, and aging, although they are essential micronutrients that support many regular processes in plants (Peng and Shahidi, 2021).

Heavy metal stress in plants causes the formation of reactive oxygen species (ROS) such as superoxide radicals (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH), leading to the oxidation of proteins, lipids, and nucleic acids in the cell and peroxidation in cell membranes, protein denaturation and oxidative changes in the structure of DNA (Shoaib and Javaid, 2021). Due to its high mobility and bioavailability, the accumulation of Cd

in plants poses a significant threat to the ecosystem. Cadmium is mainly found in soils bound to solid phases, and it is rapidly released into the soil and becomes available for plant uptake (Ondrasek et al., 2020). Cadmium is released into the environment by the Zn, Pb, and Cu industries, used in phosphate fertilizers, urban composting, wastewater irrigation, and metal processing industries. It is one of the most toxic and major environmental pollutants in the world and represents a serious problem in agriculture due to its detrimental effects on crops. When plants grow in soils contaminated with Cd, their roots uptake the heavy metal, accumulate in various organs, and ultimately reduce plant growth (Bruno et al., 2017). Cadmium toxicity induces oxidative stress, damages cell membranes and electron transport, inhibits enzymes, and impairs nucleic acids, photosynthesis, and growth (Lu et al., 2013; Waheed et al., 2022).

Various methods are available for treating soil contaminated with heavy metals. Physical, chemical, and biological processes can be used to remove and control pollutants. Although these methods are effective, they have negative aspects such as high cost, long time, and environmental damage. Phytoremediation, which has recently been widely used due to its low cost, is a method for removing pollutants from contaminated environments. This technique involves growing hyperaccumulator plants in areas contaminated with heavy metals (Laghlimi et al., 2015; Karakas et al., 2021). Phytoextraction is a soil treatment method classified under phytoremediation. It removes heavy metals that cause soil and water pollution from the environment by storing the pollutants in their roots, stems, or leaves using hyperaccumulator plants (Dağhan et al., 2012; Aybar ve ark., 2023).

The mustard plant (*Brassica juncea*) is considered an effective species for the phytoremediation of soils contaminated with heavy metals such as cadmium (Cd). Research has shown that mustard plants can absorb Cd through their roots and accumulate it in their upper parts, such as leaves and stems. This characteristic enhances their potential for cleaning Cd-contaminated soils. In particular, their high Cd tolerance and substantial biomass make them a preferred species in phytoremediation studies (Doe ve Smith, 2023).

This study aimed to evaluate the plant's phytoremediation capacity by exposing *Brassica juncea* to different concentrations of cadmium (0 [control], 25, 50, 100, 200, and 300 ppm), a significant heavy metal pollutant. Additionally, it assessed the plant's growth, physiological and biochemical responses, tolerance levels, and phytoextraction potential in leaves and roots.

MATERIALS AND METHODS

Experimental design

The mustard plants were grown with different doses of Cd $(0.0 \text{ (control)-}, 25\text{-}, 50\text{-}, 100\text{-}, 200\text{-}, and 300 ppm)}$ as CdNO₃ in 8 L pots or a randomized block design with 5 replicates under greenhouse conditions. The pots were filled with air-dry soil (clay texture), and mustard plant seeds were sown and thinned, leaving 10 plants in a pot. After four weeks, the pots were irrigated three times per week at pot capacity with irrigation water containing Cd at different doses. The mustard plants were harvested after a 12-week growing period. Physiological and biochemical analyses were made to determine the responses and tolerance of mustard plants. The Cd content of the leaves and roots was also determined to evaluate the phytoextraction potential of the plant (Figure 1).



Figure 1. The mustard plant growth with different Cd doses.

Physiological parameters

After harvest, physiological measurements (plant length, shoot fresh and dry weights, and root fresh and dry weights) were taken on plants. Plant length was measured from the soil surface to the top of the plant shoot. Shoot and root fresh weights were determined using a sensitive scale. When the samples were dried in an oven at 70 ° C until constant weight and dry weights were determined.

Biochemical parameters

Mustard plant chlorophyll-a (Chl a) and chlorophyll-b (Chl b) contents were determined according to Arnon (1949). Carotenoids were determined via the method suggested by Rajput and Patil (2017) Fresh leaf samples (0.5 g) were homogenized in 10 mL 80% acetone: water (80:20, v:v), then filtered were read for Chl a, Chl b, and

carotenoid contents at 663, 645, 480, and 510 nm, respectively in a UV microplate spectrophotometer (Epoch, SN: 1611187, manufactured in the USA). The results were calculated mg g⁻¹ Fwt.

Proline content was determined according to the method of Bates et al. (1973). Fresh leaf tissue (0.5 g) was homogenized in 3% w/v sulfosalicylic acid and the homogenate was filtered through Whatman No. 1 filter paper. Then, 2 mL of filtrate, 2 mL of acid-ninhydrin reagent (1.25 g of ninhydrin in 30 mL of glacial acetic acid, and 20 mL of 6 mol L⁻¹ phosphoric acid) were mixed in a tube and boiled at 100 °C for 1 hour. The mixture was completed in an ice bath. Then 5 ml of toluene was added to the mixture. The solution was then shaken thoroughly for 20 seconds and then left at room temperature for 20 minutes to achieve a two-layer separation. Then the absorbance of the solution was read at 515 nm using a toluene blank. L-proline prepared in different toluene concentrations was used for a standard curve. The results were reported as mol g⁻¹ Fwt.

MDA contents were measured using the method of Sairam and Saxena (2000). A fresh leaf sample (0.5 g) was homogenized with 0.1% (w/v) trichloroacetic acid (TCA). After the homogenate was centrifuged at 10,000 g for 5 min, 1 mL of the supernatant was mixed with 4 mL of 20% v/v TCA containing 0.5% v/v thiobarbituric acid (TBA). The mixture was heated in boiling water for 30 minutes, after which the process was stopped by immersing the tubes in an ice bath. The mixture was measured at 532 and 600 nm.

Hydrogen peroxide (H_2O_2) content was determined using the method of Velikova et al. (2000). Fresh leaf tissue (0.5 g) was extracted with 5 mL of 0.1% (W: V) trichloroacetic acid (TCA) and centrifuged at 12,000 g for 15 min at 4 °C. The supernatant (0.5 ml) was added to 0.5 ml of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) and 1 ml of 1 mol L^{-1} potassium iodide. The reaction was measured at 390 nm in a UV microplate spectrophotometer (Epoch, SN: 1611187, USA). The H_2O_2 content was expressed in mol g^{-1} Fwt.

Peroxidase (POX) enzyme activity (E.C.1.11.1.7) was determined using the method of Cvikrova et al. (1994). Fresh leaf samples (0.5 g) were homogenized in 10 mL of 50 mmol L^{-1} Na-phosphate buffer solution (pH 7.0). Then, $10\,\mu\text{L}$ of the supernatant was added to 290 μL of the reaction mixture containing 5 mmol L^{-1} H₂O₂, 13 mmol L^{-1} guaiacol, and 50 mmol of L^{-1} Na-phosphate. Thereafter, the oxidation of guaiacol was carried out by increasing the absorbance at 470 nm using a UV microplate spectrophotometer (Epoch, SN: 1611187, manufactured in the USA) at intervals of 1 to 3 minutes. One unit of POX enzyme activity is defined as the activity that results in an increase in absorbance of 0.1 units per minute at 25°C. The activity is expressed as enzyme unit g^{-1} Fwt.

Catalase (CAT) enzyme activity (EC 1.11.1.6) was determined according to the method of Aebi (1984). For analysis, 5 μ L of the homogenate (as obtained above) was added to 300 μ l of the reaction mixture containing 50 mmol L⁻¹ Na-phosphate buffer, 10 mmol L⁻¹ H₂O₂, and 4 mmol L⁻¹ Na₂EDTA. Reading with a UV microplate spectrophotometer (Epoch, SN: 1611187, USA) at 240 nm for 30 s. One CAT unit (U) was defined as a 0.1 increase in absorbance at 240 nm. The activity is expressed as enzyme unit g⁻¹ Fwt.

The Cd content in leaves and roots was determined according to Kacar and Inal's method (2004). Inductively coupled plasma (ICP, Perkin Elmer) was used to measure the extract obtained after filtration.

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) (version 26.0) using Duncan's SPSS software package. Duncan's Multiple Range Test was used to differentiate the treatment means for each measured parameter at a significance level of $P \le 0.05$. Correlation Hierarchical cluster analysis (HCA) and Heatmap of Pearson's Coefficient (r) heatmap were also used to observe the relation's parameters.

RESULTS AND DISCUSSION

Impact of Cd application on physiological parameters of the mustard plant

Cd was not applied to control plants, and their physiological development was not negatively affected. However, as Cd dose applications increased, signs of stress became evident in the plants, which was reflected in their physiological development. Cd applications at 25, 50, 100, 200, and 300 ppm Cd doses reduced plant length by (6.3%, 10.2%, 14.1%, 22.7%, and 32.8%), plant Fwt by (21.8%, 41.6%, 47.2%, 53.9%, and 61.2%,) and the root Fwt by (29.3%, 34.9%, 40.0%, 40.5%, and 46.3%), respectively compared to a control (0.0 ppm Cd). The lowest plant and root DW were found at a Cd dose of 300 ppm to be 0.6 g plant⁻¹ and 0.3 g plant⁻¹, respectively (Table 1).

We found that Cd stress negatively affected the physiological growth of mustard plants. Cd toxicity reduces root development in plants and causes growth recession (Waheed ve ark., 2022). The study, we determined that increasing Cd levels inhibited root development and restricted overall plant growth. The same results were found for *Eruca sativa* (Waheed et al., 2022), *Lantana camara* (Liu et al. 2019), and soybean (Xue et al., 2013).

Impact of Cd application on biochemical parameters of the mustard plant

In plants, responses of stress markers such as biochemical parameters chlorophyll (Chl a and Chl b), carotenoid, proline, MDA, H₂O₂, POX, and CAT antioxidant enzymes were determined in the harvested mustard plant. Cadmium stress causes a decrease in chlorophyll levels in plants by suppressing chlorophyll synthesis, increasing its degradation, and inducing oxidative stress. This leads to chlorosis, reduced photosynthetic capacity, and growth retardation. The study showed that Cd stress significantly decreased Chl a, Chl b, and carotenoid levels with a Cd dose of 100 ppm. The highest reductions in Chl a, Chl b, and carotenoid contents were 48.4%, 50.0%, and 30.1%,

respectively, at 300 ppm Cd ($P \le 0.05$, Figure 1). The content of chlorophyll a, chlorophyll b, and carotenoids decreased with increasing Cd dose. Numerous studies have reported the reduction of chlorophyll and carotenoid under Cd stress in barley, tomato, maize, *Lepidium sativum*, *Gossipium hirsutum*, strawberry, and *Carpobrotus acinaciformis* (Vassilev et al., 2002; Ammar et al., 2008; Ekmekci et al., 2008; Gill et al., 2012; Karanlık et al., 2013; Muradoglu et al., 2015; (Karakas et al., 2021).

Table 1. Physiological properties of the mustard plant at different Cd doses.

Cd Doses (ppm)	plant length (cm plant ⁻¹)	Plant Fwt (g plant ⁻¹)	Plant DW (g plant ⁻¹)	Root Fwt (g plant ⁻¹)	Root DW (g plant ⁻¹)
Control (0.0)	25.6±0.9a	16.5±0.71a	1.6±0.06a	4.7±0.14a	$0.5\pm0.02a$
25	24.0±0.4a	$12.7 \pm 0.47b$	$1.2\pm0.04b$	3.3±0.08b	$0.4\pm0.01b$
50	23.0±0.3b	9.6±0.39c	1.1±0.02c	3.0±0.04c	$0.4\pm0.02c$
100	22.0±0.5c	$8.68 \pm 0.58 d$	1.0±0.02d	2.8±0.06d	0.3±0.01d
200	19.8±0.9d	7.6±0.20d	$0.9\pm0.03d$	2.8±0.04d	$0.3\pm0.01d$
300	17.2±0.6e	$6.4\pm0.30e$	$0.6\pm0.01e$	2.5±0.02e	$0.3\pm0.01e$

^{*}Different letters (a,b,c,d, and e) indicate different means in the same column. P<0.05

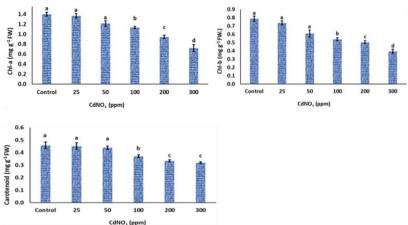


Figure 1. Chl-a, Chl-b, and carotenoid contents in the mustard plant at Cd applications.

The proline content increased significantly at a Cd concentration of 100 ppm. However, no significant increase in proline content was observed at Cd doses of 200 and 300 ppm. The MDA and H_2O_2 contents increased significantly with increasing Cd doses, 12 and 10 times, respectively, at 300 ppm Cd applications ($P \le 0.05$, Figure 2).

In our study, increasing Cd stress resulted in increased proline, MDA, and H₂O₂ concentrations in the mustard plant. Similar findings were seen in other studies such as strawberry (Doğan et al., 2022), *Arachis hypogaea* (Dinakar et al., 2008), *Lantana camara* (Liu et al., 2019) under Cd conditions.

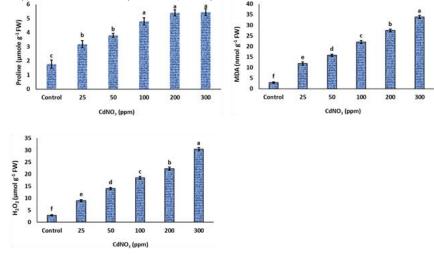


Figure 2. Proline, MDA, and H₂O₂ contents in the mustard plant at Cd applications.

POX and CAT increased significantly with increasing Cd exposure. The content of antioxidant enzymes POX and CAT were found to be 13 and 14 times higher, respectively, at the highest dose of 300 ppm Cd compared to the control plant. ($P \le 0.05$, Figure 3).

Cd stress causes oxidative stress in plants. The effect of this stress is caused by radical oxygen species (ROS). Plant cells can be protected from the harmful effects of ROS by antioxidant enzymes (Lakhdar et al., 2010). Catalase antioxidant enzyme plays an important role in the control of hydrogen peroxide, one of the ROS types caused by Cd in the environment in the plant (Martins et al., 2011). Boysan Canal et al. (2018) determined that Cd application increased CAT enzyme activity in lettuce plants due to the effect of stress. Yu et al., (2013) stated that increasing Cd applications increased CAT enzyme activity in rice (*Oryza sativa* L.) plants. Similar findings were obtained in this study.

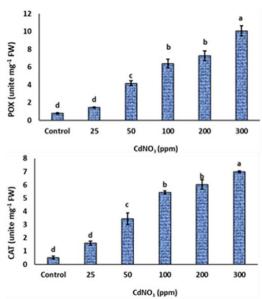


Figure 3. POX and CAT antioxidants enzymes in the mustard plant at Cd applications.

The Cd content increased with increasing Cd content in leaves and roots. The highest amount of Cd was found in leaves and roots at 53.78 and 67.71 mg kg⁻¹ DW, respectively, when mustard plants were exposed to 300 ppm Cd ($P \le 0.05$, Figure 4).

Cd hyperaccumulation refers to plant species that are capable of accumulating more than 100 mg kg⁻¹ Cd DW in plants (Baker et al., 2000). The mustard plant can be used as a phytoremediation plant in Cd-polluted soils as a hyperaccumulator plant because it accumulates large amounts of Cd in its leaves and roots.

Heavy metal ions can accumulate in the roots, leaves, and stems of the plant or be excreted from the leaves through transpiration (Ximenez et al., 2002). The roots of the plant act as a barrier to heavy metal transport and could be a potential tolerance mechanism operating in the roots (Bonnet et al., 2010). It has been reported that increased Cd content leads to an increase in the amount of Cd in the leaves and roots, and a large part of the Cd absorbed by the plant is stored in the roots while a very small part is transported to the green parts of the plant (Tiryakioglu et al., 2006). Cd hyperaccumulation refers to plant species that can accumulate more than 100 mg kg⁻¹ Cd in dry weight in plant parts (leaves and stems) (Baker et al., 2000). In this study, the mustard plant is a good hyperaccumulator plant for the toxic Cd element by accumulating Cd in its leaves and roots.

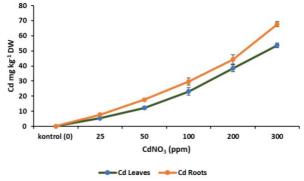


Figure 4. Leaves and roots Cd content in mustard plants with Cd applications.

Hierarchical cluster analysis (HCA) and Heatmap of Pearson's Correlation Coefficient (r) the heatmap were performed using physiological, biochemical, and Cd accumulation determinations (Figure 5). There were negative correlations between Cd content and biochemical components such as chlorophyll and carotenoid while there were negative correlations with stress parameters such as plant weight, proline, MDA, Cd accumulation in roots and leaves, and H₂O₂ suggesting that Cd stress triggers oxidative damage in plant cells. The positive correlation between Cd content and POX and CAT reflects the potential of plants to combat Cd stress. These analyses help us understand the effects of Cd on mustard and the plant's responses

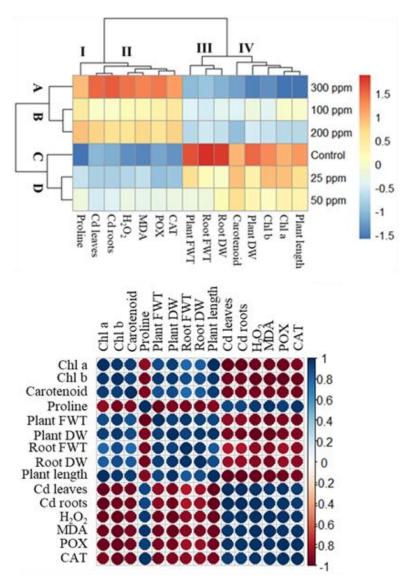


Figure 5. Hierarchical clustering analysis (HCA) and Heatmap of Pearson's Correlation Coefficient (r) showing the scores for physiological, biochemical, and Cd accumulation in mustard plants grown under different Cd levels (control, 25-, 50-, 100-, 200-, and 300 ppm).

CONCLUSION

This study demonstrated that the mustard plant ($Brassica\ juncea\ L$.) exhibited strong potential for the phytoremediation of Cd-contaminated soils, with significant relationships observed between cadmium exposure and key physiological parameters. Among these, chlorophyll content, antioxidant enzyme activity, and biomass accumulation emerged as critical indicators for tolerance and phytoextraction efficiency. The plant accumulated Cd in its leaves and roots, leading to decreases in Fwt, DW, and chlorophyll content compared to the control plants. However, despite exposure to high Cd levels, the plant survived, highlighting its tolerance to cadmium stress and ability to withstand harsh environmental conditions. In response to Cd-induced oxidative stress, proline, MDA, and H_2O_2 levels increased while the activities of antioxidant enzymes such as CAT and POX were elevated, suggesting an adaptive defense mechanism against ROS. The significant accumulation of Cd in mustard leaves and roots further emphasized that the plant has a strong phytoremediation potential. By effectively accumulating

Cd from the soil while maintaining its tolerance to this heavy metal, the mustard plant emerged as a promising candidate for the remediation of Cd-polluted lands, offering an eco-friendly and sustainable method of soil detoxification. Our future study, therefore, will focus on optimizing the growth conditions of the plant to enhance Cd uptake, exploring the molecular mechanisms underlying metal tolerance, and investigating genetic modification changes to improve phytoremediation efficiency further. The cultivation of Mustard and the improvement of methods such as the use of amino acids, plant growth regulators, and signaling molecules are also on our agenda.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author has no conflict of interest to declare.

Author contribution

Conceptualization, R.A., S.K., M.D., and F.U.; methodology, S.K., R.A.; software, S.K., and F.U; validation, R.A., S.K., F.U., and M.D.; formal analysis, R.A., and S.K.; investigation, R.A., and S.K.; resources, R.A., and S.K.; writing original draft preparation, S.K.; writing review and editing, R.A., F.U., and M.D., supervision, S.K.; project administration, S.K. All authors have read and agreed to the last version of the manuscript."

Funding

This research was funded by the Harran University Scientific Research Project (HUBAP), number 20132.

Acknowledgments

This article was published as a MSc thesis "Investigation on physiological, biochemical and photoextraction effects of mustard (*Brassica juncea* L.) under cadmium stress", Rahime ALTINTAS, Harran University, Institute of Science, 2022, Şanlıurfa/Turkey.

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