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Research Article
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INVESTIGATION OF AGRO-MORPHOLOGICAL CHARACTERISTICS IN OAT

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Abstract: In this study in which agricultural characters and morphological traits were observed, 8 oat varieties were investigated. It aim was to determine the relationship between all traits examined. The study was conducted in Türkiye (under Kahramanmaraş conditions) for 2 years between 2014 and 2016 with 3 replications according to the randomized block design. As a result of the observations, the following ranges were recorded panicle length (20.8-27.4 cm), plant height (113.4-143.3 cm), peduncle length (28.8-37.2 cm), flag leaf width (1.46-1.83 cm), flag leaf length (15.8-21.3 cm), grain number per panicle (59.7-118.6 seeds), grain weight per panicle (2.57-3.33 g), panicle number/m^2 (328.1-489.7 pcs/m^2), biomass yield (1926-3031.7 kg/ha), harvest index (13.6-25.1 %) and protein yield (59.7-86.0 kg/ha). The findings are anticipated to support breeding programs and aid in enhancing agricultural productivity.

Keywords: Oats, Agro-morphological characteristics, Panicle

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1. Introduction

Oat (*Avena sativa* L.) is a winter cultivated cereal used for animal feed and human nutrition worldwide (Hoffmann, 1995; Peterson et al., 2005). The main oat growing areas are the Americas, Europe and Asia, and to a lesser extent South America, Australia and New Zealand (Forsberg and Reeves, 1992). In contrast to the low fat and high β-glucan ratios desired for humans, oats have high protein and fat and low in β-glucan to provide maximum energy (Peterson et al., 2005). Millers prefer varieties with high groat percentage, easy threshability, white flour and high grain quality (GanBmann and Vorwerck, 1995).

In the world, 22.5 million tons of production, 9.5 million hectares of cultivation area and 2360 kg/ha yield are obtained (FAO, 2021). In Türkiye, production is 365,000 tons, the cultivation area is 137.6 hectares and the yield is 2660 kg/ha (TÜİK, 2022). Oats are significantly damaged by cold. In summer plantings in barren areas, low yields are obtained due to the high moisture requirement of the oat plant. Therefore, oats cannot compete with wheat and barley in terms of yield and price. Oat production is limited in Türkiye due to oat's low cold and drought resistance, problems such as grain shedding, lodging and failure to mature simultaneously. The main objective of plant breeding programs is to increase the yield potential. In cereals, it has been reported that traits such as number of plants per unit area, number of spikes per plant, grain weight per spike and grain number per spike are considered as selection factors in achieving high yield (Hsu and Walton, 1971). Plant biomass yield and grain yield were reported to be selection criteria. It has been reported that high yielding oat lines can be obtained with a homogeneous field trial in selection based on biomass yield or grain yield (Robertson and Frey, 1987). In this study, an attempt was made to define diversity in populations consisting of cultivated oats, wild introductions of *A. fatua* L. and natural hybrids of *A. sativa* and *A. fatua*. Very wide genetic variation was observed for these two genotypes between the species. High plant height and earliness were observed in the wild forms of *A. fatua* L., while shorter plant height and earliness were found in the hybrid populations. It was also determined that wild introductions of *A. fatua* L. can be used in oat breeding programs to increase genetic diversity by transferring special characters to cultivated oats (Matiello et al., 1999). In other a study using 12 oat cultivars, it was reported that there were significant differences in plant height, leaf area, green herbage yield and dry matter yield, but there were no differences in number of tillers per plant and number of leaves per tillers (Zaman et al., 2006). Five early oat varieties (S-2000, Fulgrain, Kent, Early Miller and Avon) were evaluated in terms of grain characteristics, green grass yield potential and quality and it was reported that S-2000 variety obtained better results in terms of plant height, stem thickness, leaf area, number of tillers per plant, number of leaves per tillers and average grain yield and S-2000 oat variety was recommended to farmers according to 4-year results (Ahmad et al., 2008). Using Seri-82, Balatilla and Golia varieties in Kahramanmaraş, a total of 24 kg/ha nitrogen was divided on the basis of sowing time (Zadoks, 00), 3-4 tillering period (Zadoks, 23-24), beginning of stem elongation (Zadoks, 31) and booting stage (Zadoks, 45) and spike number/m^2, grain number per spike, grain weight per spike, harvest index and grain yield were examined and the effect of nitrogen application times on harvest index and grain yield in the first year and grain number per spike in the second year were significant, while the effect on other characters was insignificant. In terms of nitrogen application time, it was concluded that sowing time and the beginning of stem elongation were more critical and application times including these two periods provided more grain yield (Evlice et al., 2008). In a study conducted to investigate the response of oat varieties to nitrogen fertilization in terms of grain yield, nitrogen use and yield characteristics, 6 oat varieties (Seydişehir, Apak, Yesilköy-330, Yesilköy-1779, Amasya and Checota) and 3 nitrogen doses (0, 10 and 20 kg) were used. It was reported that there were significant differences among the varieties in terms of panicle number/m^2, panicle length, grain number per panicle, grain weight per panicle, grain yield and harvest index (Maral, 2009). In a study conducted to define the general characteristics of oat germplasm adapted to the Mediterranean climate zone, plant height of 109 oat genotypes was 107.5 - 162.5 cm, grain yield 118.0 - 606.0 kg/da, harvest index 5.1 - 42.6 %, grain number per panicle 19.7-133.8 seeds and grain weight per panicle between 0.26 - 2.99 g. It was reported that a high harvest index, high 1000-grain weight and hectoliter weight were important for grain yield, tall, leafy plants and a low harvest index were important for green fodder (Iannucci et al, 2011).

This study aimed to determine the relationships between agro-morphological traits in different oat genotypes under Kahramanmaraş conditions.

2. Materials and Methods

The research was carried out by using (8 oat) varieties, according to the randomized complete block design with 3 replicates and in winter in Kahramanmaraş the 2014-2015 and 2015-2016 growing seasons, in order to determine the indirect or direct effects of the studied traits on grain yield using path analysis and correlation relationships among traits.

The province of Kahramanmaraş has a Mediterranean climate at altitudes up to 800 meters and a continental climate at higher altitudes. The temperature differences between day and night are low (Anonymous 2016a, 2016b). Climate values are given below (Figures 1 and 2). In soil of the research, calcium is too much, potassium and phosphorus are moderately sufficient and magnesium is too much. Micronutrients except manganese are insufficient. Lime content is high and slightly alkaline (Anonymous, 2015).

Oats were sown on plots of 8.30 meters in length with a spacing of 20 cm (plot size 9.96 m^2= 8.3 x 1.2) using a 6-row plot seeder with 350 grains per/m^2 based on 1000-grain weight. The sowing depth was 3 - 4 cm. Fertilization was applied using commercial fertilizer (20-20-0 compound), providing 7 kg/da pure N and 7 kg pure P2O5 as base fertilizer, followed by 7 kg/da pure N 33 % Ammonium Nitrate (NH4NO3) as a top-dressing. Water needs were made rainfall and weeds were controlled with herbicide (2.4 - D Amine).

Analysis of variance was performed using the SAS software package (SAS, 1999) and Duncan multiple comparison test was applied to compare the means. The methods described by Bares et al. (1985) were used for the determination of the studied traits. Measurements and observations were taken according to developmental stages using the Zadoks Scale (ZD). Plant measurements and yield values were calculated by using the Technical Instructions for Agricultural Values Measurement Trials in with Food, Agriculture and Livestock Ministry (Anonymous, 2008).

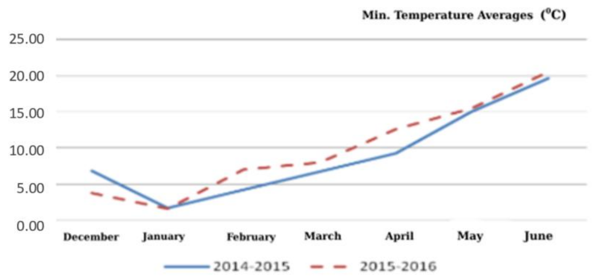

Figure 1. Graph of minimum temperature averages (°C) data for the trial years.

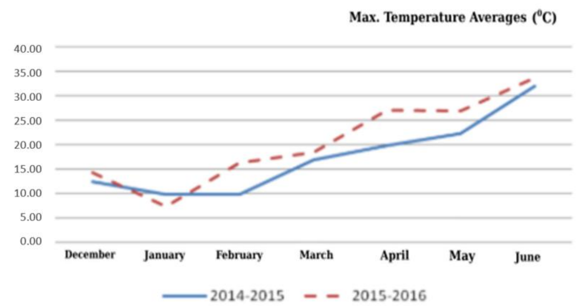

Figure 2. Graph of maximum temperature averages (°C) data for the trial years.

3. Results and Discussion

As a result of the evaluation of the varieties in terms of the traits examined by considering the two-year averages, it was determined that the varieties were significantly different in terms of panicle length, plant height, peduncle length, flag leaf width, flag leaf length, grain number per panicle, grain weight per panicle, panicle number/m^2, biomass yield, harvest index and protein yield.

When we examined the results of correlation analysis, a positive and very significant relationship was determined between grain weight per panicle and protein yield. There was also a positive and very significant relationship between grain weight per panicle and grain number per panicle (Table 1). It was stated that these two traits alone cannot be used as selection criteria (Sürek and Valentine, 1996).

There was a positive and very significant relationship between upper peduncle length and plant height, and a positive and significant relationship between upper peduncle length and panicle length. There was also a positive and very significant relationship between plant height and panicle length (Table 1).

Table 1. Correlation coefficients of examined traits of the genotypes as an averages of two years of the study

TRAITS	PY	PH	PAL	PL	FLW	FLL	PN	GNP	GWP	BIO	HI
PY	1.000										
PH	-0.514**	1.000									
PAL	-0.474**	0.640**	1.000								
PL	-0.520**	0.450**	0.345*	1.000							
FLW	0.135	0.048	0.121	0.173	1.000						
FLL	-0.408**	0.470**	0.421**	0.336*	0.384**	1.000					
PN	-0.334*	0.248	0.234	0.350*	0.159	0.420**	1.000				
GNP	0.271	-0.138	0.145	-0.163	0.105	-0.189	-0.246	1.000			
GWS	0.411**	-0.278	-0.122	-0.228	-0.102	-0.333*	-0.393**	0.659**	1.000		
BIO	-0.003	0.218	0.177	0.333*	0.601**	0.286*	0.149	-0.035	-0.224	1.000	
HI	0.534**	-0.533**	-0.509**	-0.559**	-0.383**	-0.453**	-0.211	0.151	0.379**	-0.788**	1.000

*Significant at 5 % level, **Significant at 1 % level. PY= protein yield, PH= plant height, PAL= panicle length, PL= peduncle length, FLW= flag leaf width, FLL= flag leaf length, PN= spike number/m^2, GNS= grain number per panicle, GWS= grain weight per panicle, BIO= biomass yield, HI= harvest index

The short stature of the genotype is the most important factor for the high harvest index. It has been determined that the differences among genotypes in terms of harvest index are due to differences in plant height (Balkan and Gençtan, 2008) and as a result of variations in total biomass yield and assimilate distribution, there are significant differences among genotypes in terms of harvest index (Koç and Genç, 1990; Karimi and Siddique, 1991). It was observed that the genotypes with the highest harvest index were the shortest genotypes.

Phenotypical correlation analysis was used, so it can be meaningful even at low values. When the average of two years was analyzed, the highest value in terms of panicle length was obtained from the Faikbey genotype with 27.4 cm, while the lowest value was obtained from the Kahraman genotype with 20.8 cm (Table 2). In other studies, it was reported that there may be significant differences among oat varieties in terms of panicle length (Gül et al., 1999; Yanming et al., 2006). In a study, it was determined that panicle length varied between 16.4 and 49.4 cm in oat (Çalışkan and Koç, 2017). Mut et al.(2021), in their study examining the panicle traits of different oat genotypes, reported that panicle length varied between 21.27 and 37.70 cm among the genotypes. It was also reported that panicle length is a trait that has a positive effect on plant height (Güngör et al., 2017).

Table 2. Average values of the stated traits over two years for plant height, panicle length and peduncle length

Genotypes	Plant height (cm)			Panicle length (cm)			Peduncle length (cm)		
	2014-15	2015-16	Averages	2014-15	2015-16	Averages	2014-15	2015-16	Averages
Arslanbey	125.7^{bc}	101.0^{d}	113.4^{b}	21.0^{d}	20.8^{b}	20.9^{d}	35.8^{ab}	28.7^{a}	32.2^{abc}
Kahraman	119.1^{c}	111.4^{c}	115.3^{b}	21.1^{d}	20.6^{b}	20.8^{d}	34.5^{ab}	28.4^{a}	31.5^{abc}
Faikbey	144.1^{a}	141.8^{a}	142.9^{a}	28.9^{a}	25.9^{a}	27.4^{a}	30.6^{b}	31.7^{a}	31.1^{bc}
Checota	139.4^{ab}	137.4^{a}	138.4^{a}	26.9^{ab}	22.4^{ab}	24.6^{bc}	39.8^{a}	29.7^{a}	34.8^{ab}
Kırklar	123.8^{c}	111.9^{c}	117.8^{b}	22.9^{cd}	19.9^{b}	21.4^{d}	33.4^{ab}	25.7^{a}	29.5^{bc}
Yeniçeri	125.6^{bc}	113.4^{bc}	119.5^{b}	28.1^{ab}	22.8^{ab}	25.5^{ab}	31.7^{b}	25.9^{a}	28.8^{c}
Seydişehir	149.3^{a}	137.4^{a}	143.3^{a}	29.4^{a}	23.9^{ab}	26.6^{ab}	39.3^{a}	35.0^{a}	37.2^{a}
Sebat	120.9^{c}	122.8^{b}	121.8^{b}	24.8^{bc}	20.5^{b}	22.6^{cd}	36.2^{ab}	32.6^{a}	34.4^{abc}
Averages	131.0^{a}	122.1^{b}	126.5	25.4^{a}	22.1^{b}	23.7	35.2^{a}	29.7^{b}	32.4
Mean square	399.577**	681.965**	977.380**	35.424**	12.689	41.833**	33.182	31.533	47.919*
Coeff var (CV)	5.932	4.774	5.343	7.301	9.887	8.655	11.093	16.686	13.505

When the average of the two years is evaluated, the highest value in terms of plant height was measured in the Seydişehir genotype with 143.3 cm, while the lowest value was measured in Arslanbey with 113.4 cm (Table 2). The period of rainfall is important in determining plant height and it is stated that low rainfall, especially during stem elongation, causes a decrease in plant height (Gupta et al., 2001). It has been reported that the difference in plant height is due to genotypic variation (Corville Baltenberger and Frey, 1987), very wide genetic variation was observed for plant height among species (Matiello et al., 1999) and plant height was significantly different for all varieties (Nawaz et al., 2004). It was found that yield increase in oat was negatively correlated with plant height (Redaelli et al., 2008). It was also reported that cultivars may have different plant height and there is a statistical difference between cultivars (Branson and Frey, 1989; Rocquigny et al., 2004). Plant height is also affected by different environmental factors such as water, temperature, soil nitrogen content and sunlight (Aktaş, 2017).

The highest value in terms of peduncle length was obtained in the Seydişehir variety (37.2 cm) and the lowest value was obtained in the Yeniçeri variety (28.8 cm) (Table 2). It has been reported that the differences in peduncle length between genotypes may be due to the environment (Gautam et al., 2006; Peltonen-Sainio and Rajala, 2007).

The highest flag leaf width was observed in the Sebat variety (1.83 cm) and the lowest in the Seydişehir variety (1.46 cm) (Table 3). Flag leaf width was reported to vary among genotypes (Dumlupınar, 2010). It was determined that there were significant differences between genotypes in terms of flag leaf width (Narlıoğlu, 2016). In another study, no significant difference was found between oat varieties in terms of flag leaf width (Semchenko and Zobel, 2005).

Table 3. Average values of the stated traits over two years for flag leaf length and flag leaf width

Genotypes	Flag leaf length (cm)			Flag leaf width (cm)		
	2014-15	2015-16	Averages	2014-15	2015-16	Averages
Arslanbey	22.4^{abc}	16.6^{c}	19.5^{ab}	1.49^{c}	1.52^{bcd}	1.50^{bc}
Kahraman	17.4^{c}	14.1^{c}	15.8^{d}	2.04^{ab}	1.60^{abc}	1.82^{a}
Faikbey	21.2^{abc}	20.9^{a}	21.0^{a}	1.58^{bc}	1.64^{ab}	1.61^{abc}
Checota	24.0^{a}	18.6^{ab}	21.3^{a}	1.94^{abc}	1.42^{cd}	1.68^{abc}
Kırklar	22.9^{ab}	14.5^{c}	18.7^{abc}	2.07^{a}	1.57^{bcd}	1.82^{a}
Yeniçeri	17.6^{c}	15.7^{c}	16.7^{cd}	1.93^{abc}	1.56^{bcd}	1.75^{ab}
Seydişehir	18.9^{bc}	17.1^{c}	18.0^{bcd}	1.54^{c}	1.38^{d}	1.46^{c}
Sebat	21.6^{abc}	18.2^{ab}	19.9^{ab}	1.86^{abc}	1.79^{a}	1.83^{a}
Averages	20.8^{a}	17.0^{b}	18.9	1.81^{a}	1.56^{b}	1.68
Mean square	18.413	15.333**	23.469**	0.163*	0.050**	0.128**
Coeff var (CV)	12.607	10.070	11.391	13.317	6.926	11.579

The highest flag leaf length in the Checota cultivar (21.3 cm) and the lowest in the Kahraman cultivar (15.8 cm) (Table 3). This difference between the years is thought to be due to the fact that the first year of the study was wetter than the second year. While it was reported that flag leaf length varies among cultivars (Semchenko and Zobel, 2005), it was also found to be affected by environmental conditions (Gautam et al., 2006). It was reported that there were significant differences among genotypes in terms of flag leaf length in oat (Narlıoğlu, 2016).

The highest grain number per panicle was determined in the Yeniçeri variety with 118.6 seeds and the lowest was in the Kahraman variety with 59.7 seeds (Table 4). The differences between genotypes were found to be significant in terms of grain number per panicle (Yılmaz, 1996; Topal, 1997). It was reported that the grain number per panicle varied significantly among cultivars (Kara et al., 2007). Under Adana and Kahramanmaraş conditions, grain number per panicles of oat varied between 57 and 84 (Ceyhan, 2015).

The highest value of grain weight per panicle was obtained in the Yeniçeri variety with 3.33 g, while the lowest value was measured in the Kahraman variety with 2.57 g (Table 4). Considering the two-year results, it is thought that the difference between the years is due to the different climatic conditions between the two experimental years. Grain weight per panicle was found to be between 1.57-1.95 g in oat varieties under Kahramanmaraş and Adana conditions (Ceyhan, 2015). It was reported that grain weight per panicle varies according to cultivars (Yanming et al., 2006).

In terms of the panicle number/m^2, the highest value was observed in the Arslanbey variety with 489.7 pcs/m^2 and the lowest value was obtained in the Checota variety with 328.1 pcs/m^2 (Table 4). It was reported that oat varieties were significant differences in terms of the panicle number/m^2 (Maral, 2009). It was reported that there were differences among genotypes in terms of the panicle number/m^2 in oats (Browne et al., 2006; Kara et al., 2007).

The highest biomass yield was in the Kahraman variety (3031.7 kg/ha) and the lowest in Arslanbey variety (1926.0 kg/ha) (Table 5). The differences between genotypes in terms of biomass yield were found to be significant (Shah et al., 2002; Inan et al., 2005). It was reported that year x genotype interaction was significant in terms of biomass yield (Sharma, 1992). In a study conducted in different locations, it was determined that dry grass yield varied between 848 - 1078 kg/ha (Mut et al., 2018).

The highest harvest index was observed in the Arslanbey variety (25.0 %) and the lowest in the Faikbey variety (13.6 %) (Table 5). It was reported that grain yield increased with the years of registration of the varieties from 1940 to 1970, the progress after that was not much and the reason for the increase in harvest index was due to the progress in yield (Acreche et al., 2008). In another study, in oat genotypes under Yozgat conditions, harvest index was found to vary between 22.8 % and 47.1 % (Sobayoğlu, 2017).

Table 4. Average values of the stated traits over two years for panicle number/m², grain weight per panicle and grain number per panicle

Genotypes	Panicle number/m² (panicles/m²)			Grain weight per panicle (g)			Grain number per panicle (spices)		
	2014-15	2015-16	Averages	2014-15	2015-16	Averages	2014-15	2015-16	Averages
Arslanbey	665.4ᵃ	314.1ᵃᵇ	489.7ᵃ	2.04ᵇ	3.52ᵃ	2.78ᵃ	51.0ᶜ	70.2ᶜ	60.6ᵇ
Kahraman	376.6ᶜ	300.8ᵃᵇ	338.7ᶜ	1.87ᵇ	3.27ᵃ	2.57ᵃ	53.4ᶜ	66.0ᶜ	59.7ᵇ
Faikbey	380.8ᶜ	327.9ᵃ	354.3ᶜ	1.83ᵇ	3.34ᵃ	2.58ᵃ	66.9ᵇᶜ	90.9ᵃᵇᶜ	78.9ᵇ
Checota	390.8ᶜ	265.4ᵇ	328.1ᶜ	1.94ᵇ	3.66ᵃ	2.80ᵃ	49.6ᶜ	74.5ᵇᶜ	62.1ᵇ
Kırklar	393.3ᶜ	317.9ᵃᵇ	355.6ᶜ	2.65ᵃᵇ	2.69ᵃ	2.67ᵃ	69.4ᵇᶜ	67.8ᶜ	68.6ᵇ
Yeniçeri	434.1ᶜ	297.0ᵃᵇ	365.6ᶜ	3.58ᵃ	3.08ᵃ	3.33ᵃ	123.3ᵃ	114.0ᵃ	118.6ᵃ
Seydişehir	548.7ᵇ	297.0ᵃᵇ	422.9ᵇ	1.72ᵇ	3.67ᵃ	2.69ᵃ	57.6ᶜ	89.5ᵃᵇᶜ	73.6ᵇ
Sebat	422.0ᶜ	293.7ᵃᵇ	357.9ᶜ	2.80ᵃᵇ	3.13ᵃ	2.97ᵃ	107.6ᵃᵇ	103.5ᵃᵇ	105.5ᵃ
Averages	451.5ᵃ	301.7ᵇ	376.6	2.30ᵃ	3.30ᵇ	2.80	72.3ᵃ	84.5ᵇ	78.4
Mean square	31731.984**	1083.742	17265.806**	1.267	0.329	0.374	2323.900*	950.378*	2919.967**
Coeff var (CV)	12.506	9.709	12.274	31.804	25.120	27.058	32.458	19.977	25.424

Table 5. Average values of the stated traits over two years for harvest index, protein yield and biomass yield

Genotypes	Harvest index (%)			Biomass yield (kg da⁻¹)			Protein yield (kg da⁻¹)		
	2014-15	2015-16	Averages	2014-15	2015-16	Averages	2014-15	2015-16	Averages
Arslanbey	23.9ᵃ	26.2ᵃ	25.0ᵃ	2016.3ᵈ	1835.7ᵇ	1926.0ᶜ	60.2ᵇᶜ	73.2ᵇ	66.7ᶜᵈ
Kahraman	10.5ᵈ	27.4ᵃ	18.9ᵇᶜ	4041.3ᵃ	2022.0ᵃᵇ	3031.7ᵃ	68.8ᵇ	99.8ᵃ	84.3ᵃᵇ
Faikbey	10.2ᵈ	17.0ᵇ	13.6ᵈ	2672.0ᵇᶜᵈ	2800.0ᵃ	2736.0ᵃᵇ	40.6ᵈ	78.7ᵇ	59.7ᵈ
Checota	11.7ᶜᵈ	20.8ᵃᵇ	16.2ᶜᵈ	2697.0ᵇᶜᵈ	2436.0ᵃᵇ	2566.5ᵃᵇ	49.2ᶜᵈ	74.3ᵇ	61.8ᵈ
Kırklar	14.1ᵇᶜᵈ	25.8ᵃ	20.0ᵇᶜ	3469.3ᵃᵇ	1991.3ᵃᵇ	2730.3ᵃᵇ	88.1ᵃ	83.8ᵃᵇ	86.0ᵃ
Yeniçeri	17.6ᵇ	26.7ᵃ	22.2ᵃᵇ	2935.7ᵇᶜ	1857.7ᵇ	2396.7ᵇᶜ	82.8ᵃ	79.3ᵇ	81.0ᵃᵇ
Seydişehir	13.8ᵇᶜᵈ	22.5ᵃᵇ	18.1ᵇᶜᵈ	2511.0ᶜᵈ	1988.7ᵃᵇ	2249.8ᵇᶜ	49.8ᶜᵈ	70.7ᵇ	60.2ᵈ
Sebat	16.0ᵇᶜ	20.7ᵃᵇ	18.3ᵇᶜᵈ	2931.0ᵇᶜ	2583.0ᵃᵇ	2757.0ᵃᵇ	69.5ᵇ	79.7ᵇ	74.6ᵇᶜ
Averages	14.7ᵃ	46.3ᵇ	30.5	2909.2ᵃ	1951.1ᵇ	2430.1	63.6ᵃ	79.9ᵇ	71.8
Mean square	61.560**	3104.279	1470.626	1137307.613**	588091.810	881869.93*	844.653**	246.537	735.799**
Coeff var (CV)	18.948	120.964	141.205	15.003	38.193	24.667	9.985	13.528	12.047

The highest protein yield was observed in the Kırklar variety (86.0 kg/da) and the lowest in the Faikbey variety (59.7 kg/da) (Table 5). In a study conducted to determine the suitable corn for silage varieties under Van conditions, the crude protein yield of the varieties was found to be between 45.7 - 97.9 kg/da in the first year of the experiment and between 57.7 - 98.7 kg/da in the second year, which is similar to this study (Akdeniz et al., 2004). In a study conducted in Ordu conditions in oats, it was recorded as 44.88 - 59.09 kg/da (Eğritaş, 2014).

4. Conclusion

It is estimated that the differences between the examined traits and varieties are due to the fact that both years have different ecological and environmental conditions, the varieties have different adaptation abilities and there are genetic differences between the varieties. It can also be said that genotypes responded differently to ecological conditions. Panicle length, plant height, peduncle length, flag leaf width, flag leaf length, grain number per panicle, grain weight per panicle, panicle number/m², biomass yield, harvest index and protein yield traits examined in the research can be used as selection criteria and it is very important for future breeding studies.

According to the results of the correlation analysis, there is no statistically significant difference. The reason for this is thought to be the genetic structure, the inability to mature simultaneously and problems such as lodging.

Considering this study, it can be recommended that agricultural production activities go further in this field by using other plants, locations other locations and different varieties. Local varieties fully adapted to the ecological conditions of the regions where they grow assure of the future of agriculture and humanity (Özgen et al., 2000).

Yeniçeri variety was one of the highest genotypes in terms of grain number per panicle, grain weight per panicle and harvest index, therefore Yeniçeri can be recommend for researchers and farmers.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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PLANNING SUSTAINABLE FOOD SYSTEMS: GIS BASED GREENHOUSE SITE SELECTION AND GREEN LOGISTICS INTEGRATION IN THE EASTERN BLACK SEA REGION

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Abstract: The sustainable development of food production systems in ecologically sensitive regions necessitates infrastructure planning that is spatially optimized, low-emission and logistically efficient. This study presents an integrated Geographic Information Systems (GIS)-based framework for the optimal siting of greenhouse facilities in the Eastern Black Sea region of Türkiye. The approach synthesizes spatial analysis with principles of green logistics and origin-destination modeling to identify environmentally and socio-economically viable locations. Utilizing ArcMap 10.5, the analysis incorporates diverse datasets, including transportation networks, population density, climatological variables (temperature, precipitation, solar exposure, wind speed) and topographic parameters (altitude, slope, aspect). Land suitability is evaluated using weighted overlay, weighted sum and fuzzy logic techniques, allowing for the integrated assessment of environmental, infrastructural and socio-economic dimensions. Additionally, travel distance, transport-related emissions and labor accessibility are modeled to inform the logistical sustainability at prospective sites. The findings highlight high-potential zones predominantly situated in the coastal lowlands and transitional inland areas of Ordu, Giresun and select parts of Trabzon and Gümüşhane. The study contributes a scalable spatial decision support model that integrates rigorous geospatial analytics with sustainability imperatives, thereby fostering the advancement of resilient and low-carbon agricultural infrastructure.

Keywords: Greenhouse mapping, Green logistics, Sustainable agriculture, Eastern Black Sea region, Spatial decision support

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1. Introduction

The logistics sector has become a key sector, particularly in a country's export-import transactions (Sarı and Demir, 2016). Sustainable development has emerged as a key goal in the face of increasing environmental challenges that have led to transformation in many sectors, including agriculture and logistics. Green logistics, a practice that aims to reduce environmental impacts in the supply chain, plays a key role in this transformation. It contributes to climate goals and environmental management by including environmentally friendly transportation, energy-efficient storage and resource-optimized distribution (Kurbatova et al., 2020). The integration of green logistics into agriculture is crucial because the sector is not only energy-intensive but also highly sensitive to climate variability and land-use restrictions (Onukwulu et al., 2022).

The imperative to ensure global food security amid intensifying environmental degradation and socio-economic volatility has brought sustainable agricultural practices to the forefront of scholarly and policy debates. Greenhouse farming, particularly when integrated with green logistics systems, has emerged as a strategic approach for promoting efficient, resilient and environmentally responsible food production networks. This study seeks to contribute to this emerging field by applying Geographic Information Systems (GIS) to the problem of greenhouse site selection in Türkiye's Eastern Black Sea region, with an emphasis on spatial and logistical sustainability.

Over the past decades, GIS has emerged as a pivotal tool in environmental planning, enabling stakeholders to analyze spatial relationships, assess environmental constraints and model land suitability with remarkable precision. GIS supports sustainable decision-making by integrating diverse datasets—such as topography, land use, climate and soil quality—into unified analytical frameworks that guide the optimal site selection for agricultural and environmental infrastructures. These include greenhouses, vertical farms, irrigation networks, composting facilities, cold storage depots and renewable energy-powered agricultural hubs, all of which depend on spatial suitability, resource availability and logistical efficiency for sustainable operation. (Le Hoang et al., 2023; Davidson, 1992). When combined with remote sensing, GIS can monitor land changes, model solar and wind energy potential and estimate the environmental impact of development scenarios.

The Eastern Black Sea region is characterized by complex topography, climatic heterogeneity and infrastructural constraints that render traditional agricultural planning insufficient. Therefore, spatial decision support systems (SDSS) and GIS in particular are critical for enabling data-driven site selection that integrates environmental, logistical and socio-economic parameters. In this context, the concept of green logistics—which aims to minimize the ecological footprint of transport, storage and distribution systems—becomes highly relevant. The interaction between site suitability for greenhouse development and logistical efficiency forms the core problem that this research aims to address.

In the context of the Eastern Black Sea region, which is characterized by diverse microclimates, steep terrain and ecological richness, the use of GIS to identify suitable greenhouse sites is quite useful. Site selection based on proximity to renewable energy sources such as solar or wind farms can reduce greenhouse gas operational emissions, lower energy costs and increase regional food security. Studies have shown that integrating renewable energy into green logistics systems, such as solar-powered transportation or wind-powered storage facilities, not only reduces greenhouse gas emissions but also strengthens the resilience of agricultural supply chains (Mamaev et al., 1999; Saltuk and Artun, 2019; Song et al., 2024).

The principal objective of this study is to develop an integrated analytical framework that combines environmental suitability assessment with green logistics principles. Particularly, the study aims to:

• Identify suitable locations for greenhouse development in the Eastern Black Sea region using GIS;
• Incorporate both environmental (e.g., temperature, precipitation, solar exposure) and infrastructural (e.g., road network, proximity to labor) datasets into the site selection model;
• Evaluate how origin-destination characteristics and travel-related emissions impact the greenness of proposed sites;
• Propose a replicable methodology for regional-scale spatial planning that aligns with global goals for climate-resilient agriculture and logistics.

This study moves beyond technical GIS analyses by grounding spatial evaluations in principles of sustainability science and logistics. Rather than remaining an internal technical report, this study positions itself as a scholarly contribution to the domains of environmental planning, sustainable logistics and agricultural systems modeling.

Agricultural green logistics systems supported by GIS analysis can provide significant economic and environmental benefits. For example, optimized routing and distribution routes can reduce fuel use, while greenhouse positioning based on GIS data can increase crop yields in line with favorable climate conditions (Indrianti et al., 2025; Nomura et al., 2024). Moreover, spatial integration of logistics with energy infrastructure (e.g., planning solar-compatible roofs or locating greenhouses near wind corridors), is compatible with circular economy principles and supports broader sustainability goals (Malá et al., 2017; Canıtez, 2009; Bilal et al., 2024; Bingöl et al., 2023).

However, this integrated approach can sometimes present challenges. Implementing GIS-based green logistics requires access to high-resolution environmental data, skilled personnel and adequate technological infrastructure. In addition, high initial investment costs, especially for renewable energy systems, may discourage small-scale agricultural enterprises from adopting these strategies. Regulatory fragmentation and lack of standardization in data formats may also complicate regional planning efforts (Khayyat et al., 2024; Yigezu et al., 2018; Kalmenovitz et al., 2025).

This paper investigates how GIS can be applied to identify suitable greenhouse areas in the Eastern Black Sea region by analyzing environmental parameters and their compatibility with renewable energy potential. By doing so, it highlights a path to achieve sustainable, energy-efficient and environmentally friendly agricultural practices, contributing to both regional development and global sustainability goals.

The research questions of this study are as follows:

How can GIS-based spatial analysis be used to determine the most environmentally and logistically suitable locations for greenhouse development in the Eastern Black Sea region?

What is the emission reduction potential of spatially optimized greenhouse locations when assessed through hub-and-spoke modeling?

The remainder of the article is structured as follows: Section 2 details the conceptual and methodological framework, including data acquisition, GIS techniques and analytical processes; Section 3 presents the empirical findings based on three spatial modeling techniques; Section 4 discusses the results in light of sustainable development and policy implications; and Section 5 concludes with future research directions and potential applications of the proposed model.

1.1. Previous Studies

As shown in Table 1, a review of the existing literature reveals that a large number of studies have addressed greenhouse site selection and others have explored the integration of GIS in agricultural planning, but a significant gap remains at the intersection of these fields, particularly in the context of international sustainable logistics. Most prior research has focused on greenhouse site selection based on agricultural or climatic factors alone, often without incorporating broader logistical considerations such as transportation accessibility, renewable energy integration and labor distribution. Moreover, studies that do apply GIS methodologies typically limit their scope to regional land use planning or environmental assessments, without explicitly addressing the supply chain and sustainability dimensions.

This study aims to bridge this gap by developing a GIS-based framework for greenhouse area mapping that explicitly integrates principles of green logistics. By combining data on terrain, population and infrastructure were evaluated collectively to model environmental suitability within a spatial analytical environment and applying advanced techniques such as weighted overlay, weighted sum and fuzzy overlay analyses, this research provides a comprehensive, data-driven approach to sustainable greenhouse planning. As such, it offers a novel contribution to the literature by linking spatial agricultural development with logistics optimization—an essential yet underexplored intersection in the pursuit of climate-resilient and resource-efficient food systems at both national and international levels.

Table 1. Previous studies

Author(s)	Methodology	Key Findings	Results
(Garg and Vemaraju, 2025)	Quantitative survey with validated scales (AVE, Cronbach's alpha > 0.70)	Green logistics management (GLM) significantly improves sustainable logistics performance (SLP). Green innovation enhances logistics efficiency.	High firm commitment to eco-friendly packaging (mean 5.35) and clean technologies (mean 5.14). Variability in adoption exists across firms.
(Kurbatova et al., 2020)	Conceptual analysis of green logistics within sustainable development frameworks	Green logistics integrates economics, ecology and logistics technology. Interdisciplinary collaboration enhances effectiveness.	Highlights the need for scientific support and human capital to advance green logistics.
(Onukwulu et al., 2022)	Review of green logistics in energy supply chains	Machine learning and renewable energy enhance green logistics. Blockchain promotes transparency.	Circular economy and tech integration reduce emissions and costs.
(Davidson, 1992)	Case studies in Scotland and Greece	GIS supports strategic land use and environmental assessment.	Demonstrates GIS flexibility in ecological monitoring and decision-making.
Saltuk and Ozan (2018)	GIS-based MCDA (multi-criteria decision analysis) with expert judgment and suitability modeling	Identified high, medium and low suitability zones for greenhouse expansion in Türkiye	Validated using ground truthing and spatial overlays; model proved transferable to other regions
Albuja-Illescas et al. (2025)	GIS with AHP and MaxEnt modeling comparison for agricultural suitability analysis	Combining expert and statistical models improves spatial accuracy and validation	AHP offered structured expert input; MaxEnt refined results using environmental correlations
Kil et al. (2023)	AHP-GIS model for rooftop greenhouse placement based on spatial and socio-economic factors	Rooftop greenhouse potential is high in transit-accessible, low-cost zones of Seoul	Decision maps produced to rank rooftop spaces for policy-based greening initiatives
Wang (2024)	Low-carbon logistics network modeling integrated with GIS spatial optimization	Minimizing carbon footprint requires alignment of logistics nodes with green energy clusters	Suggested integration of carbon data layers in site ranking significantly reduced emissions
Feng et al. (2023)	Multi-criteria decision-making with GIS using AHP, WLC and fuzzy logic for logistics facility siting	GIS-MCDM enhances responsiveness and spatial equity of emergency logistics deployment	Demonstrated that flexible GIS layers improve emergency planning under uncertainty

1.2. Conceptual Framework

This study is grounded in interdisciplinary concepts drawn from sustainable agriculture, green logistics, and spatial decision-making. The conceptual framework integrates three key domains: (1) agro-climatic feasibility, (2) spatial multi-criteria evaluation (MCE), and (3) logistics optimization. The interplay between these domains informs the methodological design and indicator selection. Agro-climatic feasibility ensures alignment with regional ecological conditions, while spatial MCE—supported by GIS—facilitates weighted analysis of diverse site characteristics. Simultaneously, green logistics introduces a life cycle perspective, emphasizing reduced emissions, minimized transport costs, and efficient distribution planning. This integrative framework supports site selection that is not only geographically and economically rational but also ecologically resilient. It reflects a holistic approach to spatial planning, incorporating technical, environmental, and logistical criteria.

1.3. Green Logistics and Sustainability

As outlined in Section 1.1, green logistics centers on reducing the environmental impact of supply chains, including transportation, warehousing and distribution (Kurbatova et al., 2020). It encompasses energy-efficient route planning, the use of alternative fuels, infrastructure siting and supply chain redesign to achieve lower carbon emissions (Onukwulu et al., 2022). In this study, green logistics principles guide the evaluation of greenhouse sites by emphasizing proximity to transport corridors, minimizing travel distances and optimizing connectivity with labor and markets.

1.4. ODM and Travel Emissions

ODM is a fundamental technique in transportation and spatial planning used to evaluate travel patterns between production sites (origins) and consumption or processing locations (destinations). It provides critical insights into emissions generated through freight movement, travel time and fuel efficiency (Indrianti et al., 2025). In the context of sustainable greenhouse planning, ODM allows assessment of travel-based carbon footprints and logistical efficiency for moving agricultural produce.

1.5. SDSS and GIS-Based Site Selection

SDSS are integrative analytical frameworks that combine spatial databases, visualization tools and decision-making models to solve complex location problems. GIS constitutes the technological core of SDSS by enabling data acquisition, geospatial analysis and scenario modeling. For greenhouse planning, GIS facilitates the integration of environmental, infrastructural and socio-economic data layers to identify optimal locations. This study applies a GIS environment to balance competing priorities such as solar exposure, slope stability, accessibility and labor availability.

1.6. Green Infrastructure and Socio-Environmental Externalities

Green infrastructure refers to site planning that harmonizes built structures with ecological processes to produce environmental benefits. In greenhouse farming, this includes selecting sites that promote short transport cycles, renewable energy use and minimal disruption to local ecosystems. It also encompasses consideration of indirect impacts, such as worker commute distances and access to services. A site's "greenness" therefore extends beyond technical factors and includes qualitative considerations related to its effect on the surrounding community and landscape.

1.7. Integrated Perspective

Bringing together these concepts, this study conceptualizes greenhouse site suitability as a multidimensional construct shaped by environmental constraints, logistical pathways, socio-economic interactions and policy objectives. Rather than treating GIS merely as a technical tool, the study emphasizes its role as a decision-support environment where sustainability principles, qualitative assessments and stakeholder interests converge. This integrated perspective informs both the methodological choices and the interpretation of spatial patterns identified through the analysis.

2. Materials and Methods

This study employs a spatial decision-making approach using GIS to identify optimal greenhouse site locations in Türkiye's Eastern Black Sea region. The methodological framework integrates environmental, topographic, infrastructural and socio-economic parameters with sustainability-focused criteria, including origin-destination assessments and green logistics indicators. Through the use of advanced spatial modeling techniques—weighted overlay, weighted sum and fuzzy logic—the analysis enables a comprehensive evaluation of suitable areas, informed by both quantitative data and qualitative insights.

2.1. Study Area

The Eastern Black Sea region encompasses the provinces of Rize, Trabzon, Artvin, Giresun, Ordu, Gümüşhane and Bayburt. Characterized by mountainous terrain, dense forest ecosystems and a humid climate with high rainfall, the region offers both opportunities and challenges for sustainable agricultural planning. Moderate temperatures, access to water resources and a developing transport network enhance its potential for climate-resilient greenhouse agriculture. However, logistical constraints resulting from topographical complexity and spatially dispersed population centers necessitate data-driven spatial strategies.

2.2. Relevance to Green Logistics and Spatial Decision Support

Integrating greenhouse development with green logistics principles enhances the environmental and economic efficiency of supply chains. GIS enables the visualization and synthesis of complex spatial relationships, supporting data-driven site selection that minimizes transport distances, reduces emissions and improves accessibility to labor and energy infrastructure. The strategic mapping of greenhouse zones, when aligned with renewable energy corridors and existing logistics networks, facilitates low-carbon agricultural development.

2.3. Data Collection and Dataset Identification

Multiple geospatial and environmental datasets were sourced from national meteorological archives, remote sensing platforms, governmental GIS repositories and census data. These datasets were selected for their relevance to agricultural productivity, infrastructural accessibility and sustainability performance. Table 2 presents an overview of the key datasets utilized.

Table 2. Datasets

Dataset	Source	Purpose
Digital Elevation Model (DEM)	Satellite imagery	Derive slope, aspect and altitude for topographic evaluation
Temperature Data	Meteorological databases	Identify thermal suitability for greenhouse crops
Precipitation Data	Meteorological databases	Detect high-rainfall or flood-prone areas
Sunny Days Data	Meteorological archives	Estimate solar energy potential and crop development
Wind Speed Data	Meteorological archives	Assess exposure and potential for wind-based energy integration
Road Network Data	National transport databases	Evaluate logistical connectivity and travel distance to market hubs
Population Density (15–60 age group)	Census data	Determine labor accessibility
Land Use / Land Cover	CORINE/national land use maps	Identify available or convertible land
Soil Suitability Maps	Ministry of Agriculture	Assess soil fertility and crop support capacity
Hydrological Data	National water authority	Evaluate access to water and risk of erosion or flooding

2.4. Data Preprocessing

All spatial layers were standardized to a resolution of 30 meters and projected into the WGS 84 UTM coordinate system to ensure compatibility. Data preprocessing included:

- Clipping to the regional boundary;
- Interpolation of missing climatic data using Inverse Distance Weighting (IDW);
- Min-max normalization of all raster layers to a 0–1 scale.

Given the diverse nature of the datasets—each measured in different units and scales—normalization was essential to ensure analytical compatibility. This step enabled an integrated evaluation by ensuring consistent input formats across all geospatial analyses.

2.5. Criteria Weighting and Classification

Three strategies were used to determine the weight of each criterion:

- Review of methodological literature on greenhouse and logistics site selection;
- Sensitivity analysis to test the robustness of weight allocations.

Each factor was classified into five levels of suitability and assigned a score. Higher weights were given to temperature, road proximity and population density, reflecting their strong influence on greenhouse performance and green logistics integration. Suitability scoring was designed to ensure consistency across models while capturing real-world conditions.

2.6. Analytical Techniques

Three spatial modeling techniques were used to evaluate greenhouse suitability:

- Weighted Overlay Analysis: Reclassified raster layers were combined to generate a composite suitability index. This method provided categorical distinctions between zones of varying suitability levels.
- Weighted Sum Analysis: Maintained the original value range of each layer and produced a continuous suitability surface. This allowed for finer differentiation and cumulative assessment of location desirability.
- Fuzzy Overlay Analysis: Applied fuzzy logic membership functions and a gamma operator (γ = 0.9) to model gradual transitions in suitability. This method captured spatial uncertainty and interdependencies among criteria.

2.7. ODM and Emission Analysis

To assess logistical sustainability, ODM was conducted using ArcGIS Network Analyst. Travel distances and durations from each site to regional market centers were calculated and carbon emissions were estimated based on standard vehicle CO_2/km coefficients. OD outputs were cross-referenced with suitability results to prioritize sites offering both environmental and logistical advantages.

2.8. Qualitative Assessments and Indirect Sustainability Factors

To enrich the model with socio-environmental insights, indirect factors were also evaluated:

- Proximity to settlements to reduce commuting needs;
- Avoidance of protected ecological zones;
- Alignment with regional development goals and landscape aesthetics.

These qualitative filters were implemented through rule-based spatial queries and validated by domain experts.

2.9. Mapping, Visualization and Validation

The final outputs consisted of three principal maps:

- A categorical suitability map (weighted overlay);
- A continuous gradient map (weighted sum);
- A transitional suitability map (fuzzy overlay).

Each map was enriched with road networks, hydrologyand administrative boundaries to contextualize spatial relationships. Multi-scale visualization (micro, meso and macro levels) enhanced interpretability. Suitability maps were validated through:

- Ground-truth visits to high-ranking zones;
- Overlay with known greenhouse infrastructure;
- Expert review workshops.

2.10. Policy and Planning Implications

The integrated spatial decision framework supports informed policymaking by:

- Recommending site clusters for greenhouse investment near renewable energy hubs;
- Aligning agricultural development with green logistics networks;
- Offering a scalable model for sustainable regional planning.

2.11. Contribution to the Literature

The reviewed literature highlights considerable progress in GIS applications and green logistics. However, the synthesis of these fields—particularly for greenhouse site selection considering both environmental and socio-logistical dimensions—remains limited. This study contributes to the scholarly discourse by:

- Integrating environmental, logistical and socio-economic data into a unified GIS platform;
- Employing ODM to quantify travel-based emissions;
- Proposing a replicable and flexible model that accommodates both quantitative and qualitative data.

By advancing a comprehensive, multidimensional GIS-based methodology, this study addresses the methodological and conceptual gaps in sustainable agricultural planning and logistics-informed site selection.

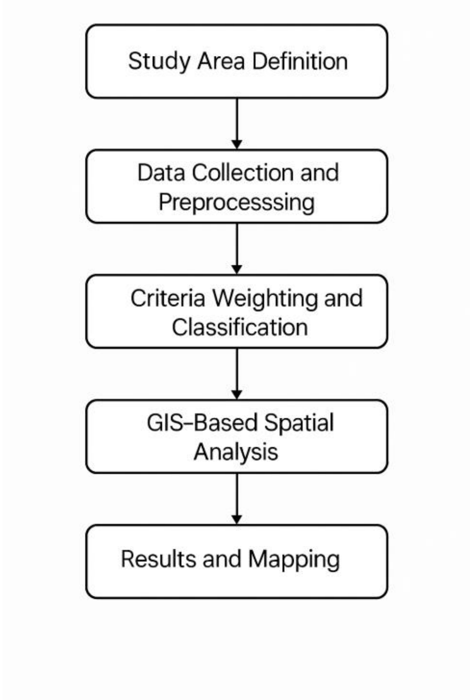

Figure 1. Flow chart of the study.

3. Results

This section presents the results obtained through the application of three spatial modeling techniques—namely, Weighted Overlay Analysis, Weighted Sum Analysis, and Fuzzy Overlay Analysis. These geospatial outputs are further complemented by origin-destination travel evaluations and qualitative assessments related to sustainability. The findings are discussed within the conceptual framework of green logistics, with particular emphasis on the environmental compatibility and logistical feasibility of the identified greenhouse site locations.

3.1. Weighted Overlay Analysis

The Weighted Overlay Analysis yielded a spatially explicit evaluation of greenhouse site suitability, generating a composite suitability map that classified the study area into six distinct categories ranging from very low to very high potential. The analysis identified high suitability zones predominantly within the provinces of Ordu and Giresun, especially in coastal regions and adjacent low-elevation inland areas. These zones were characterized by a favorable confluence of key environmental and logistical factors, including optimal solar radiation levels, moderate precipitation, accessible terrain, and proximity to major transportation corridors.

A total of nine spatial datasets were integrated into the model, each pre-processed and reclassified in accordance with defined suitability thresholds derived from relevant literature and expert consultation. The model employed a percentage-based weighting scheme, wherein each layer was assigned a relative importance value summing to 100. These weights were determined through a combination of expert judgment and benchmarking against established studies, ensuring that each variable's influence accurately reflected its contribution to greenhouse viability.

To enhance comparability and analytical rigor, all input layers were normalized into six suitability classes. A predefined scoring scale—1, 2, 2, 3, 4, and 4—was applied across layers, with higher scores corresponding to more favorable conditions. In this schema, zones identified as highly suitable received a score of 4, moderately suitable areas were assigned a score of 3, and less suitable or unsuitable areas were given scores of 1 or 2. This classification and weighting approach enabled a structured and interpretable representation of spatial heterogeneity, supporting both methodological transparency and policy-relevant insights for sustainable site selection.

It is noteworthy that road network data, despite its central relevance to logistical planning, was deliberately excluded from the Weighted Overlay Analysis to prevent redundancy and the overemphasis of accessibility-related variables. Including it within the same module would have risked inflating the influence of transportation infrastructure, thereby biasing the composite suitability scores. Instead, the outputs of the weighted overlay analysis were employed as a spatial baseline to inform a subsequent and distinct assessment focused on road alignment and logistical connectivity. This sequential analytical approach ensured that transportation planning was anchored in the core suitability zones derived from environmentally and socio-economically driven criteria, thereby maintaining methodological integrity and enhancing the coherence between ecological suitability and infrastructural development.

selection, particularly within the broader paradigm of green logistics and spatial sustainability.

To operationalize spatial suitability, all datasets were reclassified into six ordinal categories based on a predefined scoring scheme of 1–2–2–3–4–4. This scoring system enabled the stratification of land parcels according to their relative suitability, wherein values of 4 corresponded to highly suitable zones, a score of 3 denoted moderate suitability, and scores of 1 or 2 captured marginal or unsuitable conditions. The use of repeated scores (e.g., 2 and 4) permitted a nuanced yet analytically consistent representation of spatial gradients without compromising interpretability.

Within the ArcGIS environment, the weighted overlay procedure systematically applied the assigned percentage-based weights alongside the standardized scoring matrix. These inputs were documented in a tabular graphical format, ensuring transparency and reproducibility of the analytical process. Moreover, the visualized structure of the weights and scores facilitated both internal validation and external communication of the spatial decision-making rationale underpinning the site selection model.

To prevent overrepresentation, road network data—while critical to logistics—was excluded at this stage and evaluated separately in the logistical module. Instead, the results generated from the weighted overlay analysis were used as a spatial foundation for identifying and evaluating potential road alignments in a separate logistical assessment. This approach ensured that transportation planning was guided by the core suitability zones identified through environmental and socio-economic criteria.

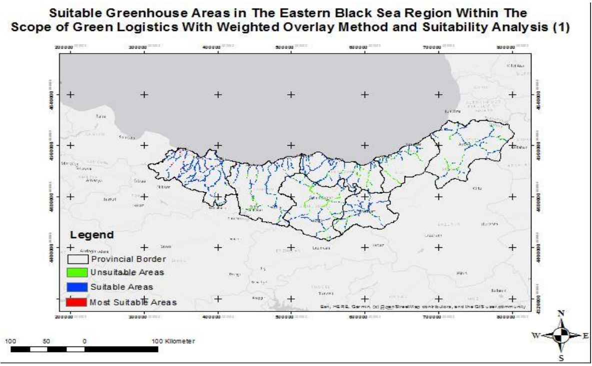

Figure 2. Map produced through weighted overlay spatial analysis.

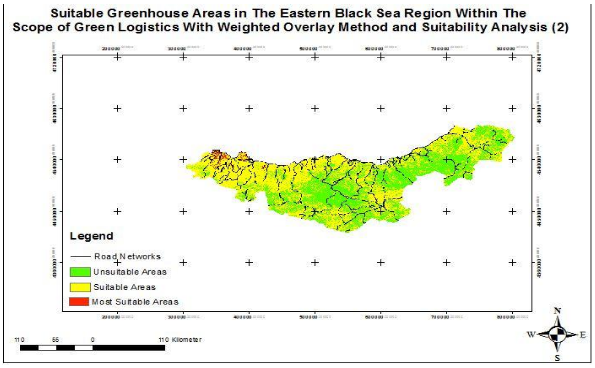

Figure 3. Map of the analysis performed with weighted overlay without including road data.

3.2. Weighted Overlay Analysis 2

The Weighted Overlay module was utilized to synthesize eight spatial datasets, each of which had been pre-processed and classified in accordance with predetermined suitability criteria. Within this integrative analytical framework, each dataset was assigned a weight on a scale from 0 to 100, reflecting its proportional influence within the overall model. These weight values were informed by expert elicitation and grounded in the thematic relevance of each criterion to greenhouse site

3.3. Weighted Sum Analysis

The Weighted Sum Analysis was employed to preserve the continuous nature of input variables, thereby enabling a more granular and nuanced representation of spatial suitability across the study area. In contrast to the categorical output of the Weighted Overlay Analysis, this method captured cumulative effects and revealed additional patterns of viability. Notably, areas previously classified as moderately suitable—particularly in parts of Trabzon and Artvin—demonstrated enhanced suitability when all spatial factors were aggregated as continuous inputs. This broader interpretive lens underscored a more spatially balanced distribution of greenhouse potential across the region, expanding the scope of viable planning zones.

Eight pre-classified datasets were imported into the Weighted Sum module for evaluation. To maintain methodological coherence, the weight values assigned to each dataset mirrored the percentage-based impact values previously utilized in the weighted overlay model. These weights were normalized to ensure that their collective sum equaled 100, thereby preserving analytical integrity and facilitating cross-method comparability. The results of this analysis provided a complementary perspective and served as a strategic reference point in the subsequent identification of optimal road alignments, offering valuable input for integrated spatial planning and sustainable infrastructure development.

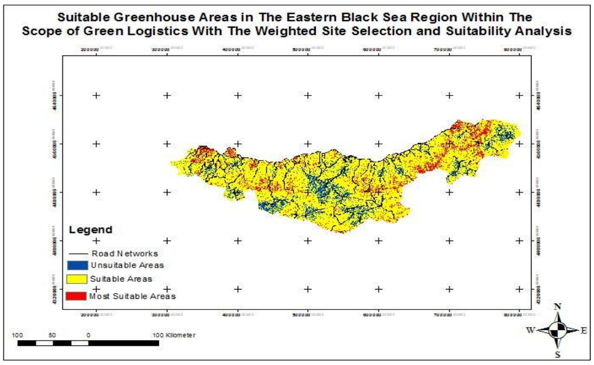

Figure 4. Map generated through weighted sum analysis.

3.4. Fuzzy Overlay Analysis

The Fuzzy Overlay Analysis was applied to incorporate uncertainty tolerance and to better capture transitional zones exhibiting moderate yet meaningful suitability potential. Unlike deterministic models, this approach was particularly effective in accounting for ecological gradients and spatial heterogeneity, thereby enhancing the representation of landscape complexity. The analysis revealed that several mid-altitude inland areas—particularly in Gümüşhane, Bayburt, and eastern parts of Trabzon—demonstrated higher suitability values than previously indicated by the Weighted Overlay and Weighted Sum models. These findings highlighted the model's capacity to identify emergent suitability patterns in areas that were otherwise underestimated.

A total of nine spatial datasets, previously categorized into six suitability classes, were transferred to the fuzzy overlay module for further processing. Data entry was conducted manually by the analyst to ensure accurate classification and proper integration within the tabular graphical interface of the GIS environment. During the modeling process, the fuzzy combination method was configured to the logical "AND" operator, meaning that only those areas satisfying all established suitability conditions were retained in the final output. This conservative approach enhanced the analytical precision of the model, particularly in the context of multi-criteria environmental assessments.

Although road network data was intentionally excluded from this module to maintain analytical independence, the resulting spatial outputs were subsequently used as a reference layer for optimizing road routing strategies. Thus, the fuzzy overlay results contributed substantively to the spatial decision-making process by providing an ecologically attuned basis for infrastructure alignment within a green logistics planning framework.

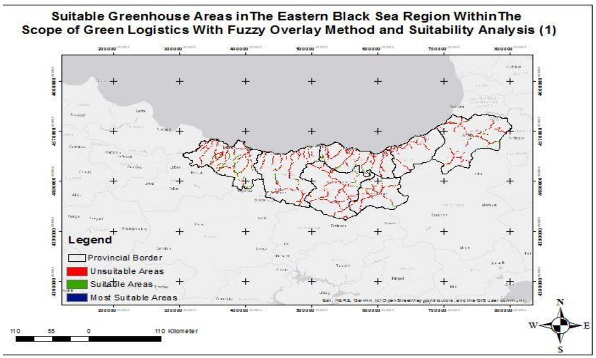

Figure 5. Map of the analysis performed with fuzzy overlay method.

3.5. Fuzzy Overlay Analysis 2

Eight classified spatial datasets were integrated into the Fuzzy Overlay module for processing. Each dataset, previously reclassified into six ordinal suitability categories, was manually entered by the analyst to ensure that the predefined suitability conditions were accurately reflected within the tabular graphical interface of the GIS platform. This step ensured methodological transparency and consistency in the application of the fuzzy logic framework. During the assignment phase, the overlay combination type was set to the logical "AND" operator. This configuration ensured that only areas simultaneously satisfying all specified criteria were retained in the final output, thereby allowing for a more conservative and refined evaluation of spatial suitability. The use of the "AND" operator enhanced the model's discriminative capacity by prioritizing locations that demonstrated comprehensive compliance with all environmental and socio-economic conditions. Importantly, road network data was excluded from this module to maintain analytical independence and avoid redundancy with previous models. Nonetheless, the output of the Fuzzy Overlay Analysis provided critical spatial insight and was subsequently utilized as a foundational reference for identifying optimal road alignments. In this way, the fuzzy model contributed meaningfully to integrated spatial decision-making processes within the context of sustainable logistics planning.

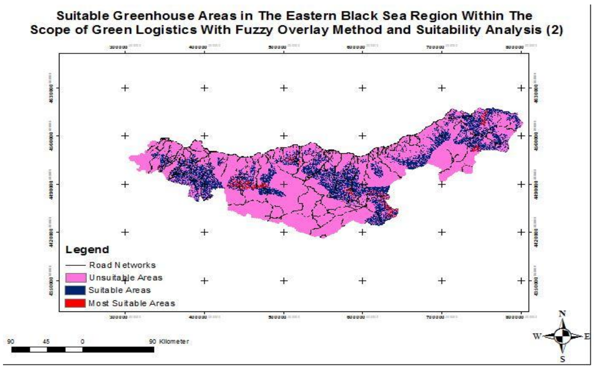

Figure 6. Map of the analysis performed with fuzzy overlay without including road data.

3.6. ODM and Emission Reduction Potential

The Origin-Destination (OD) analysis demonstrated that the top-ranked greenhouse sites identified through each spatial modeling approach yielded notably reduced average travel distances to regional market hubs—ranging from 15% to 28% shorter than those of randomly selected alternative locations. This spatial efficiency was most pronounced in areas characterized by dense transportation infrastructure and high concentrations of available labor. Furthermore, estimated carbon dioxide (CO_2) emissions associated with logistics operations were significantly lower for these optimal sites, with reductions ranging between 18 and 32 kg CO_2 per week per greenhouse unit, contingent upon both travel distance and the quality of road infrastructure.

These findings substantiate the utility of incorporating OD modeling into site selection procedures and underscore the critical role of spatial proximity to distribution centers in enhancing green logistics performance. By minimizing travel time and emissions, the analysis reinforces the environmental and operational value of geospatial decision support in sustainable agricultural planning.

3.7. Qualitative and Indirect Sustainability Insights

Incorporating qualitative criteria refined the interpretation of technical outputs. Sites adjacent to settlements with a high density of working-age population offered dual benefits: operational efficiency due to local labor availability and social sustainability through reduced commuting emissions and enhanced worker well-being.

Furthermore, the exclusion of ecologically sensitive zones and culturally significant landscapes ensured that recommended sites align with sustainable land stewardship principles. These findings reinforce the necessity of embedding qualitative judgments in technical planning.

3.8. Policy and Practical Implications

The multi-model results suggest that effective greenhouse siting in the Eastern Black Sea region must balance environmental suitability with logistical accessibility and socio-environmental integration. From a policy standpoint, this calls for:

- Prioritizing infrastructure investment in high-suitability areas identified in all three models;
- Designing agricultural logistics corridors that connect production zones with regional markets using low-carbon transport systems;
- Developing site-specific guidelines that incorporate both quantitative metrics and local stakeholder input.

3.9. Limitations and Future Directions

While the analysis produced detailed insights, some limitations remain. The absence of real-time traffic data and vehicle-specific fuel efficiency estimates constrained the precision of emission modeling. Additionally, the integration of qualitative data was limited to expert consultation and spatial proxies.

Future research should aim to:

- Integrate participatory GIS approaches involving farmers, workers and community members;
- Employ high-resolution, real-time mobility and emissions datasets;
- Explore the economic feasibility of greenhouse development in each identified zone.

Overall, the findings affirm that combining GIS-based spatial analysis with green logistics and sustainability principles yields a robust, actionable framework for climate-smart agricultural planning.

4. Conclusion

This study developed and applied a comprehensive GIS-based framework to identify optimal greenhouse locations in Türkiye’s Eastern Black Sea region (Rize, Trabzon, Artvin, Giresun, Ordu, Gümüşhane and Bayburt.), integrating principles of sustainable agriculture and green logistics. By employing a spatial analysis approach—including weighted overlay, weighted sum and fuzzy overlay techniques—alongside ODM and qualitative sustainability criteria, the research offers a robust, multidimensional methodology for informed spatial planning.

The findings demonstrate that high-suitability zones are not limited to traditional lowland regions such as Ordu and Giresun, but also extend to inland and mid-altitude areas like Trabzon, Gümüşhane, Bayburt and Artvin. Each analytical technique contributed unique insights: the weighted overlay emphasized distinct high-suitability zones based on environmental constraints; the weighted sum analysis revealed a more balanced spatial distribution by aggregating moderate conditions; and the fuzzy overlay model excelled in identifying transitional suitability areas sensitive to both ecological gradients and spatial heterogeneity.

A key innovation of the study lies in the integration of ODM, which quantified the logistical performance of selected sites by evaluating travel distances, transportation emissions and market accessibility. Sites with proximity to existing road networks and labor markets demonstrated a marked reduction in estimated carbon emissions—thus reinforcing the relevance of logistical efficiency in greenhouse site selection. Additionally, qualitative assessments ensured that the final recommendations avoided ecologically sensitive zones, minimized worker commute distances and aligned with local development plans.

From a theoretical perspective, this study contributes to the growing literature at the intersection of GIS, green logistics and sustainable agriculture by operationalizing a spatial decision-support system that is replicable, scalable and adaptable. Practically, it provides actionable insights for regional planners, agricultural investors and policymakers seeking to align greenhouse development with environmental conservation, economic efficiency and social inclusion.

Nevertheless, the study acknowledges certain limitations. Medium-resolution datasets may obscure fine-grained spatial variability and while factor weighting was informed by expert judgment and prior studies, it retains a degree of subjectivity. Future research should incorporate high-resolution environmental data, real-time transportation analytics and machine learning algorithms to further automate and refine suitability assessments. The inclusion of economic feasibility analysis and participatory GIS frameworks involving stakeholders could enhance the model's practical application and social relevance.

In conclusion, this research demonstrates that the convergence of geospatial technologies, decision-making and green logistics principles can yield a sophisticated, actionable foundation for developing low-emission, resource-efficient and socially responsive agricultural systems. The Eastern Black Sea region, with its climatic complexity and logistical opportunities, stands as a prime example of how integrative spatial planning can support resilient, climate-smart food production strategies that contribute to both regional development and global sustainability objectives.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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EVALUATION OF FOLIAR PACLOBUTRAZOL APPLICATION ON GROWTH SUPPRESSION, YIELD, RESIDUE LEVELS AND FRUIT QUALITY IN GREENHOUSE TOMATO CULTIVATION

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Abstract: This study investigated the effects of foliar paclobutrazol (PBZ) applications at varying doses and intervals on the vegetative growth, yield, fruit quality, and residue accumulation in greenhouse-grown tomatoes. The experiment was conducted from April to November 2021 in a controlled greenhouse environment at Tokat Gaziosmanpaşa University, Türkiye. Indeterminate beef tomato cultivar Bellfort F_1 (Enza Zaden) was used in the study. PBZ was applied foliarly at concentrations of 10, 20, and 40 mg/L at 7-day and 14-day intervals. Results indicated that PBZ significantly reduced plant height and internode length, with the most compact plants observed under the 40 mg/L 14-day treatment. However, yield-related parameters responded more favorably to lower PBZ doses. The highest marketable yield (175.59 t/ha) and total yield (178.27 t/ha) were obtained under the 10 mg/L 14-day treatment, which also supported greater fruit number and higher dry matter content. Moderate PBZ doses improved fruit weight and chlorophyll index, whereas higher doses tended to suppress yield and quality. While fruit soluble solids and titratable acidity were not significantly affected, slight improvements were noted under moderate PBZ treatments. Importantly, no PBZ residues were detected in fruit samples. These findings demonstrate that foliar PBZ application at low doses is effective for controlling vegetative growth without compromising fruit quality or leaving detectable residues. The results support the use of foliar PBZ as a safe and practical growth regulation strategy for high-density tomato production under greenhouse conditions.

Keywords: Plant height control, Internod length, Solanum lycopersicum, PBZ residue, Yield, Growth retardan

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1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops worldwide and in Türkiye in terms of both production and consumption. According to 2023 data, World tomato production reached approximately 192.32 million tonnes across an area of about 5.12 million hectares. China (70.21 million tonnes) and India (20.43 million tonnes) lead in production, followed by Türkiye in third place, with 13.30 million tonnes cultivated over 166.32 thousand hectares (Food and Agriculture Organization (FAO, 2023). In Türkiye, tomato cultivation is widely practiced in both open-field and greenhouse conditions. Notably, greenhouse tomato production accounts for a significant share, with approximately 4.16 million tonnes produced under protected cultivation as of 2024 (TSI, 2025).

Indeterminate tomato cultivars, commonly grown in greenhouses, exhibit continuous and prolonged vegetative growth. These cultivars are typically planted at a density of 2–3 plants per square meter, and individual plants can reach heights of 7 to 10 meters. The excessive vegetative growth complicates the use of support systems and increases labor demands, which in turn raises production costs. Consequently, there is a growing interest among producers in developing alternative methods to control plant height. Both physical and chemical strategies are employed for this purpose, with plant growth regulators (PGRs) playing a central role.

Paclobutrazol (PBZ), a member of the triazole chemical group, is a widely used PGR known for its efficacy in restricting plant height, particularly under greenhouse conditions. As an antagonist of gibberellins (GAs) and auxins, PBZ inhibits GA_3 biosynthesis, thereby limiting cell elongation and division and ultimately suppressing shoot elongation (Kumar, 2021). Gibberellins are known to promote internode elongation; however, the inhibition of their biosynthesis by PBZ results in plants with compact growth due to continued cell division but reduced cell elongation (Dayan et al., 2012; Kalra and Bhatla, 2018; Chen et al., 2020). This feature has made PBZ widely applicable in vegetable and ornamental seedling production. Numerous studies have demonstrated the beneficial effects of PBZ on seedling height control and quality. Research on various crops such as lettuce (Geboloğlu et al., 2016), cucumber (Aktaş et al., 2024), tomato (Kum and Geboloğlu, 2024; Brigard et al., 2006), pepper (Silva et al., 2021), and eggplant (Geboloğlu et al., 2015) has shown that PBZ effectively restricts seedling height and contributes to the development of more compact and healthier seedlings.

PBZ is typically applied via two main methods: foliar spraying and drenching the growing medium. Both application techniques have been reported to yield effective results (Rademacher, 2015). Due to its limited mobility in the phloem and upward (acropetal) movement through the xylem, PBZ tends to accumulate in leaves (Witchard, 1997; Rademacher, 2000; Singh and Ram, 2000; Kumar, 2023). As a result, PBZ residue levels in fruit and seeds remain low (Davis et al., 1988, Kumar, 2023).

In tomato cultivation, PBZ application shortens internode length, resulting in compact plant architecture. This allows the production of sturdier plants without negatively affecting flowering quality (Mansuroglu et al., 2009; Currey and Lopez, 2010). Similar growth-regulating effects of PBZ have also been reported in various fruit species (Ghosh et al., 2022). Depending on the application dose and crop species, PBZ may delay or accelerate flowering (Desta and Amare, 2021). Previous studies have shown that PBZ can significantly influence several morphological and physiological traits in tomatoes, including plant height, fruit shape, pericarp cell structure, flowering time, and fruit yield (Chen et al., 2020; Li et al., 2022).

The aim of this study is to determine the effects of foliar-applied paclobutrazol on plant height, yield, quality parameters, and residue levels in fruits of tomato plants grown under greenhouse conditions. The study also aims to evaluate the feasibility of controlling plant height by reducing internode length and to assess the potential implications of this application on the greenhouse production process.

2. Materials and Methods

This study was conducted between April 20 and November 10, 2021, in the greenhouse of Tokat Gaziosmanpaşa University Research and Application Center, located in Tokat province, which lies between the Central Black Sea and Central Anatolia Regions of Türkiye. The research site is situated between 39°51'–40°55' North latitude and 35°27'–37°39' East longitude.

2.1. Experimental Conditions and Plant Material

In the experiment, plants were grown in six-legged pots measuring 75×25×21 cm with a volume of 24 liters. The pots were placed on a ground surface covered with white plastic mulch. The study was carried out in a 2000 m² greenhouse equipped with heating and semi-automated environmental controls, with a height of 5 meters. A sterilized mixture of peat moss and perlite in a 2:1 ratio was used as the growing medium.

The plant material used was the indeterminate tomato cultivar "Bellfort F_1" (Enza Zaden). The seedlings were transplanted approximately 40 days after sowing, at the 4–5 true leaf stage. Plants were spaced at 1.20 m between rows and 0.40 m within rows, with two plants placed per pot.

2.2. Irrigation and Fertilisation Practices

Irrigation was carried out four times per day using Hoagland nutrient solution, with a daily total of 500 ml of solution applied per plant. The nutrient solutions were prepared in 1000-liter tanks. Fertilisation and irrigation were conducted simultaneously using a fully automated fertigation system.

Fertiliser concentrations were determined based on the method developed by Hoagland and Arnon (1950), with modifications made according to the developmental stages of the plants. Accordingly, the nutrient solution was prepared with an elemental ratio of N:P:K:Ca:Mg = 2:1:2:1.5:1 until the flowering stage, and N:P:K:Ca:Mg = 2:1:3:1.5:1 from flowering until the end of the harvest. While preparing the nutrient solution, fertilisers from Peters Professional containing N:P:K+trace elements (TE) were used, which include a full range of micronutrients. Calcium was supplied through calcium nitrate, and magnesium through magnesium nitrate. Fertigation began on the fourth day after transplanting, following an initial period of four days during which the seedlings were irrigated with plain water only. The electrical conductivity (EC) of the solution was maintained at 2.0 dS/m before flowering and increased to 2.2 dS/m after the onset of flowering. Fertilisation was applied during each irrigation cycle. Regular measurements were taken from drainage water to monitor and prevent salt accumulation in the growing medium. Irrigation was performed six times daily at equal intervals, with each cycle lasting two minutes. The experiment was concluded on November 20, following the completion of all observations and recordings of treatment effects on tomato plants.

2.3. Paclobutrazol (PBZ) Applications

Paclobutrazol applications were performed using Cultar 25SC (Active Ingredient: 250 g/L paclobutrazol; Formulation: suspension concentrate; Syngenta). Prior to application, PBZ solutions were prepared at concentrations of 10, 20, and 40 mg/L. Applications were conducted in two different intervals: every 7 days and every 14 days. PBZ was applied as a foliar spray, specifically targeting the shoot apex, using a hand-held sprayer. In each application, 5 mL of the prepared PBZ solution was sprayed per plant. The first PBZ application was initiated either 7 or 14 days after transplanting, depending on the treatment schedule. Plants receiving the first application 7 days after transplanting were treated at 7-day intervals thereafter, while those treated at 14 days after transplanting were subsequently treated at 14-day intervals. The final PBZ application was conducted on October 30, 2021. Control plants were sprayed similarly with 5 mL of distilled water per plant onto the shoot apex to ensure uniform handling across treatments.

2.4. Observations and Measurements

Plant Height (m): After the completion of all harvests, the plant height, defined as the distance from the root collar to the apical meristem, was measured using a measuring tape. The average plant height for each plot was subsequently calculated and expressed in meters.

Internode Length (cm): Following the completion of the harvests, the internode length for each plant within the plot was measured using a measuring tape. The mean internode length per plant was then calculated to determine the average internode length in centimeters.

Marketable Yield (t/ha): During each harvest, the collected fruits were categorized as either marketable or non-marketable. Marketable fruits were weighed using a precision scale with an accuracy of 0.01 g, and the corresponding fruit weights for each plot were recorded. Upon completion of all harvests, the total marketable fruit weight per plot was converted to yield values, which were then expressed in tons per hectare.

Total Yield (t/ha): For each harvest, both marketable and non-marketable fruits were weighed together using a precision balance with an accuracy of 0.01 g, and the weights were recorded. After all harvests were completed, the total fruit weight per plot was calculated and subsequently converted to yield values, expressed in tons per hectare.

Marketable Fruit Number (fruits/plant): The marketable fruits collected during each harvest were divided by the number of plants in the plot to calculate the number of fruits per plant. Following the completion of all harvests, the total number of fruits harvested per plant was calculated.

Marketable Fruit Weight (g): The weight of the fruits harvested during each collection was divided by the number of fruits, and the average marketable fruit weight for each harvest was calculated. After all harvests were completed, the average weight of the total harvest was calculated, and the marketable fruit weights were determined.

2.5. Physicochemical Fruit Quality Analysis

Fruit samples were collected during the fourth and sixth harvests. The samples were homogenized using a blender, and the resulting pulp was filtered through Whatman No. 42 filter paper. The filtrate was used for the determination of pH, soluble solids content (SSC, %), and titratable acidity (TA, %).

The pH was measured using a digital pH meter (Hanna HI-9812-5N, USA). Soluble solids content was determined with a digital refractometer and expressed as a percentage (% Brix). Titratable acidity was calculated as citric acid equivalent. For TA measurement, 20 mL of fruit juice was titrated with 0.1 N NaOH solution until reaching a pH of 8.1. TA was determined following the pH-metric method described by Cemeroğlu (2010) and expressed as a percentage.

2.6. Physiological Measurements

Chlorophyll Index (SPAD): The chlorophyll index of the leaves was measured eight weeks after transplanting. Measurements were conducted on all plants within each plot, targeting the fourth and fifth fully expanded leaves from the apical meristem downward. A portable SPAD chlorophyll meter (Minolta SPAD-502, Osaka, Japan) was used for non-destructive assessment of chlorophyll content.

Leaf Dry Matter Content (%): For each plot, the fourth and fifth fully expanded leaves were collected from each plant. Fresh weights were immediately recorded using a precision analytical balance (±0.001 g accuracy). The leaf samples were then dried in a forced-air oven at 65°C until reaching a constant weight. Dry weights were subsequently measured, and leaf dry matter content was calculated as the ratio of dry weight to fresh weight, expressed as a percentage.

Fruit Dry Matter Content (%): At the fourth and sixth harvests, two representative fruits were randomly selected from each plot. Fresh weights were determined using a precision analytical balance (±0.001 g accuracy). The fruits were quartered and oven-dried at 65°C until a constant weight was achieved. After drying, dry weights were measured, and fruit dry matter content was calculated as the ratio of dry weight to fresh weight, expressed as a percentage.

2.7. Residue Analysis

The analysis of PBZ was conducted using a Shimadzu® LC-MS 8050 system, which possesses advanced UPLC, and MS/MS capabilities. The chromatographic separation was achieved using a HPLC column (Inertsil ODS IV). The multiple reaction monitoring (MRM) transitions employed for the detection, and quantification of PBZ in LC-MS/MS analysis are enumerated below: The precursor ion at m/z 294.10 was monitored with product ions at m/z 125.10, 130.10, and 134.80. The collision energies applied for these transitions were -15.0 eV, -15.0 eV, and -28.0 eV, respectively. The declustering potential were set at -38.0 V, -33.0 V, and -35.0 V, while the entrance potentials were maintained at -21.0 V, -22.0 V, and -27.0 V, respectively.

A gradient elution method was employed, using eluent A (distilled water with 5 mM ammonium formate), and eluent B (methanol with 5 mM ammonium formate). The gradient elution program was structured as follows: Initially, the mobile phase consisted of 10% eluent B. The concentration of eluent B was then increased to 99% over a period of 7 minutes (from 1.00 min to 8.00 min), and maintained at this level until 12.00 min. At 13.00 min, the concentration of eluent B was reduced back to 10%, and held at this level until 13.01 min. The total runtime was 15.00 min, at which point the system was programmed to stop. Instrument parameters were controlled using LabSolution® software (version 5.118). The retention time (RT) for PBZ was determined to be 9.983 minutes, as shown in the chromatogram (Figure 1). This confirms the accurate identification of PBZ under the specified chromatographic conditions.

Tomato fruit samples were collected after on 1st, 3rd, 5th, 7th, and 10th treatments after spraying PBZ. The sample preparation procedures followed the QuEChERS AOAC Method 2007.01, by Lehotay (2007). The verification of the method adhered to SANTE guidelines (SANTE, 2021; Balkan and Yılmaz, 2022). According to SANTE guidelines, the method meets the required validation criteria of 70-120% recovery, and ≤20% RSD values. The method verification parameters in Table 1 demonstrate high sensitivity, accuracy, and precision, aligning with SANTE guidelines, and previous literature (Balkan and Kara, 2021).

2.8. Experimental Design and Statistical Analysis

The trial was conducted in a randomized plot design with three replications. Each replication consisted of five pots, with two plants per pot. Data were statistically analyzed using one-way analysis of variance (ANOVA) in SPSS 20.0. Mean separations were performed using Duncan's Multiple Range Test at significance levels of 5%.

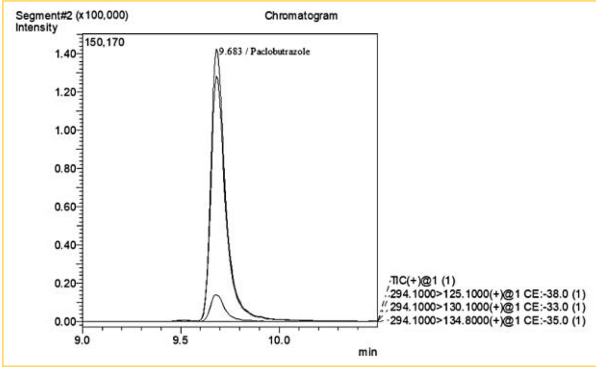

Figure 1. Chromatogram of paclobutrazole obtained from LC-MS/MS analysis after injection of a 25 µg/kg standard solution.

Table 1. Method optimization, and verification parameters

Analyte	RT (min)	Linear regression equation	Correlation coefficient (R^2)	LOD (µg/kg)	LOQ (µg/kg)	Fortification (µg/kg)	Repeatability Recovery % (RSD, %)	Reproducibility Recovery % (RSD, %)	U %
Paclobutrazol (PBZ)	9.683	Y= (24402.8)X + (44949.2)	0.99947	1.09	3.64	10	90.62 (4.10)	91.46 (7.24)	20.62
						50	103.1 (3.91)	102.08 (5.82)	
						100	104.39 (1.45)	100.88 (4.32)	

3. Results and Discussion

3.1. Plant Height and Internode Length

In this study, the findings clearly demonstrated that PBZ treatments significantly reduced both plant height and internode length. Plant height was significantly influenced by PBZ doses and application frequency (P ≤0.001). The shortest plants were recorded in the 40 mg/L 14-day PBZ treatment (3.37 m), closely followed by 20 mg/L 14-day (3.38 m) and 40 mg/L 7-day (3.44 m) treatments. All PBZ-treated plants were significantly shorter than the control plants (3.93 m). Particularly, the 40 mg/L 14-day application emerged as highly effective in achieving a compact plant architecture, which is crucial for optimizing space and improving plant management in greenhouse tomato production. Internode length was also significantly reduced by PBZ treatments (P ≤ 0.001). The shortest internodes were observed in plants treated with 40 mg/L 14-day PBZ treatment (19.77 cm). Similarly, 20 mg/L 7-day and 40 mg/L 7-day treatments also resulted in compact internode lengths (19.79 cm and 20.73 cm, respectively). In contrast, control plants exhibited the longest internodes (23.14 cm). The effects of PBZ applications on plant height and internode length in tomato are presented in Table 2.

A strong positive relationship was observed between plant height and internode length. Applications that reduced plant height simultaneously shortened internode length. Notably, the 40 mg/L 14-day treatment consistently resulted in both the shortest plant height and the most condensed internode structure, making it the most effective strategy for promoting a compact growth habit. This parallel reduction suggests that PBZ regulates vertical growth primarily by shortening internodal segments rather than reducing the number of internodes. Such coordinated growth control is ideal for intensive cultivation systems aiming for high planting densities, better canopy management, and improved operational efficiency. The literature supports these findings. Carr and Jaffe (1995) reported that PBZ applications decreased internode length in tomato plants. Flores et al. (2018) observed significant reductions in plant height in cucumber (24%), zucchini (34.7%), melon (16.3%), and watermelon (23.4%) following PBZ treatments. Studies across different species such as tomato (Banoo et al., 2020), canola (Hua et al., 2014) and bean (Bekheta and Talaal, 2009) have consistently reported similar trends. The underlying mechanism involves the inhibition of gibberellin biosynthesis. PBZ inhibits the enzyme ent-kaurene oxidase, a key enzyme in the gibberellin biosynthetic pathway, thereby restricting cell elongation while cell division continues (Kondhare et al., 2014; Banoo et al., 2020). As a result, plant height is significantly reduced due to the inhibition of gibberellin biosynthesis in the subapical meristem (Meena, 2014). Tesfahun and Menzir (2018) further emphasized that plant height reduction was mainly associated with reduced elongation of internodes rather than a decrease in the number of internodes. Similarly, Novita (2022) demonstrated that PBZ significantly reduced plant height in tomato plants. The results of this study clearly show that PBZ application at 40 mg/L with a 14-day interval is the most effective strategy for achieving compact plant growth in greenhouse tomato production. Through growth regulation, PBZ-treated plants became shorter, more compact, and easier to manage, enhancing spatial efficiency and operational practicality in intensive greenhouse systems.

3.2. Yield and Fruit Characteristics

The effects of PBZ treatments on yield and fruit-related characteristics are presented in Table 3. Marketable yield was significantly influenced by both the dose of paclobutrazol and the application interval (P≤0.01). The highest marketable yields were recorded under the 10 mg/L PBZ treatments, particularly at the 14-day (175.59 t/ha) and 7-day (174.51 t/ha) intervals, both of which were significantly superior to most other treatments (Table 3). In contrast, the 20 mg/L 14-day application resulted in the lowest marketable yield (151.22 t/ha). Notably, the 10 mg/L treatments outperformed the control treatment (159.66 t/ha), indicating that lower PBZ doses can enhance marketable yield under greenhouse conditions. Total yield followed a similar pattern (P≤0.01), with the highest values observed in the 10 mg/L 7-day (177.13 t/ha) and 10 mg/L 14-day (178.27 t/ha) PBZ treatments. Conversely, the 20 mg/L 14-day application produced the lowest total yield (154.86 t/ha), suggesting that higher PBZ concentrations, particularly when applied less frequently, may exert an inhibitory effect on yield formation. The control group (163.20 t/ha) yielded moderately but was consistently surpassed by all 10 mg/L treatments. Marketable fruit weight was also significantly affected by PBZ application (P≤0.01). The heaviest fruits were obtained with the 20 mg/L 7-day (174.49 g) and 10 mg/L 7-day (172.86 g) treatments, demonstrating that moderate PBZ doses can positively influence fruit weight. In contrast, the control group produced the lightest fruits (152.72 g). Moreover, the 40 mg/L 14-day treatment yielded relatively smaller fruits (157.32 g), suggesting that excessive PBZ application may restrict individual fruit growth despite controlling vegetative development. Regarding the number of marketable fruits per plant, significant differences among treatments were evident (P≤0.01). The highest fruit number was recorded under the 10 mg/L 14-day treatment (55.13 fruits/plant), closely followed by the control (53.34 fruits/plant) and the 40 mg/L 7-day treatment (52.57 fruits/plant). The 20 mg/L 14-day treatment resulted in the lowest fruit number (46.15 fruits/plant), reinforcing the notion that higher doses combined with extended application intervals may negatively impact reproductive performance.

Collectively, these findings suggest that PBZ applications at moderate doses (10 mg/L) effectively optimize both canopy structure and yield outcomes. Higher doses (40 mg/L) successfully enhanced plant compactness but led to reductions in yield parameters. In contrast, the 10 mg/L treatments, particularly when applied at a 14-day interval, achieved a favorable balance between vegetative growth regulation and high productivity, supporting their use as an efficient management strategy in greenhouse tomato production. These results are consistent with previous studies emphasizing the benefits of PBZ on crop yield and morphology. Rai et al. (2002) reported improved yield and quality parameters in various vegetable crops following PBZ application, while Jyothsna et al. (2022) observed enhanced yield in okra due to improved physiological traits and plant compactness. Similarly, Berova and Zlatev (2000) demonstrated that PBZ improved early fruit yield and water-use efficiency in tomatoes, aligning with the findings of this study that moderate PBZ doses enhance yield without compromising plant vigor. Conversely, Souza-Machado et al. (1999) observed no significant differences in total tomato yield despite accelerated maturity, suggesting that environmental conditions and cultivar characteristics may modulate PBZ efficacy. Meanwhile, studies by Giovinazzo et al. (2001) and Mohamed et al. (2011) corroborated the yield-enhancing potential of PBZ under specific production systems. Furthermore, Baloch et al. (2019) demonstrated that higher PBZ concentrations increased both fruit number and size in ornamental pepper, paralleling the present findings where moderate PBZ doses enhanced fruit size and number in tomatoes. Villavicencio et al. (2015) also indicated that while growth retardants significantly reduced plant height, they did not necessarily diminish fruit yield, highlighting the complex, species-specific responses to plant growth regulators.

3.3. Chlorophyll Index, Fruit Dry Matter and Leaf Dry Matter

In the study, leaf chlorophyll index among treatments ranged from 55.41 ± 5.65 to 60.13 ± 6.43 SPAD units. Although statistical analysis revealed no significant differences among treatments (p=0.528), numerical trends were evident. The highest chlorophyll index was recorded in the 20 mg/L 7-day PBZ treatment, while the lowest was observed in the 40 mg/L 14-day PBZ group. The control plants exhibited an intermediate value of 57.50 ± 6.31 SPAD units (Fig. 2). Notably, moderate PBZ doses (10–20 mg/L) tended to slightly enhance chlorophyll content relative to both the control and high-dose (40 mg/L) treatments, albeit without statistical significance. These findings align with prior research demonstrating that PBZ can promote higher chlorophyll concentrations. Berova and Zlatev (2000) reported increased photosynthetic activity following PBZ application in tomatoes, and similar enhancements of chlorophyll content in triazole-treated plants were documented by Xia et al. (2018) and Amooaghaie and Shariat (2014). Increased chlorophyll content is often attributed to PBZ's role in reducing gibberellin synthesis, leading to denser and darker green leaves. Although no significant enhancement was detected statistically in the present study, the numerical increases in SPAD values at moderate doses suggest a potential physiological benefit of PBZ in improving chlorophyll retention and leaf greenness.

Fruit dry weight percentages were significantly influenced by PBZ treatments (p ≤ 0.01). The highest fruit dry weights were recorded in the 10 mg/L 7-day (6.32% ± 0.39) and 10 mg/L 14-day (6.24% ± 0.31) treatments, closely followed by the 20 mg/L 14-day treatment (6.23% ± 0.39). In contrast, the 40 mg/L treatments produced notably lower fruit dry weights: 5.35% ± 0.32 (7 days) and 5.45% ± 0.29 (14 days). Control plants exhibited the lowest fruit dry weight at 5.12% ± 0.29 (Fig. 2). These results clearly demonstrate that lower PBZ doses, particularly at 10 mg/L, and shorter application intervals are more effective in enhancing fruit dry matter accumulation. Similar effects of PBZ in promoting dry matter partitioning towards reproductive organs have been reported in other crops. For instance, Huang et al. (1995) observed enhanced dry matter allocation to fruits in PBZ-treated apple trees, while Setia et al. (1996) and Tekalign and Hammes (2005) noted improved assimilate partitioning towards economic yield organs in Brassica and potato, respectively. The observed suppression of vegetative growth by PBZ likely redirects assimilates towards reproductive sinks, thus improving fruit dry weight percentage. Conversely, high PBZ doses (40 mg/L) negatively affected fruit dry weight, indicating potential over-suppression of physiological processes necessary for optimal fruit development. This outcome aligns with previous findings where excessive PBZ concentrations led to decreased biomass and compromised growth (Khalil and Rahman, 1995; Rigsby et al., 2025).

Leaf dry weight percentages varied between 14.31% ± 0.81 and 15.49% ± 0.89 across treatments, but these differences were not statistically significant (p=0.243). The highest leaf dry weight was recorded in the 10 mg/L 7-day treatment, followed by 10 mg/L 14-day and 20 mg/L 7-day applications. The lowest value was found in the 40 mg/L 14-day treatment (Fig. 2). Although not statistically meaningful, the numerical trend suggests that lower PBZ doses may slightly enhance leaf dry matter content. This is consistent with Senoo and Isoda (2003), who found no significant changes in leaf dry matter in PBZ-treated peanut plants. However, minor increases in leaf dry matter could be attributed to a thicker, more compact leaf structure induced by PBZ, resulting in heavier leaves per unit area. These structural changes are consistent with PBZ's known effects on plant morphology, including reduced leaf expansion and thicker mesophyll tissue (Berova and Zlatev, 2000; Xia et al., 2018).

Table 2. Effect of PBZ treatments on plant height and internode length of tomato

PBZ Doses	Application Interval	Plant Height (m)	Inter-node Length (cm)
10 mg/L	7 day	3.56 ± 0.22[b]	21.48 ± 1.86[ab]
10 mg/L	14 day	3.41 ± 0.20[b]	20.32 ± 1.83[b]
20 mg/L	7 day	3.46 ± 0.21[b]	19.79 ± 1.78[b]
20 mg/L	14 day	3.38 ± 0.21[b]	20.56 ± 1.67[b]
40 mg/L	7 day	3.44 ± 0.23[b]	20.73 ± 2.08[b]
40 mg/L	14 day	3.37 ± 0.24[b]	19.77 ± 2.08[b]
Control		3.93 ± 0.26[a]	23.14 ± 2.22[a]
Significance			
P-value		0.003	0.009
Sig.		**	**

Means in the same column followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test.
** Represents statistically significant difference at P≤0.01.

Table 3. Effect of PBZ treatments on yield and fruit characteristics in Tomato

PBZ Doses	Application Interval	Marketable Yield (t/ha)	Total Yield (t/ha)	Marketable Fruit Weight (g)	Marketable Fruit Number (fruits/plant)
10 mg/L	7 day	174.51 ± 9.42[a]	177.13 ± 9.67[a]	172.86 ± 14.86[a]	51.54 ± 3.31[ab]
10 mg/L	14 day	175.59 ± 9.41[a]	178.27 ± 10.43[a]	162.65 ± 13.42[bc]	55.13 ± 3.22[a]
20 mg/L	7 day	166.37 ± 9.78[ab]	173.14 ± 9.19[ab]	174.49 ± 12.46[a]	48.72 ± 3.22[bc]
20 mg/L	14 day	151.22 ± 8.31[c]	154.86 ± 8.96[d]	167.22 ± 11.87[ab]	46.15 ± 3.00[c]
40 mg/L	7 day	168.03 ± 8.58[ab]	172.12 ± 9.63[abc]	163.23 ± 10.25[bc]	52.57 ± 4.94[a]
40 mg/L	14 day	158.69 ± 8.09[bc]	162.77 ± 8.92[cd]	157.32 ± 9.54[cd]	51.54 ± 4.78[ab]
Control		159.66 ± 8.53[bc]	163.20 ± 8.82[bcd]	152.72 ± 9.78[d]	53.34 ± 5.18[a]
Significance					
P		0.008	0.001	0.002	0.002
Sig.		**	**	**	**

Means in the same column followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test.
** Represents statistically significant difference at P≤0.01.

3.4. Soluble Solid Content , Titratable Acidity and pH

In the study, soluble solids content in tomato fruits exhibited numerical variations among treatments, although these differences were not statistically significant (p=0.056). SSC values ranged from 5.83% ± 0.35 in the 10 mg/L 7-day PBZ treatment to 6.13% ± 0.55 in the control group. The highest SSC values were recorded in the control and 20 mg/L 7-day PBZ treatment groups (6.13% ± 0.44), suggesting that moderate doses of PBZ might support higher soluble solids accumulation. In contrast, the lowest SSC values were associated with the 40 mg/L PBZ treatments, particularly at the 14-day interval (5.93% ± 0.55) (Fig. 3). These findings align partially with previous research. For instance, Sarker and Rahim (2018) reported that PBZ application increased TSS in mango fruits, suggesting an enhancement in fruit quality at moderate PBZ doses. However, in other studies, such as those by Sansavini et al. (1986) and Deigado et al. (1986), PBZ application did not significantly affect SSC in apple and citrus fruits, respectively. This discrepancy could be attributed to species-specific responses, application methods, or environmental conditions during fruit development. The slight numerical increase in SSC observed at moderate PBZ doses in this study may indicate a potential, although limited, role of PBZ in improving fruit sweetness.

Titratable acidity (TA) values ranged narrowly from 0.181% ± 0.010 (40 mg/L 7-day PBZ) to 0.193% ± 0.011 (20 mg/L 7-day PBZ), and no significant differences were detected among treatments (p = 0.661) (Fig. 3). The control group exhibited a TA value of 0.185% ± 0.012, which was comparable to the PBZ-treated groups. Previous studies offer mixed results regarding PBZ's effects on fruit acidity. For example, Suja and Anusuya (2018) observed varying acid ratios following PBZ treatments in tomato, while Jain et al. (2002) and Burondkar et al. (2013) reported increased TA content in mango and lemon. Conversely, Samaan and Nasser (2020) and Sha et al. (2021) highlighted PBZ's role in reducing fruit acidity. In the current study, the negligible impact of PBZ on TA is consistent with findings in other crops such as grape (Christov et al., 1995) and strawberry (Lolaei et al., 2012), suggesting that the effect of PBZ on acidity might be minor in tomato or strongly influenced by cultivar-specific and environmental factors.

The pH values across all treatments ranged from 4.53 ± 0.40 (control) to 4.73 ± 0.36 (40 mg/L 14-day PBZ). Although no statistically significant differences were found (p = 0.184), a slight trend towards higher pH values with increasing PBZ doses, particularly at 40 mg/L, was observed (Fig. 3). These findings are in line with the results of Sansavini et al. (1986), who reported no significant effect of PBZ on pH in apple fruit quality studies. Similarly, Banoo et al. (2020) found that PBZ treatment did not substantially alter the internal quality parameters in tomatoes, including acidity and sweetness, despite improving yield attributes. The small pH increases observed at higher PBZ concentrations in this study might reflect subtle shifts in fruit chemical composition, possibly linked to altered metabolic processes under PBZ-induced growth regulation.

3.5. Effect of Paclobutrazol Applications on Residue Levels in Tomato Fruit

No paclobutrazol residues were detected in fruit samples collected at various time points, including one day after application. According to previous studies, PBZ applied to the soil is absorbed by the roots and transported via the xylem to apical meristems, leaves, and fruits (Early and Martin, 1988; Rademacher, 2000; Mahesvari et al., 2022). In the present study, however, lower doses were used, and applications were conducted foliarly, which may have limited the translocation of PBZ to the fruits and prevented residue accumulation. Fletcher et al. (2000) have reported that PBZ is not phloem-mobile, although some evidence suggests that it may exhibit limited movement in the phloem (Witchard, 1997; Singh and Ram, 2000). Given that PBZ is predominantly transported through the xylem and tends to accumulate in the leaves, with minimal phloem mobility, its low residue levels in fruits—supplied mainly via the phloem—are consistent with expectations (Davis et al., 1988.

These findings suggest that foliar application may reduce PBZ efficacy compared to soil application due to limited systemic movement. However, one of the primary objectives of this study—plant height reduction—was successfully achieved, and no residue was detected in the fruits. Therefore, foliar application of PBZ may be considered an effective strategy for growth regulation when residue management is a priority. Future research should explore the systemic behavior and residue dynamics of PBZ by comparing different application methods (foliar vs. soil) and dose levels in tomatoes and other vegetable crops.

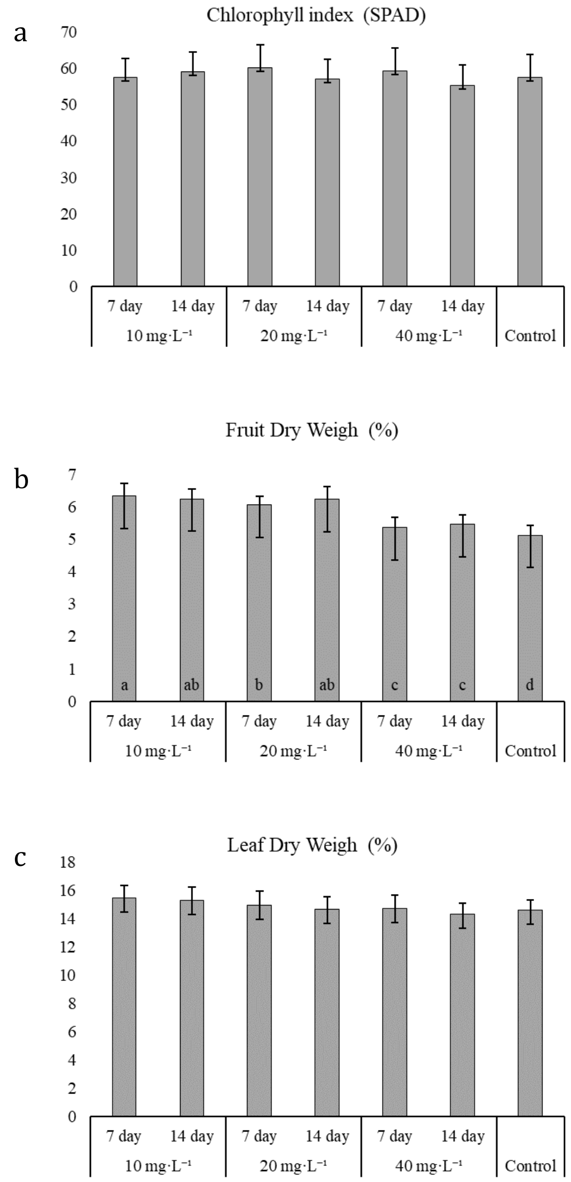

Figure. 2. Effect of PBZ treatments on chlorophylle index (a), fruit (b) and leaf dry weight (c) of tomato.

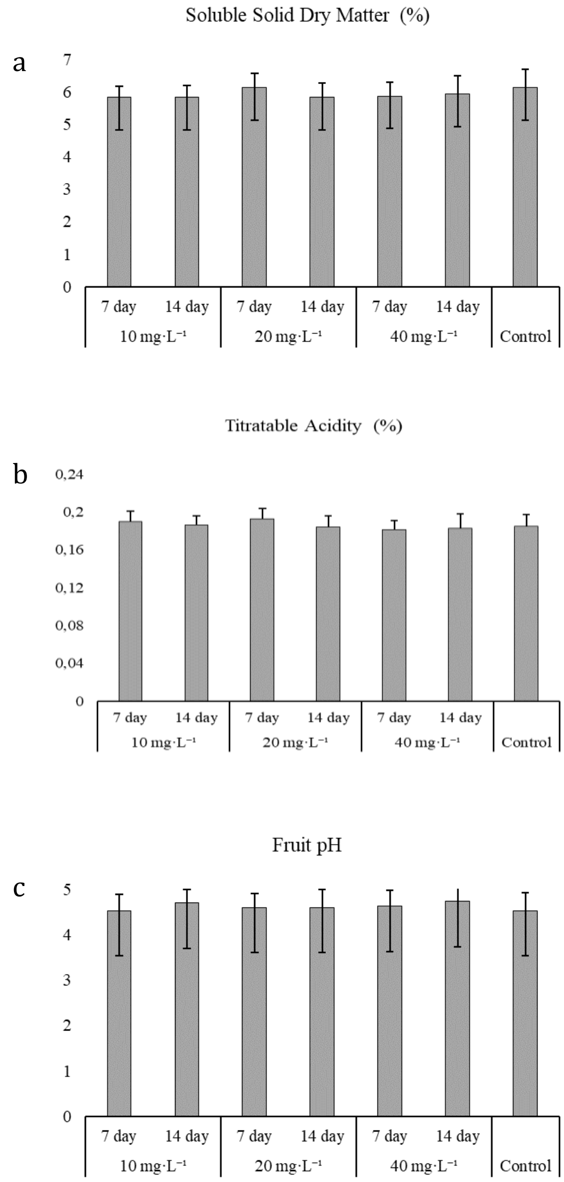

Figure. 3. Effect of PBZ treatments on soluble solid dry matter (a), titretable acidty (b) and fruit pH (c) of tomato.

4. Conclusion

This study comprehensively evaluated the effects of foliar paclobutrazol applications at different doses and intervals on plant growth, yield, fruit quality, physiological characteristics, and residue levels in greenhouse-grown tomatoes with a long production cycle. The results clearly demonstrated that PBZ significantly reduced plant height and internode length, with the most compact plant architecture achieved under the 40 mg/L dose applied every 14 days. This application was particularly effective for managing vertical growth, which is crucial for optimizing plant spacing and improving operational efficiency in high-density greenhouse systems.

In terms of yield, the 10 mg/L PBZ treatment, applied at both 7-day and 14-day intervals, resulted in the highest marketable and total yields, suggesting that lower PBZ doses effectively regulate vegetative growth while enhancing reproductive development. In contrast, higher doses (especially 20 and 40 mg/L) negatively impacted yield parameters, including fruit number and total yield. Regarding fruit weight, moderate doses of PBZ (10–20 mg/L) were found to positively influence fruit size, whereas excessive doses (40 mg/L) led to smaller fruits.

Physiological assessments indicated a numerical increase in chlorophyll index and dry matter content in both fruit and leaves, although these differences were not statistically significant. Quality parameters, including soluble solids content, titratable acidity, and pH, showed no significant differences across treatments. A key finding was the absence of detectable PBZ residues in fruit samples, indicating that foliar applications do not lead to residue accumulation in the harvested fruits.

In conclusion, foliar applications of PBZ at lower doses (10 mg/L), particularly at 14-day intervals, proved to be an effective strategy for regulating growth, enhancing yield, and ensuring residue-free fruit production in greenhouse tomato cultivation. These findings support the use of PBZ as a growth regulator, offering both agronomic and food safety benefits. Further studies should investigate the systemic behavior and residue dynamics of PBZ in different application methods (foliar vs. soil) and across various vegetable crops.

Author Contributions

Percentages of the authors' contributions are present below. All authors reviewed and approved final version of the manuscript.

	Ö.Y.	E. P.	T. B.	N. G.
C	30	30	20	20
D	50	40	10	
S			50	50
DCP	30	30	20	20
DAI	20	20	30	30
L	20	20	30	30
W		20	30	50
CR		20	30	50
SR		20		80
PM	20	30	20	30
FA	25	25	25	25

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

Conflict of Interest

The authors declare no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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AGRONOMIC CHARACTERISTICS OF TURKISH MAIZE LANDRACES HAVING THE LEVEL OF DIFFERENT OPACITY

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Abstract: Maize (*Zea mays* L.), a warm season cereal with high adaptability, can grow under various climatic conditions and has been cultivated in Türkiye for many years to meet diverse needs. While opacity is closely linked to protein quality in maize, studies evaluating agronomic performance in Turkish landraces with varying opacity levels are limited. This study utilized six local maize populations with opaque endosperm types and three standard genotypes, with field trials conducted in 2021 and 2022 using a randomized block design with three replications. During the first year, agronomic traits of the genotypes were examined, and harvested seed samples were categorized into five opacity levels (0%, 25%, 50%, 75%, and 100%) using a light table. In the second year, these samples were subjected to field trials, and agronomic measurements were repeated. Two-year averages revealed significant variations among genotypes in plant height (165.33–224.92 cm), first ear height (75.03–127.95 cm), ear length (15.69–19.56 cm), ear diameter (34.11–44.99 mm), number of rows per ear (10.43–21.33), number of grains per row (20.75–40.72), ear weight (88.76–258.37 g), and ear grain weight (64.12–204.91 g). Variance analysis showed significant effects of opacity level, genotype, and their interaction on the traits examined. An increase in opacity level was associated with decreases in plant height, first ear height, and ear weight. Compared to standard genotypes, populations POP2 and POP6 demonstrated promising agronomic traits at high opacity levels, indicating potential for breeding genotypes with high-quality protein. It has been evaluated that these populations can be used as source material in the development of genotypes with high quality protein.

Keywords: Opaque, Agronomic measurement, Light table, Protein quality, *Zea mays*

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1. Introduction

Maize (*Zea mays* L.), native to the Mexico-Guatemala region in Central America, has been cultivated as a staple grain in these areas for thousands of years. Globally, it is the third most cultivated crop after rice and wheat, with its cultivation area and production volume increasing annually. Approximately 70 million farming families worldwide rely on maize farming for their livelihood (Dowswell et al., 1996). Depending on its maturity period, maize plants can grow to a height of 2.5–4.5 meters within 4–6 months and produce 600–1000 seeds per ear. Its high yield potential compared to other cereals is attributed to the grain's efficient energy storage capacity and the plant's ability to utilize natural energy factors effectively through its roots, stems, leaves, and reproductive organs (Kırtok, 1998). Additionally, maize plays a critical role in meeting global demands for food, animal feed, industrial raw materials (e.g., starch, oil, sugar, protein, cellulose, and ethanol), and energy (Cerit et al., 2016).

Maize grain is composed of approximately 80–82% endosperm, 12–14% embryo, 5–6% shell, and 1% pedicel (Kırtok, 1998; Hallauer, 2001). Its biochemical composition includes about 70% starch, 10% protein, 5% oil, 2% sugar, and 2% ash (Öztürk, 2017). While maize shares a similar protein content with other cereals, this characteristic significantly influences its grain quality. The protein content of normal maize varieties typically ranges between 8% and 11%. Despite being adequate in quantity for nutritional purposes, the protein quality of maize is considered poor. This deficiency is primarily attributed to the proportional distribution of its protein fractions, which include albumin, globulin, glutelin, and zein (Osborne, 1987). Among these fractions, zeins are the predominant type and are notably deficient in essential amino acids such as lysine and tryptophan (Vasal, 2000). Consequently, normal maize genotypes exhibit low protein quality. However, reducing the proportion of zein fractions in the grain increases the levels of lysine and tryptophan, enhancing its protein quality (Prasanna et al., 2001). Thus, most research aimed at improving maize protein quality focuses either on directly increasing essential amino acid levels or on decreasing zein content.

Efforts to enhance the protein quality of maize began in the mid-1960s with the introduction of the opaque-2 mutant, which significantly increased lysine and tryptophan levels amino acids that are deficient in maize endosperm proteins. However, the use of the opaque-2 mutant was hindered by adverse pleiotropic effects, which limited its practical application (Krivanek et al., 2007). Subsequent interdisciplinary research addressed these challenges by improving the negative traits associated with the opaque phenotype, resulting in the development of Quality Protein Maize (QPM) varieties. These varieties are characterized by higher levels of lysine and tryptophan and enhanced protein quality compared to conventional maize (Atlin et al., 2010; Bressani, 1991; National Research Council, 1998). Research has shown that the protein in QPM has a nutritional value approximately 90% that of breast milk, compared to just 40% in conventional maize (Twumasi-Afriyie et al., 2016). Consequently, QPM varieties have proven beneficial for humans, pigs, and other monogastric animals, including poultry, due to their improved nutritional profile.

Existing literature highlights several studies focusing on various agronomic traits of maize. Özata and Kapar (2014) examined changes in grain yield, grain moisture at harvest, plant height, first ear height, protein content, and oil content in 20 hybrid dent maize varieties. Similarly, Çağlar (2016) investigated the grain yield and quality of maize genotypes grown across different locations. Yılmaz and Han (2016) analyzed the yield and yield components of eight maize varieties (TK6063, Calcio, Hido, Everest, Carella, Cadiz, Sagunto, and Tavascan), while Yılmaz et al. (2020) evaluated plant characteristics in silage maize varieties. Despite these contributions, studies focusing specifically on protein quality in local maize genotypes in Türkiye remain limited. Notably, agronomic traits have not been comprehensively studied in materials categorized by different opacity levels. Moreover, most existing research has been conducted for screening purposes rather than detailed characterization. To date, no studies have systematically separated local maize populations based on opacity levels to identify promising materials for both protein quality and agronomic traits. Consequently, there is a lack of source materials tailored for breeding efforts aimed at improving maize protein quality. In Türkiye, breeding studies predominantly focus on yield per unit area, often overlooking protein quality critical factor given maize's substantial role in human and animal nutrition. Addressing this gap by enhancing protein quality in maize is essential to meet nutritional demands effectively.

The aim of this study is to evaluate local maize populations collected from various regions of Türkiye which were previously characterized as having opaque features, in terms of their agronomic traits. Additionally, the study seeks to develop breeding materials with enhanced protein quality.

2. Materials and Methods

2.1. Material

2.1.1. Field experiment

The experiments were conducted in accordance with the randomized block trial design with 3 replications during the 2021 and 2022 summer main crop growing periods. The field experiment was carried out in Sarıcaeli Village, Çanakkale Province. The study utilized six opaque maize landraces (POP1, POP2, POP3, POP4, POP5, POP6) and three standard genotypes, including two normal and one opaque genotype (Table 1).

Table1. Six opaque local maize populations and three standard genotypes were used in the study

Genotypes	Characteristic	Source
POP1	Opaque population	ÇOMU
POP2	Opaque population	ÇOMU
POP3	Opaque population	ÇOMU
POP4	Opaque population	ÇOMU
POP5	Opaque population	ÇOMU
POP6	Opaque population	ÇOMU
*CADIZ (STD1)	Normal standard	Semilas Fito
*BODEGA (STD2)	Normal standard	May Tohum
*PI608781 (STD3)	Opaque standard	ÇOMU

ÇOMU: Çanakkale Onsekiz Mart University

2.1.2. Soil and Climatologic Features of Experimental Area

Soil analysis results, presented in Table 2, were obtained from the experimental field prior to the start of the study. The analysis revealed that the soil was poor in organic matter but high in clay content (Table 3). The soil analyses were performed in COBİLTUM (Çanakkale Onsekiz Mart University Science and Technology Application and Research Center).

The field experiment was designed using a randomized complete block design with three replicates. Each genotype was planted in two-row plots with a spacing of 70 × 20 cm and a row length of four meters. Sowing was performed manually on May 18, 2021, for the first year and on May 18, 2022, for the second year. Weed control was carried out manually in the experimental area during both years. Irrigation was applied weekly using a drip irrigation system installed at the site, and fertilization was delivered through the same system based on the results of soil analysis.

Table 2. The experimental material used in this study

Soil Analysis	Results	Class
pH	7.39	Neutral
Soil Properties (%)	67.70	Clay-loam
E.C. (mS/cm)	89.30	Low
Organic matter (%)	1.87	Low
Lime (%)	8.50	Medium chalky
P (kg/da)	2.83	Low
K (kg/da)	80.80	Low

Table 3. The climatological conditions of the experimental field (*1:2021, 2:2022)

Months	May		June		July		August		September		October	
Years	1	2	1	2	1	2	1	2	1	2	1	2
Average Temperature (°C)	19.9	18.4	24.1	24.1	28.2	26.2	28.3	26.6	23.1	22.0	18.1	17.6
Total Rainfall (mm)	57.3	7.6	57.1	7.4	2.0	44.8	0.0	111.6	8.9	1.2	75.9	0.6
Highest Temperature (°C)	36.6	33.1	38.5	33.4	39.1	35.4	39.7	36.1	32.8	31.8	24.6	28.5
Lowest Temperature (°C)	11.2	7.8	13.8	17.3	19.8	17.7	21.5	18.8	12.0	9.2	11	7.3
Temperature for Many Years (°C)	17.5		22.2		25.0		24.9		21.05		16.18	
Long Years of Rainfall (mm)	30.1		24.6		11.7		6.6		22.8		54.1	

The Çanakkale province, located in the northwest of Türkiye, shares similar climatic characteristics with the central areas of the province where the experiment was conducted. The average temperatures during the experimental period were comparable to the long-term averages; however, they were slightly higher than historical values. Summers in the region are typically dry, with no precipitation recorded in August. Fertilization was carried out on June 23, 2021, for the first year and on May 7, 2022, for the second year. The experimental area was deeply ploughed with a mouldboard plough once in every two years. After ploughing, 15-15-15 compound fertilizer was applied by hand, 10 kg nitrogen, 10 kg phosphate and 10 kg potassium per decare. Then, the cultivator was pulled and mixed into the soil. Then, the test area was made ready for planting by pulling a coulter. After planting, drip irrigation system was laid lateral to each row and irrigation was carried out by drawing water from the canal next to the trial field with a motor pump. Each irrigation was done by operating the motor pump for 8 hours at 15-day intervals. According to the soil analysis results, urea was applied in addition to the base fertilizer to provide 10 kg of pure nitrogen per decare.

2.2. Method

2.2.1. Agronomic measurements

The agronomic characteristics examined in the genotypes included plant height, first ear height, ear length, ear diameter, number of rows per ear, number of grains per row, ear weight, and grain weight per ear. Measurements were performed on 10 plants per replication for each population. The evaluation of agronomic traits followed the methodologies described by Kırtok (1998) and Kün (1978).

Plant Height (cm): The height of the plants, including the top tassel, was measured at harvest.

First Ear Height (cm): Measured as the distance from the soil surface to the first ear formed on the plant.

Ear Length (cm): Determined as the length from the base to the tip of the ear after removing the husks.

Ear Diameter (mm): Measured at the midpoint of the ear using a digital caliper.

Number of Rows on the Ear (number/ear): Counted visually to determine the number of grain rows across the ear's width.

Number of Grains per Row (number): Counted visually along the length of the ear from two representative points.

Single Ear Weight (g): Determined by weighing each dehusked ear individually on a precision scale.

Grain Weight per Ear (g): Grain samples obtained after dehusking were weighed on a precision scale.

2.3. Statistical Analysis

The data collected during the study were analyzed using the R software (R Core Team, 2019). Statistical models were applied in alignment with the randomized complete block design, utilizing one-way analysis of variance (ANOVA) in the first year and two-way ANOVA in the second year. Mean differences were compared using the Least Significant Difference (LSD) test.

3. Results and Discussion

3.1. Plant Height (cm)

In the first year of the experiment, the average plant height of the genotypes ranged from 208.20 cm to 251.80 cm. The highest plant height, 251.80 cm, was recorded in the POP2 genotype. Additionally, the POP4, STD1, and STD2 genotypes were statistically grouped with the POP2 genotype. The lowest plant height was observed in the STD3 genotype at 208.20 cm (Table 4). In 2022, the average plant height values varied between 165.33 cm and 224.92 cm. The highest plant height, 224.92 cm, was measured in the STD2 genotype at 0% opacity, while the lowest, 165.33 cm, was observed in the POP2 genotype at 75% opacity (Table 4). When averages across opacity levels were considered, the highest plant height was 205.06 cm at 0% opacity, and the lowest was 189.94 cm at 100% opacity (Table 4). These results indicate that plant height decreased as opacity levels increased. The observed variation in plant height across genotypes and opacity levels suggests a potential physiological impact of endosperm opacity on overall plant vigor. Specifically, the decreasing trend in plant height with increasing opacity may be related to pleiotropic effects of the opaque gene, particularly opaque-2, which has been reported to influence not only grain quality but also plant architecture and growth dynamics (Wessel-Beaver et al., 1988). Similar reductions in plant height in opaque or modified endosperm maize have been reported by Krivanek et al. (2007), indicating that these genotypes may allocate more metabolic resources to kernel composition rather than vegetative growth. Furthermore, our findings align with previous studies on Turkish maize landraces, which showed plant height values ranging from approximately 160 to 280 cm (Ayrancı and Sade, 2004; Özata and Kapar, 2014; Kuşvuran and Nazlı, 2014), highlighting the wide genetic variability within local populations. Such variability underscores the importance of evaluating opacity-associated traits not only for kernel quality but also for their agronomic implications in breeding programs.

Table 4. Average plant height by year and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	214.22 c	209.97 a-f	199.51 d-h	200.93 c-h	211.52 a-e	198.93 d-h	204.17 ab
POP2	251.80 a	210.62 a-e	220.29 ab	206.88 b-g	165.33 j	213.83 a-d	203.39 b
POP3	209.95 c	191.85 g-i	198.70 d-h	185.23 hi	182.28 h-j	187.26 hi	190.51 b
POP4	224.40 a-c	184.85 hi	186.04 hi	185.27 hi	180.82 ij	191.49 g-i	185.69 b
POP5	219.35 bc	197.02 e-i	206.28 b-g	203.73 c-g	193.62 g-i	193.07 g-i	198.74 b
POP6	214.57 c	216.63 a-c	199.18 d-h	198.20 d-h	200.95 c-h	193.93 f-i	201.78 b
STD1	227.45 a-c	204.65 b-g	-	-	-	-	204.65 ab
STD2	246.65 ab	224.92 a	-	-	-	-	224.92 a
STD3	208.20 c	-	-	-	-	180.84 ij	180.84 b
Average	-	205.06 a	201.67 ab	197.38 abc	194.19 bc	189.94 c	-
Variance Analysis	Genotype: 975.47**	Genotype; 939.66** , Opacity: 475.24**, G × O: 314.37**					

P value <0.05*, 0.01**

3.2. First Ear Height

Table 5 presents the average first ear heights for 2021 and 2022. In 2021, the longest first ear height was recorded in the POP2 genotype at 127.95 cm, while the shortest was observed in the POP1 genotype at 75.03 cm. In 2022, no significant interaction was found between the first ear height and the opacity levels of the populations. Although numerical differences were observed across opacity levels, these differences were not statistically significant (Table 5). The variation in first ear height across genotypes and years, along with the absence of statistically significant differences among opacity levels in 2022, suggests that this trait may be influenced more strongly by genetic background and environmental factors than by endosperm opacity alone. The reduction in ear height observed in the second year could be partially attributed to differences in experimental setup, particularly the lack of genotype separation based on opacity in the first year. This observation is consistent with previous findings that emphasize the sensitivity of ear placement to planting density and genotype × environment interactions (Sofi et al., 2009). Moreover, the generally lower ear heights recorded in 2022 correspond with the reduction in plant height during the same period, reflecting the well-established positive correlation between plant height and ear height (Sofi et al., 2009; Liu et al., 2010). The values observed in this study, ranging from 75 to 128 cm, are in line with prior reports on Turkish maize populations, confirming the considerable genetic diversity and plasticity among landraces (Ayrancı and Sade, 2004; Özata and Kapar, 2014; Acar et al., 2017). Although no direct statistical link to opacity was established for this trait, the ear position may still hold indirect importance in evaluating the agronomic performance of genotypes with different kernel types. he first ear height values obtained in this study are consistent with these ranges reported in the literature. However, a significant decrease in population averages was observed in the second year compared to the first. This discrepancy may be attributed to the fact that materials were not planted separately according to their opacity levels during the first year. A similar trend was observed for plant height, which aligns with the known positive correlation between first ear height and plant height in maize.

3.3. Ear Length (cm)

In 2021, the average ear length of the genotypes ranged from 15.69 cm to 19.56 cm, with no statistically significant differences observed between populations (Table 6). In 2022, ear length values varied from 12.80 cm to 19.76 cm among the populations, but no significant differences were found based on genotypes or opacity levels. The average ear lengths according to opacity levels ranged between 15.80 cm and 17.29 cm (Table 6). The relatively stable ear length observed across genotypes, years, and opacity levels in this study highlights the low phenotypic plasticity of this trait under varying conditions. Consistent with prior reports, ear length exhibited limited variation, which is largely governed by the genetic makeup of the material rather than environmental factors or kernel opacity (Sönmez et al., 2013; Saygı and Toklu, 2017). The ear length values recorded in this study (12.80–19.76 cm) were generally lower than those reported by Sönmez et al. (2013) and Kılınç et al. (2018), but aligned with the range provided by Saygı and Toklu (2017), suggesting that Turkish maize landraces may harbor moderate to short ear types. The absence of statistically significant differences, despite some numerical variation, reinforces the notion that ear length is a relatively stable trait, as also stated by Lauer et al. (2004), and therefore may be less responsive to selection pressure when compared to traits like ear diameter or kernel number. Nonetheless, it remains an important yield component and should be considered in combination with other traits in multi-trait selection strategies.

Table 5. Averages of first ear height by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	75.03 c	72.23	64.59	70.11	68.36	61.70	67.40
POP2	127.95 a	91.66	94.59	92.83	80.17	96.17	91.08
POP3	91.20 bc	67.95	74.75	75.82	63.23	77.70	73.00
POP4	101.55 b	80.01	73.34	74.38	71.37	80.59	75.94
POP5	101.12 b	77.70	87.01	85.52	74.80	79.49	80.91
POP6	103.25 b	99.73	91.69	83.82	92.25	90.52	91.60
STD1	88.33 bc	71.77	-	-	-	-	71.77
STD2	108.98 ab	93.37	-	-	-	-	93.37
STD3	87.35 bc	-	-	-	-	63.06	63.06
Average	-	81.80	81.00	80.68	76.50	78.46	-
Variance Analysis	Genotype: 923.83**	Genotype; 1095.66**, Opacity: 103.82, G × O: 74.46					

P value <0.05*, 0.01**

Table 6. Ear length averages by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	19.56	17.46	19.76	17.77	18.27	16.57	17.97
POP2	17.81	18.23	15.88	14.17	12.80	17.40	15.70
POP3	17.32	16.90	19.76	15.57	18.07	16.58	17.41
POP4	17.29	15.22	13.38	15.53	18.10	15.74	15.60
POP5	18.56	16.00	18.47	16.21	17.57	16.38	16.92
POP6	15.69	17.81	16.50	15.50	16.80	18.08	16.94
STD1	19.45	17.04	-	-	-	-	17.04
STD2	18.55	17.58	-	-	-	-	17.58
STD3	17.18	-	-	-	-	16.20	16.20
Average	-	17.03	17.29	15.80	16.79	16.71	-
Variance Analysis	Genotype: 6.0	Genotype; 8.35, Opacity; 5.31, G × O: 7.23					

P value <0.05*, 0.01**

3.4. Number of rows on the ear

In 2021, the genotypes STD1 (16.43 rows/ear) and STD2 (16.60 rows/ear) exhibited the highest number of ear rows (Table 7). The lowest number of rows was recorded in the POP2 genotype with 12.38 rows/ear. Apart from POP2, eight other genotypes were statistically grouped together in 2021. In 2022, no statistically significant differences were observed in the population × opacity interaction data. The highest number of rows (21.33 rows/ear) was observed in the POP6 genotype at 50% opacity, while the lowest (10.43 rows/ear) was recorded in the POP2 genotype at 0% opacity. When evaluated independently, the highest average number of rows per ear in 2022 was found in the POP6 genotype (18.54 rows/ear), and the lowest was in the POP2 genotype (13.45 rows/ear). The average number of rows across opacity levels ranged from 14.46 rows/ear at 100% opacity to 16.51 rows/ear at 75% opacity (Table 7). The number of rows on the ear, a trait primarily under genetic control, exhibited moderate variability across genotypes and opacity levels. The consistency in ranking of genotypes such as POP2 for lower values and STD1/STD2 for higher values across years supports the idea that this trait is relatively stable but still responsive to genetic background. While no significant interaction was observed between genotype and opacity in 2022, the differences among genotypes remained evident, suggesting that opacity level alone may not exert a strong influence on this trait. The values obtained in this study—ranging from 10.43 to 21.33 rows/ear—are broadly in line with those reported by Bozokalfa et al. (2004) and Öner (2017), although some genotypes slightly exceeded previously reported upper limits. This can likely be attributed to the broader genetic diversity represented in the present study, including a greater number of local landraces. Similar findings have been noted by Betrán et al. (2003), who emphasized the importance of evaluating ear traits in diverse germplasm pools, as these traits significantly contribute to grain yield and kernel set. Given its moderate heritability, the number of rows per ear remains a valuable selection criterion in maize breeding, especially when combined with other yield components.

Table 7. Averages of the number of rows on the ear by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	15.85 ab	16.20	17.97	16.00	14.93	16.00	16.22 ab
POP2	12.38 b	10.43	11.94	14.56	18.00	12.33	13.45 c
POP3	15.30 ab	15.65	14.10	14.00	14.67	13.56	14.38 bc
POP4	15.75 ab	16.00	15.33	16.00	16.00	13.00	15.27 bc
POP5	15.70 ab	13.56	14.00	12.89	14.67	15.33	14.09 bc
POP6	16.22 ab	18.50	16.67	21.33	19.56	16.67	18.54 a
STD1	16.43 a	16.20	-	-	-	-	16.20 a-c
STD2	16.60 a	14.78	-	-	-	-	14.78 bc
STD3	16.00 ab	-	-	-	-	14.33 e-i	14.33 bc
Average	-	15.16	15.00	15.90	16.51	14.46	-
Variance Analysis	Genotype: 6.40*	Genotype: 32.47**, Opacity: 9.82, G × O: 8.24					

P value <0.05*, 0.01**

3.5. Number of Grains in the Row

Table 8 presents the average number of grains per row for 2021 and 2022. In 2021, the values ranged from 24.22 to 39.94, with the highest number of grains observed in the STD1 genotype (39.94 grains/row) and the lowest in the POP6 genotype (24.22 grains/row). All other genotypes, except POP2 and POP6, were grouped statistically together. In 2022, the number of grains per row varied between 20.75 and 40.72. The average values ranged from 27.36 to 40.72 grains/row, but no statistically significant differences were found between the highest and lowest values. The number of grains per row showed a wide range of variation across genotypes and years, indicating that this trait is highly influenced by genotypic potential and possibly by environmental conditions affecting pollination and kernel set. Although statistical differences were not significant in 2022, the observed numerical variation—ranging from 20.75 to 40.72 grains per row—aligns with or exceeds values reported in previous studies. For example, Bozokalfa et al. (2004) and Nar (2023) reported values within the range of 31.0–49.5 grains per row, while Albayrak (2019) noted broader variability, extending as low as 12.33 grains. These differences likely reflect the diversity in plant architecture, ear morphology, and source–sink balance among genotypes. High-performing genotypes such as STD1 consistently exhibited superior kernel number, suggesting that traits related to ear fertility and grain set are crucial for yield improvement. According to Vasal et al. (2000), kernel number per row is strongly associated with assimilate partitioning and silk pollination efficiency, which in turn are sensitive to both genetic and environmental factors. Therefore, even in the absence of strong statistical separation among opacity levels, the genotypic variability observed in this study points to promising genetic resources for improving ear productivity in local maize breeding programs.

3.6. Ear Diameter

The average ear diameter values obtained from the populations in 2021 showed significant variation, ranging from 34.11 mm to 49.99 mm. The highest ear diameter was observed in the STD2 genotype (49.99 mm), while the lowest was recorded in the POP2 genotype (34.11 mm). Genotypes with higher ear diameters were predominantly standard varieties (Table 9). In 2022, ear diameter values ranged from 35.49 mm to 49.16 mm, with statistically significant differences between populations. The highest ear diameter (49.16 mm) was observed in the STD1 genotype, while the lowest (36.56 mm) was measured in the STD3 genotype. Genotypes POP1, POP3, POP4, and POP6 were found to have high ear diameter values and were statistically grouped with the standard varieties. Although not statistically significant, the highest numerical ear diameter (49.16 mm) was recorded in the STD1 genotype at 0% opacity, and the lowest (35.49 mm) was observed in the POP2 genotype at 100% opacity. Across opacity levels, the average ear diameters ranged from 41.14 mm (100% opaque) to 43.96 mm (75% opaque) (Table 9). The significant variation observed in ear diameter across genotypes and years highlights the influence of both genetic structure and environmental conditions on this yield-related trait. Consistently higher ear diameters in standard varieties, such as STD1 and STD2, compared to local landraces suggest that commercial breeding lines have been selected for enhanced sink capacity and kernel-bearing surface, as noted in previous studies (Sönmez et al., 2013; Saygı and Toklu, 2017). The results of the present study, ranging from 34.11 mm to 49.99 mm, are in agreement with the upper values reported by Sönmez et al. (2013) and Saygı and Toklu (2017), but surpass the lower limit reported by Öner (2017), likely due to differences in genetic materials and sample sizes. Although opacity levels did not significantly affect ear diameter, a numerical trend was observed whereby the lowest mean diameter corresponded to 100% opacity. This may imply a weak negative association between kernel opacity and cob development, possibly due to pleiotropic effects influencing both endosperm structure and ear morphology (Krivanek et al., 2007).

3.7. Ear weight (g)

Ear weight is a critical parameter among yield components. In 2021, ear weights ranged from 88.76 g to 258.37 g, with the highest value recorded in the STD2 genotype (258.37 g) and the lowest in the POP2 genotype (88.76 g) (Table 10). In 2022, ear weight values varied between 91.65 g and 222.45 g. The highest ear weight was observed in the STD1 genotype, while the lowest was again recorded in the POP2 genotype. Although no statistically significant differences were observed between opacity levels, the highest ear weight was associated with 0% opacity, and the lowest with 100% opacity. Ear weight, being a direct contributor to grain yield, demonstrated notable variability across genotypes in both years of the study. As expected, standard varieties such as STD1 and STD2 outperformed local landraces in terms of ear weight, highlighting the yield potential of commercially bred genotypes. The lowest ear weight values consistently belonged to the POP2 genotype, suggesting genotypic limitations in kernel set or ear filling capacity. Although differences among opacity levels were not statistically significant, a numerical decline in ear weight was observed as opacity increased, with the highest values recorded at 0% opacity. This trend may point to an indirect association between endosperm transparency and resource allocation for ear development. Previous research has emphasized the importance of ear weight as a reliable indicator of overall productivity, especially under well-managed conditions (Bozokalfa et al., 2004; Eşiyok et al., 2004). The ear weight values reported here (88.76–258.37 g) fall within or near the ranges provided in earlier studies (e.g., 198.7–257.7 g by Bozokalfa et al., 2004; 160–320 g by Yıldız et al., 2017), but are slightly lower than those reported by Eşiyok et al. (2004), likely due to differences in genotype pools and environmental factors. Our results indicating a decline in ear weight and grain yield at higher opacity levels are in line with findings by Erdal et al. (2021), who reported that opaque-2 and DZR1 inbred lines showed reduced grain yield compared to normal endosperm types, despite improved protein quality. These findings reaffirm ear weight as a critical selection criterion in maize breeding, particularly when targeting opaque local landraces for yield improvement.

Table 8. Averages of the number of grains in the row by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	29.03 ab	30.50	35.77	30.67	32.12	20.75	29.96
POP2	25.31 b	30.10	25.64	25.25	27.00	28.83	27.36
POP3	26.32 ab	27.00	23.32	30.50	27.67	36.59	29.12
POP4	29.79 ab	27.00	21.78	29.67	31.50	31.42	28.27
POP5	32.20 ab	32.67	35.00	32.44	33.33	32.06	33.10
POP6	24.22 b	32.92	27.67	31.67	31.00	34.33	31.52
STD1	39.94 a	40.05	-	-	-	-	40.05
STD2	36.65 ab	40.72	-	-	-	-	40.72
STD3	29.73 ab	-	-	-	-	33.90	33.90
Average	-	32.62	28.20	30.00	30.78	31.13	-
Variance Analysis	Genotype 108.34*	Genotype: 124.41,Opacity: 17.77, G × O: 46.41					

P value <0.05*, 0.01**

Table 9. Averages of ear diameter by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	44.19 ab	46.86	48.68	45.47	47.09	43.75	46.37 a
POP2	34.11 c	37.89	39.16	41.47	43.26	35.49	39.45 b
POP3	42.79 a-c	43.49	42.61	39.69	42.51	45.57	43.08 ab
POP4	44.85 ab	44.87	45.99	46.89	45.36	44.54	45.53 a
POP5	42.64 a-c	38.16	40.91	39.00	40.92	43.33	40.46 b
POP6	37.46 bc	42.09	41.39	46.85	43.68	38.74	42.55 ab
STD1	48.12 a	49.16	-	-	-	-	49.16 a
STD2	49.99 a	43.70	-	-	-	-	43.70 ab
STD3	36.94 bc	-	-	-	-	36.56	36.56 b
Average	-	43.28	43.12	43.44	43.96	41.14	-
Variance Analysis	Genotype: 111.34**	Genotype: 99.14**, Opacity: 11,.7, G × O: 16.20					

P value <0.05*, 0.01**

Table 10. Averages of ear weight by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	162.55 a-c	164.12	204.31	157.31	188.33	107.17	164.25 ab
POP2	88.76 c	108.70	116.95	126.22	104.00	91.65	109.50 c
POP3	130.78 bc	146.84	124.72	135.61	136.67	134.92	135.61 bc
POP4	172.64 a-c	155.41	121.93	156.32	169.07	141.25	148.80 a-c
POP5	153.89 a-c	130.48	136.81	120.73	158.33	137.49	136.77 bc
POP6	103.98 c	151.12	105.17	134.56	127.38	122.88	128.22 bc
STD1	229.83 ab	222.45	-	-	-	-	222.45 a
STD2	258.37 a	192.04	-	-	-	-	192.04 ab
STD3	89.77 c	-	-	-	-	97.30	97.30 c
Average	-	158.90	134.98	138.62	148.63	118.95	-
Variance Analysis	Genotype: 14241.63**	Genotype: 7568.15**, Opacity: 1594.33, G × O: 1123.71					

P value <0.05*, 0.01**

3.8. Grain weight per ear

In 2021, the average grain weight per ear ranged from 64.12 g to 204.91 g. The highest grain weights were recorded in the STD1 (204.91 g) and STD2 (203.36 g) genotypes, while the lowest value (64.12 g) was observed in the POP2 genotype (Table 11). The highest grain weight values were associated with the standard varieties, and all other populations, except for POP2 and POP6, were statistically grouped with the standard genotypes. In 2022, ear grain weight values ranged from 65.27 g to 199.37 g. Among the populations, average values varied from 74.87 g to 199.37 g, with the highest weight recorded in the STD1 genotype and the lowest in the STD3 genotype. Additionally, the POP1 genotype was statistically grouped with the standard varieties for grain weight. While no statistically significant differences were found between opacity levels, the highest numerical grain weight (199.37 g) was observed in the STD1 genotype at 0% opacity, and the lowest (65.27 g) in the POP2 genotype at 100% opacity. Grain weight per ear, a primary component of grain yield, exhibited considerable genotypic variation in both study years. As expected, standard hybrid varieties such as STD1 and STD2 consistently produced the highest values, reflecting their superior sink strength and grain-filling capacity. In contrast, the consistently low grain weights recorded in the POP2 genotype suggest limited yield potential, possibly linked to reduced kernel number or poor kernel development. Although no statistically significant differences were detected among opacity levels, the numerical pattern showing decreasing grain weight with increasing opacity—especially the lowest values observed at 100% opacity—may imply a subtle physiological link between kernel opacity and assimilate partitioning efficiency. When compared to the literature, the grain weights recorded in this study (64.12–204.91 g) fall below those reported by Ayrancı and Sade (2004), who evaluated hybrid cultivars under favorable conditions (134.66–242.33 g), but are closer to the findings of Atakul (2011) (108.12–139.25 g), who worked with more diverse genotype sets. These differences reinforce the importance of both genetic structure and experimental context in determining yield performance. Despite the lower average values, the ability of some landraces (e.g., POP1 in 2022) to statistically group with high-performing standards suggests potential for selection and genetic improvement in local germplasm.

Table 11. Averages of grain weight per ear by years and maize genotypes with variance analysis results

	2021	2022					
Genotype	Average	%0	%25	%50	%75	%100	Average
POP1	123.32 ab	142.41	173.36	128.64	156.12	78.61	135.83 ab
POP2	64.12 b	98.47	98.94	89.89	91.00	65.27	88.71 c
POP3	100.55 ab	120.68	73.17	103.67	101.33	138.03	108.69 bc
POP4	143.14 ab	112.43	94.33	127.78	140.83	118.57	118.79 bc
POP5	132.98 ab	120.43	102.51	97.48	133.67	116.27	114.07 bc
POP6	74.13 b	109.09	78.83	109.35	93.90	96.11	97.46 bc
STD1	203.36 a	199.37	-	-	-	-	199.37 a
STD2	204.91 a	163.21	-	-	-	-	163.21 ab
STD3	70.00 b	-	-	-	-	74.87	74.87 c
Average	-	133.26	103.52	109.81	121.74	98.25	-
Variance Analysis	Genotype: 11414.82**	Genotype: 6919.76**, Opacity: 1226.27, G × O: 1400.99					

P value <0.05*, 0.01**

4. Conclusion

The results of this two-year field study demonstrate that increasing kernel opacity levels have a negative impact on plant height, first ear height, and ear weight, whereas traits such as ear length, row number per ear, grains per row, grain weight per ear, and ear diameter were not significantly affected. These findings are consistent with previous research indicating that opaque kernel types, particularly those associated with opaque-2 or related mutations, may alter assimilate distribution patterns and reduce vegetative growth and ear development (Wessel-Beaver et al., 1988; Krivanek et al., 2007)

Among the genotypes tested, local landraces POP2 and POP6, along with the standard genotype STD3, were identified as promising genetic resources for breeding quality protein maize (QPM). While Türkiye has a wide range of registered maize cultivars, high-protein, nutritionally enriched maize types remain scarce. This study contributes to filling this gap by identifying genotypes with both agronomic adaptability and potential for nutritional enhancement. As highlighted in previous studies (Erdal et al., 2021), enhancing protein quality through opaque-2 and related genetic mechanisms often results in yield penalties. However, the identification of landraces such as POP2 and POP6 with relatively stable agronomic traits suggests that it may be possible to select genotypes that balance yield and nutritional quality.

Furthermore, although numerous studies have been conducted in Türkiye on local maize diversity and biochemical grain quality (e.g., protein, oil, and fatty acid composition) (Cömertpay et al., 2009; Öner, 2011), there is still a lack of research exploring how endosperm opacity influences morphological traits and how this relationship can be utilized in developing improved maize cultivars.

The integration of traditional landrace diversity with modern breeding approaches offers a strategic opportunity to develop high-quality protein maize adapted to local agro-ecological conditions and nutritional needs.

Author Contributions

Percentages of the authors' contributions are present below. All authors reviewed and approved final version of the manuscript.

	G.K.B	F.K
C	50	50
D	50	50
S		100
DCP	70	30
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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BIBLIOMETRIC ANALYSIS OF POPPY ALKALOIDS AND MORPHINE BIOSYNTHESIS

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Abstract: This bibliometric analysis examines the scientific literature development in poppy alkaloids and morphine biosynthesis from 1980 to 2024. Using data from 845 Web of Science publications, we conducted comprehensive analyses including annual scientific production, international collaboration networks, keyword analysis, citation patterns, and thematic mapping. Our findings reveal steady growth in scientific output since the 1980s, peaking at 45 publications in 2016. The USA, Canada, UK, Germany, and Australia emerge as the most productive and influential countries in international collaboration networks. Citation analysis identifies Facchini (1996), Morishige (2000), Liscombe (2007), and Hagel (2010) as the field's most influential reference points. Thematic mapping identifies "opium poppy," "molecular-cloning," and "expression" as motor themes driving the field, while "biosynthesis," "alkaloids," and "morphine" constitute core themes. Word cloud analysis shows "Papaver somniferum" as the most frequently used term (frequency: 76), representing 8% of the total research focus according to treemap analysis. This study provides a comprehensive mapping of the scientific structure and developmental dynamics in poppy alkaloids and morphine biosynthesis research, offering valuable insights for identifying research gaps and predicting future directions in this interdisciplinary field.

Keywords: Poppy, Alkaloids, Bibliometric

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1. Introduction

Human activities, industrial development, urban sprawl, intensive agriculture and deforestation threaten the genetic diversity of plant species. This poses a major challenge to world agriculture and food security, as well as a critical loss of medicinal value (Çeliktaş et al., 2022). Plants have the potential to produce a large number of metabolites used in the treatment of various diseases and hence in the production of medicines, and many have been used as medicines since the beginning of life. Among these plants is *Papaver somniferum* L. (poppy), which produces phytochemicals with numerous pharmacological effects (Portakal and Tan, 2024). Poppy (*Papaver somniferum* L.) belongs to the order *Rhoedales*, family *Papaveraceae* and genus *Papaver*. Türkiye is considered very rich in *Papaver* species. ''Flora of Türkiye'' states that there are a total of 39 *Papaver* species, 19 annual and 20 perennial. Of these, 10 species, 2 subspecies and 4 varieties are endemic to Türkiye (Davis et al., 1988; Kapoor, 1997). Poppy, which is among the cultivated and medicinal plants, is of great importance in our country in terms of the alkaloids it contains. The benzylisoquinoline alkaloids (BIA) produced by the poppy plant are the most important medicinal properties of this plant. Herbal medicines are obtained from the roots, stems, bark, leaves, flowers, seeds or the whole plant (Ameri et al., 2015). Opium is obtained as a result of solidification of the liquid leaked by scratching the ripe capsule of the poppy plant (Özgen et al., 2017). The latex obtained is thoroughly dried and opium powder is prepared. Opium contains up to 25% alkaloids by weight (Masihuddin et al., 2018). Poppy plant contains various groups of alkaloids such as tebain, codeine, noscopine, papaverine and morphine which are important for the medical sector. Apart from these, capsules containing more than 30 different alkaloids are used in the production of raw materials of semi-synthetic drugs with high material value (Şahin, 2023). It also exhibits analgesic, antidiarrheal, narcotic and antitussive properties. The German researcher Sertürner's isolation of morphine from opium in 1804 was revolutionary and the scientific study of opium alkaloids increased over the years, but interest in opium has continued to the present day. It is monographed in numerous pharmacopoeias. Since Sertürner, more than 40 alkaloids have been extracted from opium. Scientific studies have proven that the activity of opium is due to its alkaloids. Five main alkaloids, namely morphine, codeine, tebain, noscapine and papaverine, are most widely used (Baser and Arslan, 2014).

Numerical analysis and statistical analysis of scientific studies can be defined as Bibliometrics. Bibliometric methods apply a quantitative approach to the identification, evaluation and interpretation of previously published research. Bibliometric analysis is one of the analysis methods used by researchers to interpret and evaluate research areas, countries, citation rates of publications or journal numbers. In bibliometric methods, researchers first explore the literature and show the researcher's work by revealing the most influential studies (Freire and Nicol, 2019; Donthu et al., 2021). At the same time, this analysis is to evaluate the historical development, current status and future trends of a particular research area with mathematical and statistical methods, as well as to obtain the findings of the researchers and the collective bibliographic data produced by other researchers working in this field and to express the results through citation or writing (Yavuz, 2023). In addition, it determines the amount of academic publications in a specific time period and also reveals the extent to which a study influences subsequent research. The goal of bibliometric methods is to obtain the findings of researchers and bibliographic data produced by other scientists operating in this field and to present the findings through reference or text. In addition to this, bibliometric methodology is attracting increasing attention in the scientific field and has come to the forefront as a field of study shaped by the rapid advancement of computer technology and internet usage. Bibliometric methodology is a fundamental method for evaluating scientific studies and is based on general librarianship and information science (Persson et al., 2009; Merigó and Yang, 2017; Derviş, 2019; Han et al., 2020). Bibliometrics is the quantitative study of certain characteristics of publications such as author, subject, publication information, and cited sources. In bibliometric analysis, quantitative and statistical approaches are used to characterize publication trends in a particular field or literature (Abdi et al., 2018). All statistical evaluations were performed using R software with the “bibliometrix” package (R Core Team, 2020). In a study, a total of 1643 documents containing the search query “Papaver somniferum” in the title, abstract and/or keywords for the period 1910-2022 were accessed from the Scopus database. Although the first document was published in 1910, the number of articles did not exceed 10 publications per year until 1970. Until the 1990s, the number of publications remained relatively stable at around 10-20 articles per year (Diaz-Bárcena and Giraldo, 2023). The data were bibliographically generated from the WoS system in plain text format. The aim of this study is to analyze the scientific publications in the field of poppy alkaloids and morphine biosynthesis indexed in the Web of Science database between 1980 and 2024 using bibliometric analysis methods. Our research aims to identify the temporal evolution of scientific production in the field, international collaboration networks, conceptual structure, as well as the most important publications of the selected time period. This analysis will contribute to assessing the current state of the field, identifying research gaps and predicting future research directions. It will also provide researchers working in the field of poppy alkaloids and morphine biosynthesis with a holistic perspective on the scientific structure and developmental processes of the field.

2. Materials and Methods

This study was conducted to determine the bibliometric analysis of scientific publications in the field of poppy alkaloids and morphine biosynthesis indexed in the Web of Science database. Within the scope of the study, articles published between 1980 and 2024 were analyzed. The keywords “poppy alkaloids”, “morphine biosynthesis”, “benzylisoquinoline alkaloids”, “Papaver somniferum”, “opium poppy”, “codeine biosynthesis”, “thebaine biosynthesis” and “noscapine biosynthesis” were used in the data collection process. These keywords were selected from the terms frequently used in the publications of leading researchers in the field in order to comprehensively search for studies in the field of poppy alkaloids and morphine biosynthesis. As a result of the search, a total of 845 scientific publications were identified and these publications constituted the data set for bibliometric analysis. VOSviewer (version 1.6.18) and Bibliometrix R-package (version 3.1.4) software were used for bibliometric analysis. VOSviewer is a particularly useful tool for visualizing and mapping bibliometric networks (van Eck and Waltman, 2010). The Bibliometrix R-package is an open source software designed for comprehensive bibliometric analysis (Aria and Cuccurullo, 2017). In the data analysis process, raw data were first cleaned and standardized. Then, by applying the bibliometric analysis methods mentioned above, the scientific structure and development of the field of poppy alkaloids and morphine biosynthesis were comprehensively mapped. The main bibliometric indicators used in the analysis are: number of publications, number of citations, h-index, co-authorship links, keywords and citation networks. These indicators were used to assess the productivity, impact, collaboration and conceptual structure of the field.

3. Results

3.1. Annual Scientific Production (1980-2024)

When the temporal development of scientific production in the field of poppy alkaloids and morphine biosynthesis is analyzed, a clear upward trend is observed between 1980 and 2024. Scientific studies, which started with an average of 2-3 articles per year in the early 1980s, reached 5-6 articles in the mid-1990s. With the first significant increase in 1995, the annual number of articles increased to 13 articles, but fluctuations were observed in the following years. In the early 2000s, annual production was around 8-10 articles, with a gradual increase to 13-14 articles between 2002 and 2006. After reaching 20 articles in 2006, there was a decline between 2008 and 2010. After 2010, a rapid increase in scientific production was observed, reaching the highest levels in the field with 25 articles in 2012, 32 articles in 2014 and 45 articles in 2016. Between 2016 and 2022, annual scientific production fluctuated between 27-40 articles, reaching a second peak of 40 articles in 2022. After declining to 27 articles in 2023, scientific production dropped sharply to 7 articles in 2024. This last decline can be explained by the fact that the data collection process was not yet complete (Figure 1).

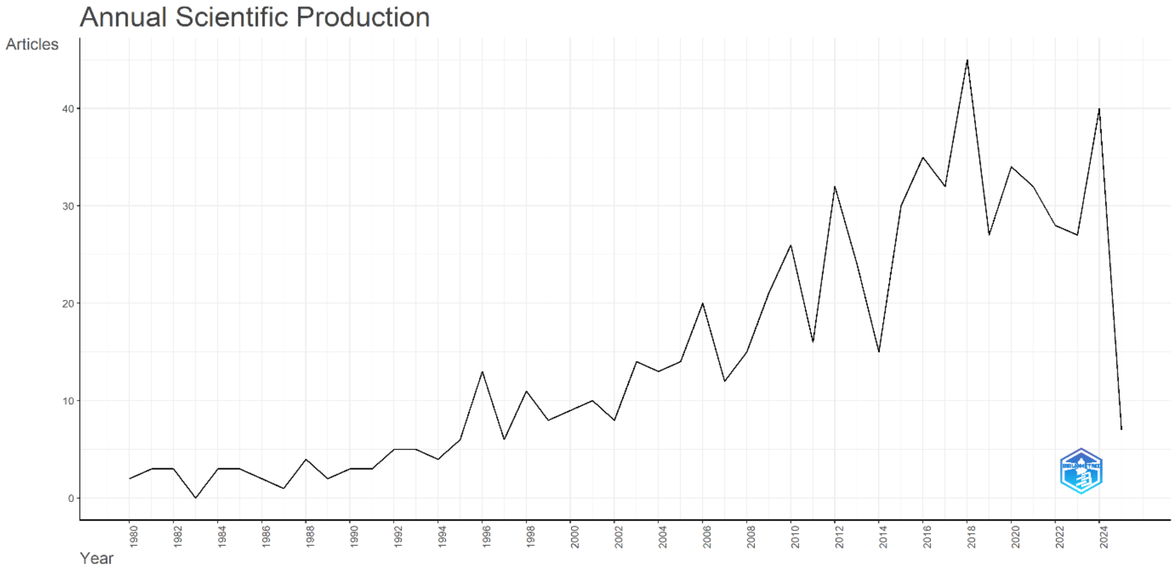

Figure 1. Annual scientific production (1980-2024).

3.2. Country Collaboration Map

The international collaboration network analysis revealed the existence of a significant international research network in the field of poppy alkaloids and morphine biosynthesis (Figure 2). The geographical collaboration map shows that there are dense connections between North America, Europe, Asia and Australia. The United States of America (USA) and Canada stand out as the countries with the highest intensity of scientific production and collaboration in the field. The United States has particularly strong collaborative links with Europe (United Kingdom, Germany, France), Asia (China, India, Japan) and Australia. The thickness of the connection lines on the map reflects the intensity of cooperation, and it was observed that the connections between the US-Australia, the US-United Kingdom and the US-China represent the most intense cooperation networks. The intensity of countries' scientific production is shown in shades of color on the map, with darker shades of blue representing higher scientific production. Accordingly, the United States, Canada, the United Kingdom, Germany, China, India and Australia are the most productive countries in the field. Some countries in the Middle East and Asia (Iran, Türkiye, Japan, South Korea) show moderate scientific production, while Latin American and African countries show limited participation in the field.

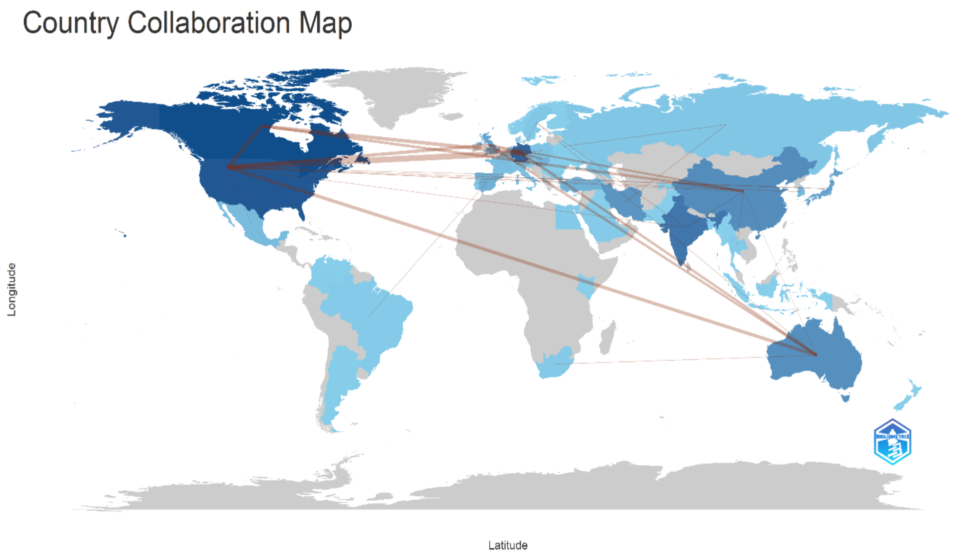

Figure 2. Country collaboration map.

3.3. The Keyword Network Analysis

The keyword network analysis (Figure 3) visualizes the main conceptual structure and research foci in the field of poppy alkaloids and morphine biosynthesis. The network map clearly shows the interrelationships and clustering trends of key terms in the field. The results of the analysis reveal the existence of five main conceptual clusters in the field. The centrally located terms “molecular-cloning” and “opium poppy” have the highest centrality values of the network and represent the main focal points of research in the field. The purple cluster formed around these two terms covers molecular biology and gene cloning studies. Within this cluster, the terms “enzyme”, “benzylisoquinoline alkaloid biosynthesis”, “norcoclaurine synthase”, “codeinone reductase” and “protein” show high link density. This cluster represents research focused on the molecular characterization of enzymes involved in the biosynthesis of poppy alkaloids. The second important cluster is the group around the term “biosynthesis”, shown in blue. Within this cluster, the terms “pathway”, “cells”, “cultures”, “poppy” and “plant” exhibit high link density. The third cluster, shown in orange, is the group centered around the term “alkaloids”. The terms “identification”, “growth” and “mass-spectrometry” stand out in this cluster. The fourth cluster, shown in green, is the group centered around the term “morphine”. Within this cluster, the terms “thebaine”, “liquid-chromatography” and “separation” show clear connections. The fifth cluster is a small group shown in red and includes the terms “codeine” and “urine”. When the links between the terms are analyzed, it is seen that the relationship between the terms “molecular-cloning” and “biosynthesis” constitutes the strongest link, followed by the link between “opium poppy” and “alkaloids” (Figure 3).

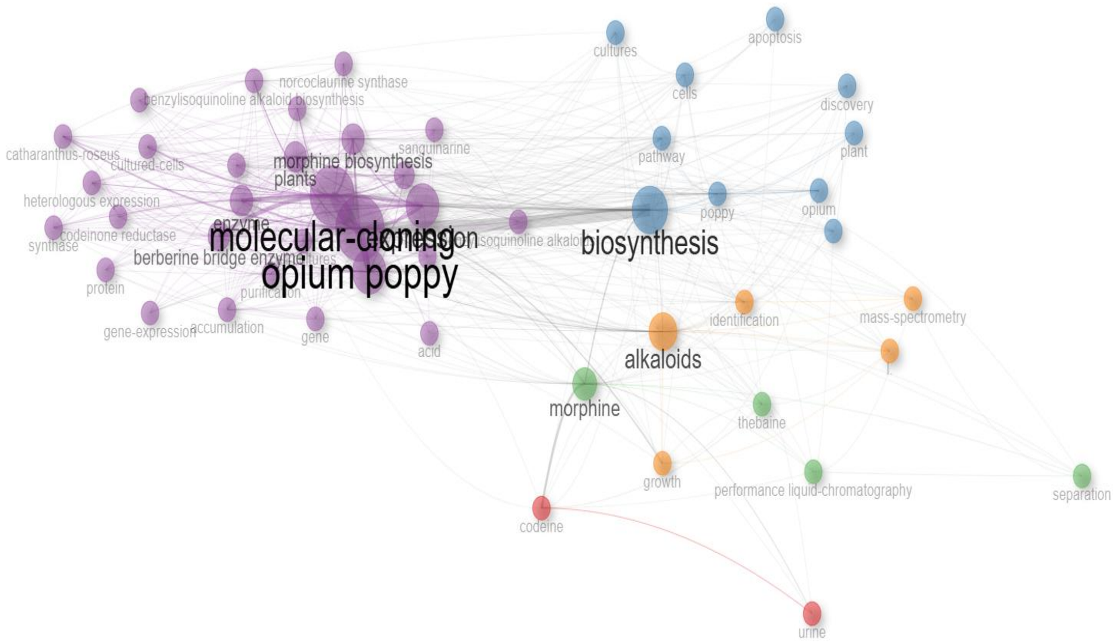

Figure 3. The keyword network analysis.

3.4. Citation Analysis And Author-Subject Relationship (1980-2024)

Citation analysis and author-subject relationship visualizes researchers and their fields of study in the field of poppy alkaloids and morphine biosynthesis. This analysis shows the source articles on the left, authors in the middle and research topics on the right. The thickness of the connecting lines represents the strength of the relationships. The results of the citation analysis reveal that the prominent researchers in the field are Facchini PJ, Hagel JM, Kutchan TM and Liscombe DK. Facchini PJ stands out as the researcher with the highest citation impact and is particularly notable for his work on “opium poppy”, “benzylisoquinoline alkaloids” and “biosynthesis”. Facchini's papers published in 1996, 2001, 2003 and 2014 are among the most influential in the field. Hagel JM was ranked as the second researcher and made significant contributions especially in the fields of “metabolic engineering”, “secondary metabolism” and “morphine”. Kutchan TM is the third ranked researcher and has conducted research on “biosynthesis” and “benzylisoquinoline alkaloids”. Liscombe DK's article published in the Journal of Biological Chemistry in 2007 stands out as the most influential work in the field. This study deals with the molecular characterization of the enzyme “norcoclaurine synthase” and was carried out in collaboration with Facchini PJ. As can be seen in the diagram, the Liscombe-Facchini collaboration stands out in terms of the number of joint publications. Other important researchers include Sato F, Chen X and Shukla S. These researchers have made significant contributions on “alkaloids”, “opium” and “thebaine”, respectively. As can be seen in the diagram, Sato F's work shows particularly strong links with “alkaloids” and "papaveraceae", Chen X's work with "opium" and "codeine", and Shukla S's work with "thebaine" and "morphine". When the research topics are analyzed, it is seen that the terms "benzylisoquinoline alkaloids" (number of links: 28), "opium poppy" (number of links: 25), "biosynthesis" (number of links: 24) and "morphine" (number of links: 22) have the highest link density. This indicates that research in the field is mainly focused on the biosynthesis of benzylisoquinoline alkaloids, poppy plant physiology and morphine metabolism (Figure 4).

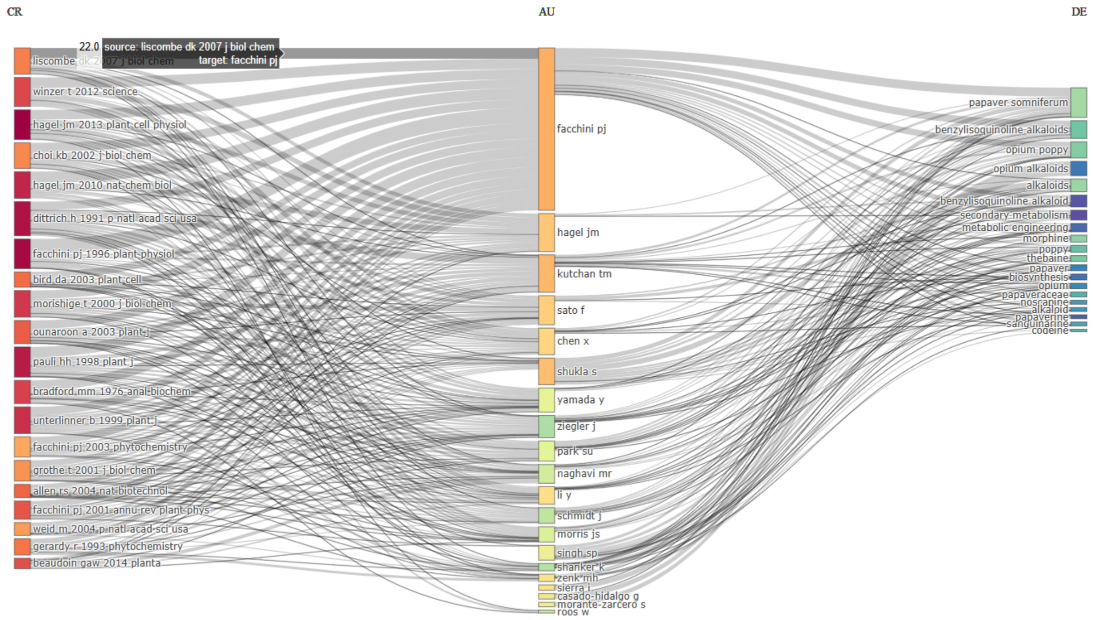

Figure 4. Citation analysis and author-subject relationship (1980-2024).

3.5. Word Cloud Analysis

The word cloud analysis in Figure 5 visualizes the most frequently used terms and key concepts in the field of poppy alkaloids and morphine biosynthesis. In the word cloud, the size of the words is proportional to the frequency of use and the color coding represents different thematic categories. In the word cloud analysis, the term "papaver somniferum" has the highest frequency of use and is the main focus of research in the field. The term "opium poppy" also exhibits high frequency of use. The term "Alkaloids" has the second highest frequency of use and refers to the general class of secondary metabolites found in the poppy plant. The term "Benzylisoquinoline alkaloids" also shows high frequency of use and represents the specific subclass of alkaloids to which poppy alkaloids such as morphine, codeine and tebain belong. The term "morphine" has the third highest frequency of use, representing the most important pharmacological component of the poppy plant and the main target of research in the field. The terms "thebaine", "codeine" and "noscapine" (frequency value: 420) also show high frequency of use and represent other important alkaloids of the poppy plant. The term "biosynthesis" had the fourth highest frequency of use. The terms "alkaloid biosynthesis" and "morphine biosynthesis" also show high frequency of use and represent research focusing on specific biosynthetic processes. The term "Papaveraceae" refers to the plant family to which the poppy plant belongs and represents taxonomic studies in the field. The terms "papaver" and "papaverine" also exhibit high frequency of use and represent other species and components of the poppy genus. The terms "secondary metabolism" and "secondary metabolites" also show significant frequency of use and represent research examining the role of alkaloids in plant metabolism.

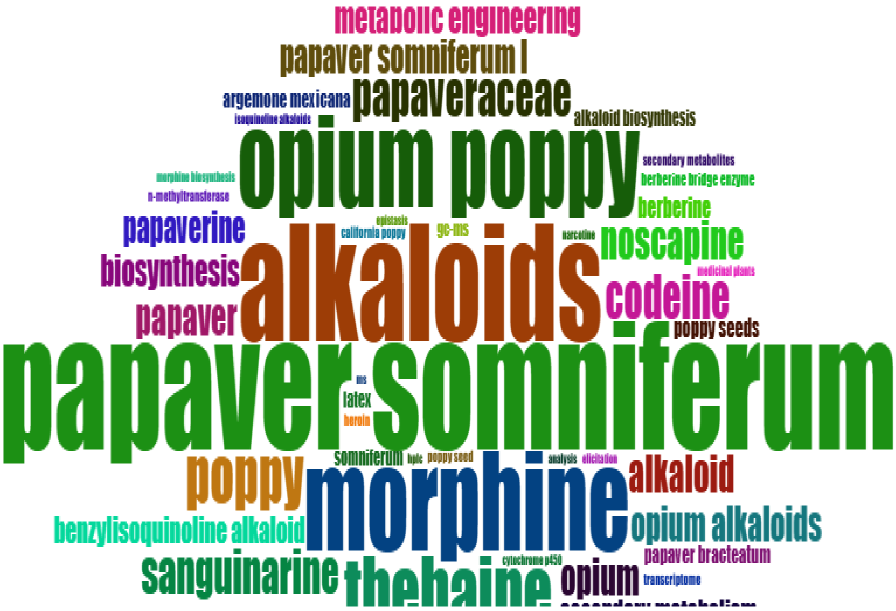

Figure 5. Word cloud analysis.

3.6. Treemap Analysis

The Treemap analysis (Figure 6) visualizes the frequency of use and hierarchical structure of key terms in the field of poppy alkaloids and morphine biosynthesis. Each rectangle represents a term and the area represents the frequency of use of the term. In the Treemap analysis, the term “papaver somniferum” covers the largest area (8% of the total area, frequency value: 76) and constitutes the main focus of research in the field. The term “Alkaloids” covers the second largest area (8% of the total area, frequency value: 69) and refers to the general class of secondary metabolites found in the poppy plant. Under this term are the terms “thebaine” (5% of total area, frequency: 41), “codeine” (3% of total area, frequency: 27) and “alkaloid” (3% of total area, frequency: 23), representing specific types of alkaloids. The term “morphine” occupies the third largest area (7% of the total area, frequency: 59) and represents the most important pharmacological component of the opium poppy plant. Under this term are the terms “opium poppy” (6% of total area, frequency: 55) and “poppy” (4% of total area, frequency: 34), which are common names for the poppy plant. The term “benzylisoquinoline alkaloids” occupies the fourth largest area (4% of the total area, frequency: 40) and represents the specific subclass of alkaloids to which poppy alkaloids such as morphine, codeine and tebain belong. The term “Papaveraceae” (3% of total area, frequency value: 27) refers to the plant family to which the poppy plant belongs. Under this term are the terms “sanguinarine” (3% of the total area, frequency: 26) and “papaver” (2% of the total area, frequency: 22), representing other species and components of the poppy family. The terms “opium” (2% of the total area, frequency value: 22), “biosynthesis” (2% of the total area, frequency value: 19), “metabolic engineering” (2% of the total area, frequency value: 19) and “papaverine” (2% of the total area, frequency value: 19) also occupy significant areas. In the treemap analysis, the terms “noscapine” (3% of the total area, frequency value: 24), “opium alkaloids” (2% of the total area, frequency value: 21) and “papaver somniferum l” (2% of the total area, frequency value: 20) also occupy significant areas and represent specific research topics in the field. Among the smaller domains, terms such as “secondary metabolism” (1% of the total domain, frequency: 15), “berberine” (1% of the total domain, frequency: 13), “eschscholzia californica” (1% of the total domain, frequency: 12), “poppy seeds” (1% of the total domain, frequency: 12) and “argemone mexicana” (1% of the total domain, frequency: 11) also have a certain importance in the domain. However, among the smallest fields are technical and methodological terms such as “transcriptome” (1% of total field, frequency: 7), “gc-ms” (1% of total field, frequency: 9), “hplc” (1% of total field, frequency: 6) and “cytochrome p450” (1% of total field, frequency: 6). The treemap analysis reveals the multidimensional and interdisciplinary nature of the field of poppy alkaloids and morphine biosynthesis, visualizing the contributions of different disciplines such as plant biology, biochemistry, molecular biology, analytical chemistry and metabolic engineering.

Figure 6. Treemap analysis.

3.7. The Conceptual Structure Map

The Conceptual Structure Map (Figure 7), created by Multidimensional Coherence Analysis (MCA), visualizes the distribution of key concepts in the field of poppy alkaloids and morphine biosynthesis in two-dimensional space and the relationships between them. This analysis reveals the conceptual structure and research clusters in the field. In the map, dots represent individual terms and colored areas represent conceptual clusters. The factorial map analysis shows that research in the field is divided into six main conceptual clusters. The first dimension (x-axis) explains 56.3% of the total variance and the second dimension (y-axis) explains 17.9%. Together, these two dimensions represent 74.2% of the conceptual structure in the field. The red cluster in the upper right of the map represents molecular biology and enzymology studies. This cluster includes terms such as “molecular-cloning”, “benzylisoquinoline alkaloid biosynthesis”, “synthase”, “enzyme”, “berberine bridge enzyme”, “heterologous expression”, “adenosyl-l-methionine” and “norcoclaurine synthase”. The blue cluster in the bottom right of the map represents plant physiology and biosynthesis studies. This cluster includes terms such as “biosynthesis”, “benzylisoquinoline alkaloids”, “opium poppy”, “pathway”, “gene-expression”, “protein”, “cells”, “plants”, “sanguinarine”, “accumulation” and “cultures”. The pink cluster in the bottom left of the map represents the study of plant growth and development.

This cluster contains the terms "growth", "plant" and "l.". The light blue cluster in the centre left of the map contains the terms "alkaloids", "identification", "mass spectrometry", "discovery" and "hashish". The yellow cluster in the upper left region of the map contains the terms "morphine", "thebaine", "separation" and "performance liquid-chromatography". The green cluster at the top of the map represents clinical and pharmacological studies. The terms "codeine" and "urine" are included in this cluster. The conceptual structure map reveals the multidimensional and interdisciplinary character of the field of poppy alkaloids and morphine biosynthesis and visualises the role and relationships of molecular biology, plant physiology, analytical chemistry and clinical research approaches in the field.

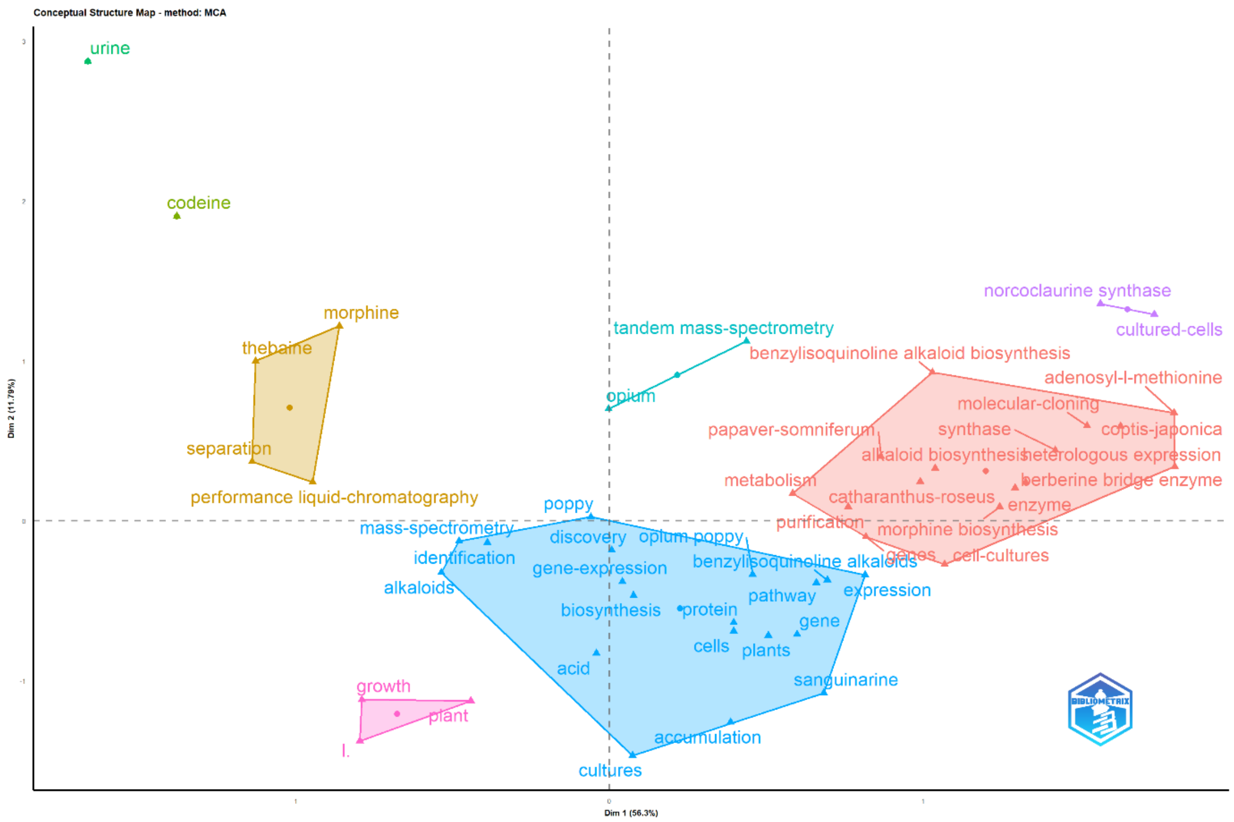

Figure 7. Multidimensional correspondence analysis (MCA).

3.8. The Thematic Map analysis

The Thematic Map analysis (Figure 8) visualises the distribution of research themes in the field of poppy alkaloids and morphine biosynthesis according to their centrality (x-axis) and density (y-axis). In the map, the circles represent the themes and the size of the circles represents the visibility of the themes in the literature. The thematic map shows that the research themes in the field are divided into four main categories: Core Themes (bottom right quadrant): These themes, which have high centrality and low density values, constitute the conceptual basis of the field. This category includes the themes of "biosynthesis", "alkaloids", "identification", "morphine", "thebaine" and "separation". These themes represent the main research areas for the biosynthesis, identification and characterisation of poppy alkaloids. In particular, the "biosynthesis" theme stands out as one of the most central themes in the field and covers studies aimed at elucidating alkaloid biosynthesis pathways. The themes "morphine" and "thebaine" represent research focussing on specific alkaloids. Motor Themes (top right quadrant): With high centrality and high intensity values, these themes represent the driving forces driving the development of the field. This category includes the themes "opium poppy", "molecular-cloning" and "expression". Niche Themes (top left quadrant): This category includes the themes "codeine", "urine", "oxidative stress", "death", "resistance", "validation", "combining ability" and "f-2 generations". Emerging or Declining Themes (bottom left quadrant): These themes, which have low centrality and low intensity values, represent research areas that are still developing or losing their importance in the field. This category includes the themes "analogues", "biological evaluation", "apoptosis", "binding" and "in-vitro". The thematic map analysis reveals the strategic research structure of the field of poppy alkaloids and morphine biosynthesis and visualises the research dynamics and potential future development areas in the field through the identification of core, engine, niche and emerging/declining themes.

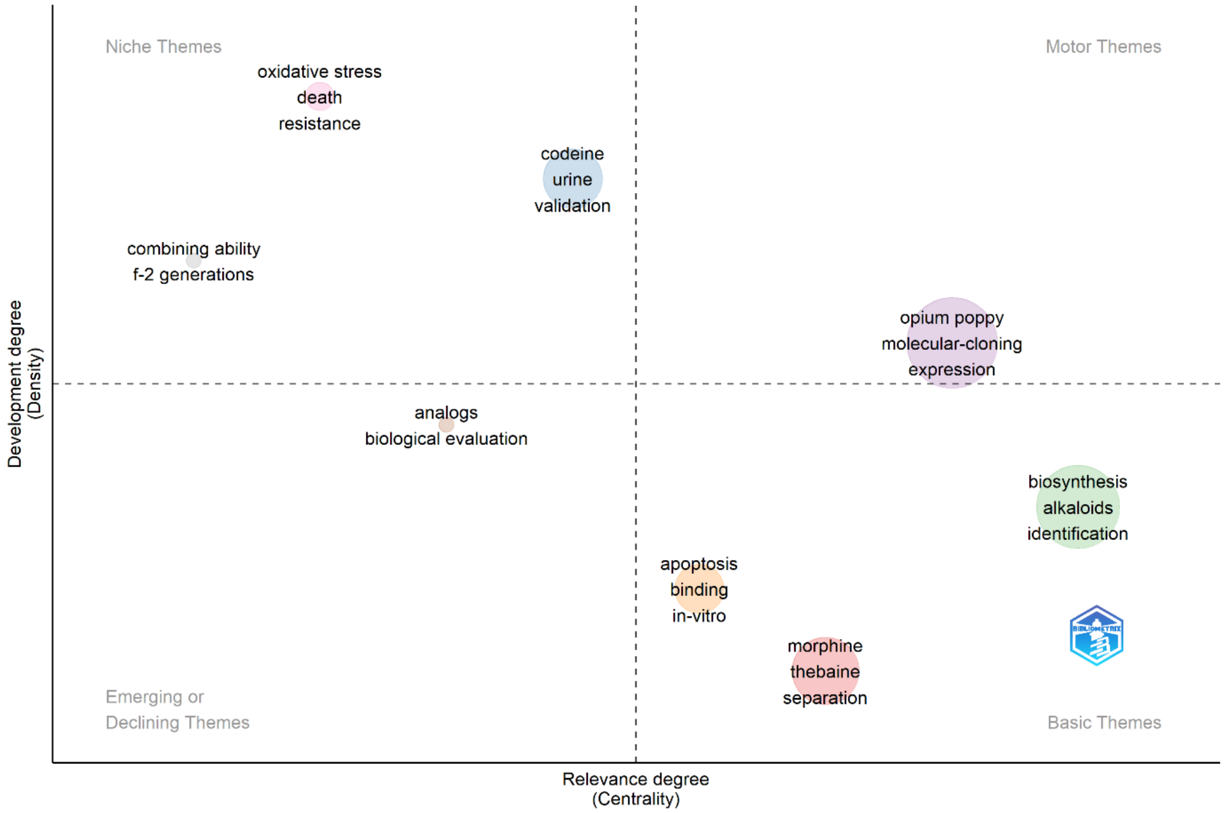

Figure 8. Thematic map analysis.

4. Conclusion

The bibliometric analysis conducted in this study provides a comprehensive assessment of the scientific literature in the field of poppy alkaloids and morphine biosynthesis. The analysis of publications between 1980 and 2024 reveals international collaboration networks, citation analysis, author-subject relationships, and the most frequently used keywords. The study shows that scientific output in the field of poppy alkaloids has increased since the 1980s and reached high levels especially between 2000 and 2016. When evaluating the international collaboration network analysis, it is determined that the United States of America, Canada, the United Kingdom, Germany, Australia, China, and Japan are among the countries that produce the most scientific publications in the field of poppy alkaloids. Keyword network analysis revealed the existence of five main conceptual clusters, with the terms "molecular-cloning" and "opium poppy" being the most central. Citation network analysis shows that studies published by Facchini PJ (1996), Morishige T (2000), Liscombe DK (2007), and Hagel JM (2010) are the most influential reference sources in the field. In the word cloud analysis, the term "papaver somniferum" has the highest frequency of use and constitutes the main focus of research in the field. The conceptual structure analysis shows that the field is divided into four main research clusters: (1) molecular biology and enzymology, (2) plant physiology and metabolism, (3) biosynthetic pathway analysis, and (4) analytical methods. The strong links between these clusters reflect the interdisciplinary integrated nature of the field. Thematic analysis shows that "opium poppy," "molecular-cloning," and "expression" are the motor themes of the field, while "biosynthesis," "alkaloids," and "morphine" constitute the core themes. The themes "codeine," "oxidative stress," and "resistance" represent niche research areas, while the themes "analogues" and "biological evaluation" reflect emerging or declining research areas. In conclusion, the bibliometric analysis reveals that the field of poppy alkaloids and morphine biosynthesis is an interdisciplinary and global research area. The future development of the field will be shaped by the advancement of engineering approaches and the expansion of biology applications. These developments will provide important opportunities for the sustainable production of poppy alkaloids, the discovery of new bioactive compounds, and the expansion of pharmaceutical applications.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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ASSESMENT OF THE AMOUNT OF PESTICIDE RESIDUES ON SPRAYER OPERATORS

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Abstract: Chemical control practices for managing harmful organisms in plant production increase agricultural productivity. However, these practices pose risks to human health, the environment, and natural ecosystems. The lack of personal protective equipment and improper sprayer calibration during pesticide applications increases health risks for operators driving cabinless tractors. Therefore, the objective of this study was to determine the amount of pesticide residues to which drivers are exposed during pesticide application while operating a cabinless tractor pulling a sprayer. Pesticide residue measurements were taken from the head (front and back), chest, back, arms, and legs. The experiments were conducted under field conditions at two spray heights (50 cm and 70 cm) and two spray pressures (5 bar and 7 bar). Water sensitive papers were used to determine the volume median diameter, surface coverage, and droplet density. The analyses were performed using the Image Tool for Windows V3 image processing software. The obtained results were evaluated through statistical analysis. Volume median diameter (µm), surface coverage (%) and droplet density (droplet/cm^2) were assessed on different parts of the tractor driver's body. In terms of volume median diameter, the minimum value of 44.20 µm was measured on the arm at a spray height of 70 cm and a pressure of 7 bar, while the maximum value of 98.79 µm was measured on the arm at 70 cm and 5 bar. Regarding surface coverage, the minimum value of 1.11% was measured on the chest at 70 cm, whereas the maximum value of 4.87% was recorded on the arm at 50 cm. In terms of droplet density, the minimum value of 17.25 droplets/cm^2 was measured on the arm at a spray height of 70 cm and a pressure of 7 bar, while the maximum value of 51.25 droplets/cm^2 was measured on the arm at 50 cm and 7 bar. Regarding the maximum values for volume median diameter, surface coverage, and droplet density, 86.53 µm was recorded on the arm, 3.87% on the leg, and 45.33 droplets/cm^2 on the chest, respectively. In the sampling areas considered as the target surfaces, the highest values of volume median diameter, surface coverage, and droplet density were recorded as 307.31 µm, 57.70%, and 405.25 droplets/cm^2, respectively.

Keywords: Surface coverage, Volume median diameter, Droplet density, Water sensitive paper, Image processing program

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1. Introduction

The world population is projected to reach 8.5 billion by 2030, 9.7 billion by 2050, and 10.4 billion by the 2080s. Due to this population growth, problems related to malnutrition and hunger are expected to increase steadily. To prevent hunger, it is essential to increase agricultural production. However, achieving this requires either expanding existing agricultural land or increasing the yield per unit area. Currently, approximately 40% of the global population is inadequately nourished, and around 20 million people die each year from hunger-related causes (FAO, 2022). In agricultural production, diseases, pests, and weeds cause a 35% loss in crop yield. Pesticides play an important role in preventing these losses. However, the use of pesticides has negative effects on human health, the environment, and natural resources, and also increases production costs. Therefore, pesticides must be applied carefully to minimize their adverse impacts (Çelen, 2013).

The most important factor for successful pesticide application is the selection of appropriate tools and equipment. Proper use of these tools and equipment plays a critical role in delivering the pesticide to the target area and is also essential for economic and operational efficiency. The choice of machinery varies depending on the requirements of the pest control method and its mode of application (Collantes et al, 2024). The suitability of spraying quality for its intended purpose can be assessed by analyzing the distribution of pesticide droplets. To evaluate spraying quality, it is necessary to determine the volume median diameter and uniformity of the droplets collected on the target surfaces after application, as well as droplet density and surface coverage.

Losses due to drift, defined as the movement of pesticide droplets through the air from the target area to non-target areas during or after spraying, can be quite significant (Özkan, 1995). Droplet size and wind speed are two of the most important factors affecting drift losses. Therefore, pesticide applications should be carried out under weather conditions where wind speed and direction are stable. It is essential to minimize spray drift near human settlements, sensitive plants, and water sources. The production, transportation, storage, and application of pesticides, as well as the consumption or use of products contaminated with pesticide residues, and the transfer of these substances to living environments through soil, water, and air, can cause undesirable effects on human health and the environment in both the short and long term. Pesticide poisoning can occur in two forms: acute and chronic. Acute poisoning occurs when a substance exerts its effect immediately after entering the body or the environment, leading to functional disturbances in organs. Chronic poisoning, on the other hand, results from the long- or short-term accumulation of substances with cumulative properties in the body or the environment. This type of poisoning can occur in production workers, applicators, and consumers who are continuously exposed to pesticides. Chronic poisoning may lead to serious health problems such as cancer, birth defects, neurological disorders, and reduced reproductive capacity. Pesticides can enter the human body through dermal absorption, eye contact, inhalation, or ingestion (Balsari, 2021).

In pesticide applications, approximately 30% of the pesticide typically drifts off target. Preventing and quantifying drift are among the most important research topics in pesticide application technology. The most commonly measured parameters used to evaluate application effectiveness include volume median diameter, surface coverage rate, and droplet density (Turgut and Duran, 2025). Image processing methods are widely used to assess these parameters (Moor et al., 2000; Duran, 2012). In this method, volume median diameter and surface coverage ratio are calculated using water-sensitive papers (Syngenta, 2024).

In pesticide applications, spray pressure influences the volume median diameter of the droplets, while spray height is a critical parameter affecting droplet deposition on the target. Since both spray pressure and spray height may impact the amount of residue on the operator's body, these factors were examined in the study.

This study was conducted to determine the amount of pesticide residues that drivers are exposed to during pesticide applications while operating a cabinless tractor pulling a sprayer.

2. Materials and Methods

Field experiments were conducted at the Ondokuz Mayıs University, Faculty of Agriculture, Bafra Agricultural Research and Application Center in 2022. Data analyses was conducted at Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering.

In the experiments, a field sprayer manufactured by CANSA Agriculturel Machinery (Figure 3) and a John Deere 5050E model tractor were used.

IDK 120-04 type air induction nozzles with a diameter of 1.2 mm (Figure 1) were used. In addition, the wind speed and air temperature during the experiments were measured with Prova AVM 05 model anemometer device.

To perform droplet analysis (volume median diameter and surface coverage), 26 × 76 mm water sensitive papers (WSP) (Syngenta) were used as the sampling surfaces (Salyani et al., 2013; Açık, 2018). For applications made on the ground, wooden wedges of 10 × 10 cm were used to secture the water sensitive papers. The water sensitive papers were attached to 10 × 2 cm slats and clamps on different parts of the opearator body (Figure 2).

Figure 1. Sprayer used in the trials.

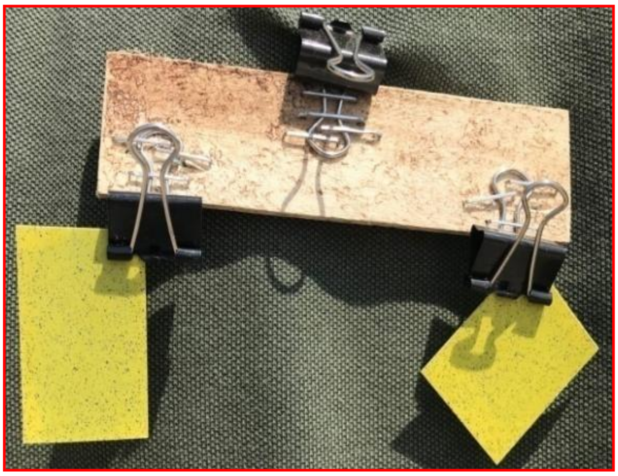

Figure 2. Clamp for water sensitive paper attached to the operator.

Figure 3. Sprayer used in the trials.

The WSPs after experiments were scanned using a Canon E414 Scanner for droplets analyses. In field conditions, during turns and normal spraying, the amount of residue was determined in different parts of the operator's body (head front, head back, chest, back, arms, legs). From the spray characteristics, volume median diameter (VMD), droplet density (DD) and surface coverage (SC) were determined. Applications were made at two spray pressures (P1: 5 bar - P2: 7 bar) and two spray heights (H1: 50 cm - H2: 70 cm) (Akkaş, 2023). The experiments were carried out with 4 replications at 23.40 L/min application rate and 8.10 km/h forward speed. The average wind speed was measured as 1.10 m/s and the air temperature as 27.70 ^{o}C. The reason for selecting 5 bar and 7 bar, and the spray height as 50 cm and 70 cm is that farmers generally use these values in spray applications. It is reported that the spray height and spray pressures affect droplet distribution uniformity (Višacki et al., 2016; Li et al., 2021).

In the applications, WSP attached to wedges and slats with clamps were used as sampling surfaces. One WSPs was attached to each wedge and 16 WSPs were placed at each breast level and 1 m apart on the concrete floor of the application area (Figure 4). Two WSPs were attached to each slat, and a total of 8 slats were placed on the operator's body (Figure 5).

Figure 4. Placement of water sensitive papers (WSP) on blocks in the experimental area.

Figure 5. The positioning of WSP on the operator.

After each application (Figure 6), the WSPs were collected, dried and placed in labeled envelopes (Özyurt et al., 2022). WSPs were then scanned with a scanner in 600 dpi in TIFF format (Jeon et al., 2011; Turgut and Duran, 2025) and transferred to a computer (Figure 7). Each scan was analyzed using the image processing program UTHSCSA Image Tool for Windows V3. The found spot diameters (Figure 8) were evaluated in the Excel program using the spreading factor coefficients and the real droplet diameter values (Duran et al., 2013). The number of droplets and surface coverage (%) values were calculated (Zhu et al., 2011; Turgut and Duran, 2025). The droplet density (number/cm^2) values were also determined by comparing the area of the WSP used in the experiments with the number of droplets.

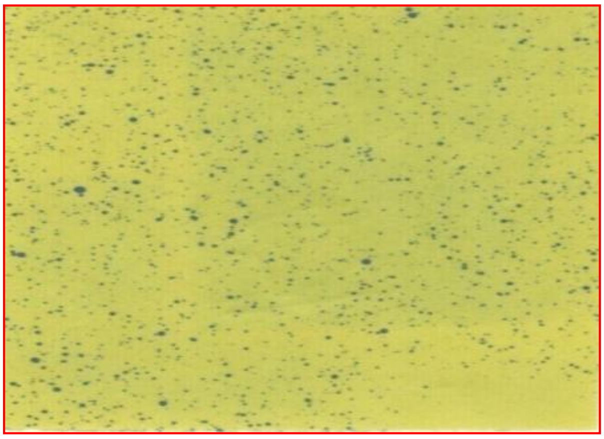

Figure 6. A water sensitive paper (WSP) after application.

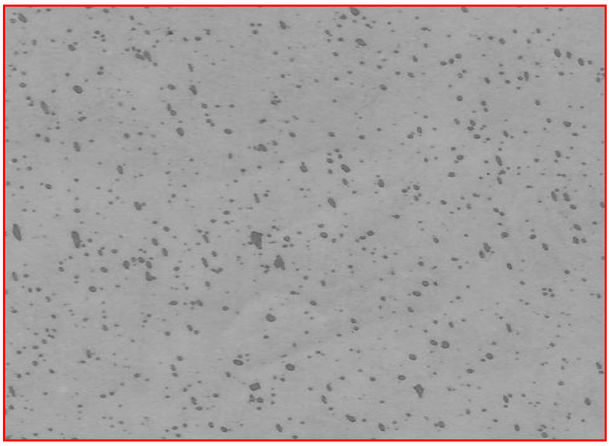

Figure 7. Gray images of the WSP transferred to the computer after the application.

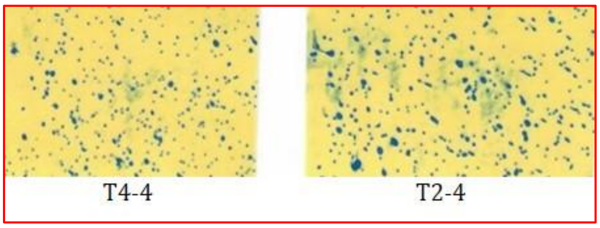

Figure 8. WSP from operator's body after the application.

Variance analysis (ANOVA) was used to determine the differences in spray pressures and spray heights in terms of volume median diameter (VMD), droplet density (DD), and surface coverage (SC) parameters. To further determine the statistical significance of the observed differences, the least significant differences (LSD) test was applied, with a significance level of P<0.05. The statistical analysis of the experimental data was performed using the SPSS software (version 15, SPSS Inc., USA), a microcomputer program commonly employed for statistical evaluations.

3. Results and Discussion

In the experiments, residue levels on the operator were determined on different parts of the body and the ground. To assess the amount of pesticide on the bodies of operators, water-sensitive papers were attached to various parts of the operator's body for measurement (Glenda et al., 2011). Following the applications, evaluations were conducted on the operator and the ground in terms of volume median diameter (VMD), droplet density (DD), and surface coverage (SC) (Güler et al., 2006). To reduce the risks associated with pesticide use, an international framework needs to be established (Shaw et al., 2023).

3.1. Operator Exposure to Pesticides During Turning

3.1.1. Volume median diameter (VMD)

During turning, the amount of pesticide deposited on the operator's body varied in terms of volume median diameter (VMD) (Table 1). Several studies have also indicated that the amount of pesticide on different parts of the operator's body may vary depending on the type of sprayer used and the application parameters.

Table 1. VMD values during turning

		Spray parameters				
Height (cm)	Pressure (bar)	Front head	Chest	Back	Leg	Arm
50	5	80.77 a	88.04 a	69.43	90.69	86.53 a
70	7	60.23 b	64.44 b	58.21	58.73	57.20 bc
70	5	56.26 b	64.40 b	85.85	80.95	66.05 b
50	7	36.50 c	50.28 c	55.38	58.59	49.69 c
	5	68.52	69.16	77.64 a	85.82 a	76.30 a
	7	58.25	65.85	56.79 b	58.65 b	53.45 b
CV (%)		8.00	9.00	12.00	7.00	9.00
LSD		9.70	12.07	11.01	7.12	11.32

*The difference between values with different letters is significant (P<0.05).

Coffman et al. (1999) reported that pesticide accumulation was highest in the neck, shoulder, and arm regions, indicating the need for additional protective measures. Kline et al. (2003) found that, in both cabbed and cabinless tractors, the highest levels of pesticide residues were observed on the steering wheel and tractor seat areas.

3.1.1.1. Frontal head region

The maximum VMD value of 80.77 µm was obtained in applications with a spray pressure of 5 bar and a spray height of 50 cm. It was observed that the VMD values were statistically different in groups with a spray pressure of 5 bar and spray heights of 50 cm and 70 cm (Table 1). Açık (2018) reported that droplet sizes varied depending on spray heights and forward speeds with different types of hydraulic nozzles. A larger droplet size indicates a higher amount of pesticide exposure for the operator (Coffman et al., 1999). No assessment was made from a VMD perspective, as there was no statistical difference between applications in terms of the amount of pesticide exposure to the back of the operator's head.

3.1.2. Chest region

The highest VMD value of 88.04 µm was recorded at a spray pressure of 5 bar and a height of 50 cm.

Statistically significant differences were observed between the VMD values at 50 cm and 70 cm spray heights under 5 bar. A similar trend was also noted at a spray pressure of 7 bar (Table 1). In a study conducted with different nozzle types, two spray heights, and three spray pressures, Turgut (2021) reported that VMD values varied depending on the spray height and pressure. This finding indicates that the VMD of pesticide droplets reaching the operator's chest region may also differ under these conditions.

3.1.1.3. Back region

The spray height was found to have no significant effect on the amount of pesticide reaching the operator's back region. At a spray pressure of 5 bar, the highest VMD value of 77.64 µm was obtained. The VMD values at the two applied pressure levels were found to belong to statistically different groups. The highest VMD value in the operator's back region, 77.64 µm, was recorded under conditions with a spray pressure of 7 bar.

3.1.1.4. Leg region

The spray height was found to have no significant effect on the amount of pesticide reaching the operator's leg region. In applications with a spray pressure of 5 bar, the highest VMD value was recorded as 85.82 µm. The VMD values obtained at spray pressures of 5 bar and 7 bar belonged to statistically different groups (Table 1). This indicates that the VMD of pesticide droplets reaching the operator's leg region varied under different pressure conditions. Moreover, Illyassou et al. (2019) reported that, in different types of sprayers, variations in spray height resulted in the highest residue occurring in the chest and leg regions. Similarly, Ceruta et al. (2018) found that, in applications using different nozzle types and two different spray pressures, the maximum amount of pesticide was deposited on the lower parts of the operator's body.

3.1.1.5. Arm region

The highest VMD value (86.53µm) was obtained in applications where the spray pressure was 5 bar and the spray height was 50 cm. At spray pressures of 5 bar and 7 bar, the VMD values at spray heights of 50 cm and 70 cm were statistically different (Table 1). The smallest VMD in the operator's arm region was obtained at a spray pressure of 7 bar and a spray height of 50 cm.

3.1.1.6. Surface coverage (SC)

No statistically significant differences were found between applications in terms of pesticide exposure, based on surface coverage (SC), in the operator's chest and arm regions.

3.1.2.1. Front head region

The highest SC value was obtained in applications with a spray pressure of 5 bar and a spray height of 70 cm. The spray pressure of 5 bar, the SC values at the two different spray heights were found to belong to statistically different groups (Table 2). According to the results, the lowest amount of pesticide exposure on the operator's front head region is expected under conditions of 5 bar spray pressure and 50 cm spray height. The use of proper spraying techniques during pesticide applications reduces the operator's exposure to pesticides (Nuytens et al., 2003).

Table 2. SC values during turning

Spray parameters					
Height (cm)	Pressure (bar)	Front head	Head back	Back	Leg
70	5	1.87 a	0.73 b	1.24	3.87 a
50	7	1.76 a	1.06 b	1.02	1.84 b
70	7	1.41 ab	1.72 a	1.43	1.40 b
50	5	0.88 b	1.13 b	1.26	3.25 a
70		1.64	1.23	1.09 a	2.64
50		1.32	1.10	0.54 b	2.55
CV (%)		25.00	0.20	26.00	13.00
LSD		0.69	0.44	0.28	0.64

*The difference between values with different letters is significant (P<0.05).

3.1.2.2. Back head region

The highest SC value was obtained in applications with a spray pressure of 7 bar and a spray height of 70 cm. The lowest pesticide deposition on the operator's back and head region was observed in the application with a spray pressure of 5 bar and a spray height of 70 cm, amounting to 0.73% (Table 2).

3.1.2.3. Back region

Spray pressure was found to have no significant effect on the amount of pesticide reaching the operator's back region. The highest SC value of 1.09% was recorded in applications with a spray height of 70 cm. Statistical analysis showed that spray height had a significant effect on SC. The lowest pesticide deposition on the operator's back region was observed at a spray height of 50 cm (Table 2).

3.1.2.4. Leg region

The highest SC value on the operator's leg region, at a rate of 3.87%, was obtained in applications with a spray pressure of 5 bar and a spray height of 70 cm. No statistically significant differences in SC were observed between the two spray heights at a spray pressure of 5 bar. A similar trend was observed for the 7 bar. The lowest SC values on the operator's leg region were recorded in applications with a spray pressure of 7 bar and spray heights of 50 cm and 70 cm (Table 2). Machado et al. (1998) stated that the highest level of contact with the operator's legs and feet occurred with the use of a backpack sprayer, reaching 92%.

3.1.3. Droplet density (DD)

No statistically significant differences were found between applications in terms of pesticide exposure, based on droplet density (DD) (number/cm²), on the operator's leg, front head, and back head regions.

3.1.3.1. Chest region

Spray height had no statistically significant effect on the DD on the operator's chest region. The highest DD value of 45.33 droplets/cm² was recorded in applications with a spray pressure of 5 bar. Statistical analysis showed significant differences in DD between the two pressure levels. The lowest DD on the operator's chest region was observed at a spray pressure of 7 bar. Additionally, spray height was found to affect DD values (Table 3).

Table 3. DD values during turning

Spray parameters				
Height (cm)	Pressure (bar)	Chest	Back	Arm
	5	45.33 a	26.33	29.50
	7	31.16 b	31.50	36.17
70		40.67	33.83 a	40.16 a
50		35.83	24.00 b	25.50 b
CV (%)		20.00	22.00	18.00
LSD		10.40	8.32	7.79

*The difference between values with different letters is significant ($P<0.05$).

3.1.3.2. Back region

Spray pressure had no significant effect on DD in the operator's back region. However, spray height influenced DD, with the highest value of 33.83 droplets/cm² recorded at a spray height of 70 cm. The lowest DD values on the operator's back region were observed at a spray height of 50 cm (Table 3).

3.1.3.3. Arm region

Spray pressure had a significant effect on DD in the operator's arm region, while spray height was found to be more influential. The highest DD value of 40.16 droplets/cm² was recorded at a spray height of 70 cm. In their study on different types of sprayers, Xuehua et al. (2020) reported that the highest pesticide amounts were found on the hands and legs of operators. The lowest DD value on the operator's back region was observed at a spray height of 50 cm (Table 3).

3.2. Pesticide Exposure of the Operator During Spraying

In the experiments, evaluations for conditions outside of turning were conducted based on volume median diameter (VMD), surface coverage (SC), and droplet density (DD) using sampling surfaces on different regions of the operator's body. These evaluations were performed according to varying spray pressures and spray heights. It was also determined that the use of properly equipped tractors by operators during pesticide application reduced their exposure levels to pesticides (Vitali et al., 2008). According to Konthonbut et al. (2020), the use of protective clothing such as long-sleeved shirts, trousers, and gloves significantly reduced operators' exposure to pesticide residues during applications with backpack sprayers. European Food Safety Authority (EFSA) data were utilized for the environmental risk assessments of pesticides (Wong et al., 2018).

3.2.1. Volume median diameter (VMD)

In the applications, the volume median diameter (VMD) values on different body regions of the operator were evaluated based on varying spray pressures and spray heights (Table 4).

Table 4. VMD values during spraying

Spray parameters						
Height (cm)	Pressure (bar)	Front head	Chest	Back	Leg	Arm
70	5	86.48 a	77.92 a	85.68 a	84.38 a	98.79 a
50	7	74.52 ab	78.60 a	83.59 a	78.46 a	79.45 b
70	7	68.32 b	52.32 b	52.24 b	44.97 c	44.20 c
50	5	65.41 b	63.60 ab	67.29 ab	58.63 b	51.57 c
CV (%)		11.00	13.00	19.00	10.00	13.64
LSD		13.80	15.13	23.22	11.38	14.95

*The difference between values with different letters is significant ($P<0.05$).

3.2.1.1. Frontal head region

The highest VMD value of 86.48 µm was obtained at a spray pressure of 5 bar and a spray height of 70 cm. Statistically significant differences in VMD values were observed at both spray heights under spray pressures of 5 and 7 bar. These findings indicate that the droplet sizes of pesticides reaching the operator's frontal head region varied across applications. Furthermore, the lowest VMD value in the operator’s frontal head region was recorded under the condition of 5 bar and 50 cm (Table 4).

3.2.1.2. Chest region

The highest VMD value of 78.60 µm was recorded under the application conditions of a spraying pressure of 7 bar and a spraying height of 50 cm. Statistically significant differences in VMD values were observed at both spraying heights for the 7 bar and 5 bar pressure levels. Furthermore, the application condition associated with the lowest risk in terms of VMD for the operator's chest region was identified as 7 bar and 70 cm, where a VMD of 52.32 µm was obtained (Table 4).

3.2.1.3. Back region

The highest VMD values were recorded under the application conditions of a spray pressure of 5 bar with a spray height of 70 cm, and a spray pressure of 7 bar with a spray height of 50 cm. It was determined that the VMD values in all combinations of spray pressures and spray heights were statistically significant. The lowest VMD value on the operator's back was observed under the condition of a 7 bar and a 70 cm, with a value of 52.24 µm (Table 4).

3.2.1.4. Leg region

The highest VMD values were obtained in applications with a spray pressure of 5 bar and a spray height of 70 cm, as well as with a spray pressure of 7 bar and a spray height of 50 cm. This indicates that both spray pressure and spray height have a significant effect on the VMD values reaching the operator’s leg region. The lowest VMD value detected on the operator’s leg region was 44.97 µm, observed in the application with a spray pressure of 7 bar and a spray height of 70 cm (Table 4).

3.2.1.5. Arm region

The highest VMD value of 98.79 µm was recorded in the application with a spray pressure of 5 bar and a spray height of 70 cm. All applications with spray pressures of 5 and 7 bar and spray heights of 50 cm and 70 cm showed statistically significant differences in terms of VMD. This indicates that the VMD values reaching the operator’s arm region are notably affected by these parameters. The lowest VMD value, representing the minimum pesticide exposure to the operator’s arm, was obtained in the application with a spray pressure of 7 bar and a spray height of 70 cm measuring 44.20 µm (Table 4).

3.2.2. Surface coverage (SC)

No statistically significant differences were found between the applications in terms of surface coverage (SC) (%) regarding pesticide exposure on the operator’s back of head and back regions.

3.2.2.1. Frontal head region

The highest SC value of 3.16 was obtained in the application with a spraying pressure of 7 bar and a spraying height of 50 cm. No statistically significant differences were found among other applications with different spray pressures and heights. The lowest SC in the operator's forehead region was recorded as 1.15% in the application with a spray pressure of 7 bar and a spray height of 50 cm (Table 5).

Table 5. SC values during spraying

Spray parameters					
Height (cm)	Pressure (bar)	Front head	Chest	Leg	Arm
50	7	3.16 a	2.56	2.04 a	4.87 a
70	5	1.41 b	1.10	1.72 ab	1.85 b
70	7	1.16 b	1.14	1.40 ab	2.48 b
50	5	1.15 b	1.91	1.27 b	1.95 b
50		2.16	2.23 a	1.66	3.41
70		1.29	1.11 b	1.56	2.17
CV (%)		23.00	25.00	29.00	20.00
LSD		65.00	48.00	76.00	92.00

*The difference between values with different letters is significant (P<0.05).

3.2.2.2. Chest region

It was determined that spray pressure did not have a significant effect on the SC of pesticides reaching the operator's chest area. However, both spray heights showed statistically significant differences in terms of SC. The lowest SC on the operator's chest was observed in the application with a spray height of 70 cm (Table 5).

3.2.2.3. Leg region

Spray height did not have a statistically significant effect on the SC in the operator's leg region; however, spray pressure was found to be significant. The highest SC in the leg region was 2.04%, recorded at a spray pressure of 7 bar and a spray height of 50 cm. Conversely, the lowest SC of 1.27% was observed at a spray pressure of 5 bar and a spray height of 50 cm (Table 5).

3.2.2.4. Arm region

The spray height was not statistically significant on the SC in the operator’s arm region. However, spray pressure was found to have a significant effect. The highest SC of 4.87% was observed in the application with a spray pressure of 7 bar and a spray height of 50 cm. No statistically significant differences were found among the other applications. The lowest risk in terms of SC was recorded as 1.85% in the application with a 5 bar and a 70 cm (Table 5).

3.2.3. Droplet density (DD)

No statistically significant differences were found between the applications in terms of pesticide exposure, based on droplet density (DD) (number/cm^2), in the operator's rear head and back regions.

3.2.3.1. Frontal head region

In the applications, it was determined that spray height had no statistically significant effect on DD when the spray pressure was 5 bar; however, spray pressure was significant at 7 bar. The highest DD value of 42 droplets/cm^2 was obtained in the application with a spray pressure of 7 bar and a spray height of 50 cm. The lowest DD value on the operator's front head region was recorded as 21.50 droplets/cm^2 under the application conditions of 7 bar and 70 cm (Table 6).

Table 6. DD values during spraying

Spray parameters					
Height (cm)	Pressure (bar)	Front head	Chest	Leg	Arm
50	7	42.00 a	33.50 a	47.25 a	51.25 a
70	5	36.25 a	31.25 a	21.50 b	26.25 b
50	5	36.00 a	19.00 b	18.75 b	17.25 b
70	7	21.50 b	27.50 ab	26.25 b	29.50 b
CV (%)		22.00	19.00	25.00	28.00
LSD		12.05	8.85	11.59	14.16

*The difference between values with different letters is significant (P<0.05).

3.2.3.2. Chest region

In the applications with spray pressures of 5 bar and 7 bar, the DD values at different spray heights were found to be statistically significant. The highest DD value on the operator's chest region, 33.50 droplets/cm^2, was recorded in the application with a spray pressure of 7 bar and a spray height of 50 cm. The lowest DD value on the chest region, 19 droplets/cm^2, was observed in the application with a spray pressure of 5 bar and a spray height of 50 cm (Table 6).

3.2.3.3. Leg region

In terms of DD, no statistically significant differences were found among spray heights in applications where the spray pressure was 5 bar on the operator's leg region. However, significant differences were observed in applications with a spray pressure of 7 bar. The highest DD of 47.25 droplets/cm^2 was recorded at a spray pressure of 7 bar and a spray height of 50 cm. The lowest DD on the operator's leg region was also observed under the same conditions of 7 bar and 50 cm (Table 6).

3.2.3.4. Arm region

The DD values obtained for the operator's leg region showed similar trends in the arm region. The highest DD value in the arm region was recorded as 51.25 droplets/cm^2 in the application with a spray pressure of 7 bar and a spray height of 50 cm, while the lowest DD value was 17.25 droplets/cm^2 in the application with a spray pressure of 5 bar and a spray height of 50 cm (Table 6).

3.3. Evaluation of characteristics on the spraying surface

The volume median diameter (VMD), surface coverage (SC), and droplet density (DD) characteristics detected on the operator's body in the experiment area are also presented alongside the values obtained from the analysis of WSP placed on blocks positioned on the ground during the application (Table 7).

Table 7. VMD, SC, and DD values surface during spraying

Spray parameters		Volume median diameter	Surface coverage	Droplet density
Height (cm)	Pressure (bar)			
70		317.30 a	48.70 b	297.25 b
50		239.92 b	57.70 a	405.25 a
	7	311.96 a	47.41 a	331.88
	5	245.26 b	58.99 b	370.63
50	5	192.02	88.04 a	420.50
70	5	298.51	67.25 a	320.75
50	7	287.84	64.44 a	390.00
70	7	336.10	50.28 b	273.75
CV (%)		11.00	9.00	15.00
LSD		36.23	5.51	61.67

*The difference between values with different letters is significant (P<0.05).

3.3.1. Volume median diameter (VMD)

The effects of spray pressures and spray heights on the volume median diameter (VMD) were examined on WSP surfaces placed on the ground outside the operator during the applications. The impacts of spray pressures and spray heights on VMD were found to be statistically significant.

The highest VMD value of 317.30 µm was obtained in the application with a spray height of 70 cm. In the application with a spray pressure of 7 bar, the highest VMD recorded was 311.96 µm (Table 7). In pesticide applications, a higher VMD value on the target surfaces indicates a greater volumetric amount of residue on the application surface. Therefore, the application with a spray height of 70 cm and a VMD of 317.30 µm may be considered suitable in terms of application efficacy. The highest VMD value was obtained in the applications with a spray pressure of 7 bar. On the target surface, the VMD value measured in the application with a spray pressure of 5 bar was 245.26 µm.

3.3.2. Surface coverage (SC)

The effects of spray pressures and spray heights on surface coverage (SC) were found to be statistically significant. The highest SC of 88.04% was obtained in applications where the spray height was 50 cm and the spray pressure was 5 bar. The lowest SC, 50.28%, was observed in the application with a spray height of 70 cm and a spray pressure of 7 bar. In pesticide applications, the target surface requiring actual treatment is the ground, where the highest SC is expected (Table 7).

3.3.3. Droplet density (DD)

Regarding droplet density (DD), spray heights were found to be statistically significant in the applications, whereas spray pressures were not significant. The highest DD value of 405.25 droplets/cm^2 was obtained at a spray height of 50 cm. In contrast, at a spray height of 70 cm, the DD value was 297.25 droplets/cm^2. This indicates that the pesticides reaching the application surface exhibit varying SC rates (Table 7). A higher DD value contributes to better coverage on the target surface, thereby enhancing the effectiveness of the application. From this perspective, selecting a spray height of 50 cm can be considered more appropriate.

4. Conclusion

The lowest VMD values on the operator's body parts, specifically the head, chest, arms, legs, and back, during turning, were recorded when the spray height was 50 cm and the spray pressure was 7 bar. In applications, the lowest VMD values on the operator's chest, back, legs, and arms were obtained at a spray height of 70 cm and a spray pressure of 7 bar. The lowest DD values on the chest, legs, and arms were recorded at a spray height of 50 cm and a spray pressure of 5 bar.

In spray applications, the highest VMD value on the target surface was obtained at a spray height of 70 cm and a spray pressure of 7 bar. The highest SC was achieved at a spray height of 50 cm and a spray pressure of 5 bar. Additionally, the highest DD value was recorded in applications with a spray height of 50 cm.

The results of this study will provide fundamental data for similar future research. Conducting studies with varying application parameters, such as nozzle types, spray pressure, spray height, application rate, and forward speed, may offer clearer insights into the amount of pesticide exposure to which operators are subjected. Moreover, the results of similar studies will also provide opportunities to identify measures that can be taken by operators.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	Ö.A	H.D.
C	30	70
D	30	70
S	10	90
DCP	40	60
DAI	30	70
L	50	50
W	30	70
CR	20	80
SR	20	80
PM	20	80
FA	40	60

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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IN VITRO DIRECT SHOOT REGENERATION OF KAMAN 1 WALNUT VARIETY AND KAMAN 5 WALNUT GENOTYPE (*Juglans regia* L.)

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Abstract: In this study, a simple and effective clonal micropropagation protocol was developed from axillary buds of Kaman 1 walnut variety and Kaman 5 walnut genotype. For surface sterilization of explants, they were first treated in 70% ethanol for 1 minute and then treated with 0.2% HgCl2 containing two drops of Tween 20 for 5 minutes. To prevent browning of the explants, 100 mg/l Ascorbic Acid, 100 mg/l Citric Acid and 100 mg/l Ascorbic Acid+100 mg/l Citric Acid were added to the MS nutrient medium. In addition, explants were subcultured at 3, 24 and 48 hour intervals. It was observed that the most effective treatment in both Kaman 1 walnut variety and Kaman 5 walnut genotype was 100 mg/l AA + 100 mg/l SA + 48 hours subculture. Within the scope of the shoot regeneration study, six different combinations of 0.5, 1.0 and 1.5 mg/l doses of BAP and 0.5 and 1.0 mg/l doses of IBA were tried. The highest average number of shoots per explant 2.67 was obtained from the Kaman 1 variety on MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l IBA. On the other hand, in the medium containing 0.5 mg/l BAP and 0.5 mg/l IBA, the average number of shoots was 2.00 for the Kaman 1 cultivar and 0.67 for the Kaman 5 genotype. After the shoots were kept in a solution containing 4 mg/l IBA for 10 minutes, they were planted in pots containing a peat: perlite (1:1) mixture, but rooting couldn't be achieved from the shoots.

Keywords: Walnut, *İn vitro*, Tissue culture, Micropropagation, Antioxidant, Subculture

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1. Introduction

Walnut (*Juglans* regia L.) is a very rich type of fruit in terms of nutritional value. It is rich in many vitamins including Thiamine, vitamin B6 and Folate. In addition to vitamins; it is rich in iron, zinc, copper, magnesium, phosphorus and potassium. Walnut is the only fruit type containing silver in fruit species**.** Another important element is selenium (Akça, 2009). Turkey is the homeland of walnuts as well as many fruit species and has an important potential in terms of walnut production. When it comes to vegetative propagation of walnut trees, the first thing that comes to mind is grafting (Şen, 2011). Another vegetative propagation method is propagation by tissue culture. Tissue culture enables intensive production of desired lines with its advantage. For this reason, it becomes an important option against traditional propagation methods such as seed, cutting, branching and grafting in various plant species. However, micropropagation of fruit species and characteristics of hard-shelled species is quite difficult compared to micropropagation of herbaceous plants. For this reason, explant selection and the physiological period of the explant, overcoming the harmful effects of tannin and other toxic compounds in cultures, ensuring an acceptable shoot growth rate and obtaining rooted shoots are extremely important for micropropagation of selected hard shellfish (Babaoğlu et al., 2002). A group of plants that are vegetatively propagated from a single plant and that carry the same genotype form a "clone". Vegetative production using very small plant parts (explants) under sterile conditions in the laboratory is called "*in vitro*" propagation or microproduction (Emiroğlu and Gürel, 2005). Since it is essential to preserve the genotype in clonal propagation, it is important to use shoot tips or axillary buds in *in vitro* production and to know that meristem tissues are stable in terms of genotype (D' Amato, 1977). The most important step in *in vitro* tissue culture is the sterilization process. Bacteria are the most common source of contamination. Acute bacterial or fungal contaminants may appear several days after culture. Therefore, the sterilization process should be handled at the entire laboratory level. Growth regulators (2,4-D, IBA, IAA, Kinetin, BAP, etc.) added to the *in vitro* medium significantly affect development (Babaoğlu ve ark., 2002). As with many woody plants, the most important problem encountered in the propagation of walnut by tissue culture is the browning that occurs in the culture medium. Browning occurs as a result of the oxidation of phenols leaking from the cut surface of the explant. To overcome this problem, antioxidant substances such as polyvinyl pyrrolidone (PVP), citric acid, ascorbic acid, activated charcoal, thiourea, L-cysteine, glutamine, asparagine, arginine are added to the medium or subculture is frequently used (Rout et al., 1999). In countries where walnut cultivation is developed, many studies have been carried out on the propagation of walnuts by tissue culture and important knowledge has been gained. Most of these studies focused on the appropriate concentration of growth regulators to be added to the culture medium (Revilla et al., 1989; Penuela et al., 1988; Rodriquez et al., 1993). Although unsuccessful results were obtained at the beginning in some countries where walnut cultivation was developed, studies were continued persistently and today a certain success has been achieved in propagating walnuts with tissue culture. In this study, it was aimed to determine the propagation possibilities of Kaman 1 walnut variety and Kaman 5 walnut genotype, which have an important place in walnut cultivation in Türkiye by tissue culture.

2. Materials and Methods

This study was carried out in the Plant Tissue Culture Laboratory of Ahi Evran University, Faculty of Agriculture, Department of Agricultural Biotechnology, in Kırşehir province. In our study, explants taken from grafted saplings of Kaman 1 walnut variety and Kaman 5 walnut genotype were used. Explants were taken from the axillary buds of 3-month-old grafted seedlings (Figure 1a). Explants were isolated and used starting from May, when shoot development in seedlings was most active. Explants approximately 1.0–3.0 cm long were used, but this caused increased secretion of phenolic compounds and negatively affected shoot regeneration. In our subsequent studies, explants containing at least two buds and approximately 6-7 cm in length were used (Figure 1b).

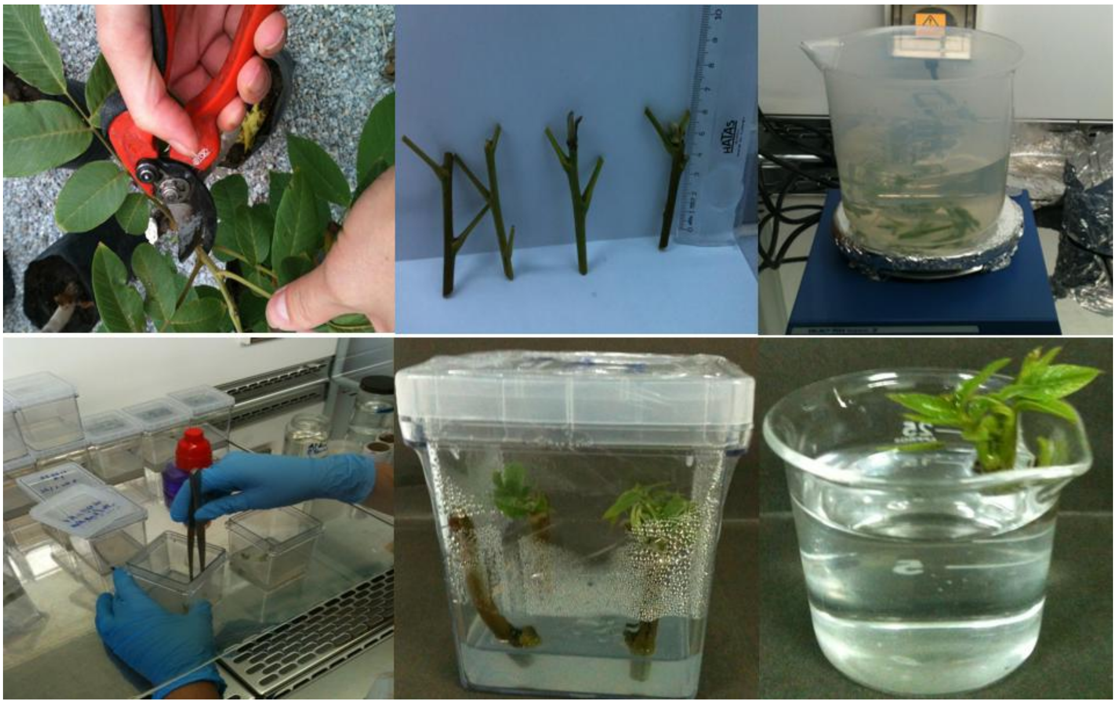

Figure 1. a) Explant isolation from three-month-old grafted walnut seedlings in the greenhouse b) Explants of 6-7 cm length carrying two nodes c) *In vitro* surface sterilization of explants with five min. in 0.2% $HgCl_2$ d) *In vitro* cultivation of explants in MS nutrient media e) Shoot regeneration of Kaman 1 walnut variety in MS nutrient media containing 0.5 mg/l BAP+1.0 mg/l IBA+100 mg/l ascorbic acid+100 mg/l citric acid+5 ml/l augmentin f) Treatment of shoots in 4 mg/l IBA solution for 10 min. before acclimatization.

2.1. Surface Sterilization of Explants

Two different methods were used for surface sterilization of explants. In the first treatment; the isolated explants were presterilized under tap water for 5 minutes. Then, sodium hypochlorite was applied at doses of 20%, 40% and 60% for periods of 10 minutes and 30 minutes. After rinsing with bidistilled water 3 times for 5 minutes, they were cultured in MS (Murashige & Skoog, 1962) nutrient medium. In the second treatment; explants were washed under running tap water for 10 minutes and with distilled water for 5 minutes. Then, presterilization was completed by keeping it in 70% ethanol for 5 minutes. In the biosafety cabinet, two drops of Tween 20 are added to 0.2% $HgCl_2$

(0.2% mercuric chloride was prepared by adding 0.2 g of mercuric chloride to 100 ml of water and mixing) and sterilized in the prepared solution for 5 minutes. Finally, the explants were rinsed with bidistilled water 3 times for 5 minutes (Figure 1 c, d).

2.2. Culturing Explants

MS nutrient medium containing 3% sucrose was used. The pH of the nutrient medium was adjusted to 5.6–5.8 the optimal value for plant growth, using 1N NaOH or 1N HCl. Before autoclaving, 0.6% agar was added to the medium and solidified. Sterilization of the environment is done in an autoclave at 1.2 atmospheres of pressure and 121 °C for 20 minutes preserved and provided. All cultures were kept under Philips white fluorescent light (Preheat daylight/42 µmol photons $m^{-2}s^{-1}$) at 24±2 °C with a photoperiod of 16 hours light and 8 hours dark.

2.3. Prevention of Browning

To prevent browning, explants were first subcultured into MS0 nutrient medium at 3, 24 and 48 h intervals. Secondly, different concentrations of antioxidants were applied. In this context, 100 mg/l Ascorbic Acid, 100 mg/l Citric Acid and 100 mg/l Ascorbic Acid+100 mg/l Citric Following autoclaving, ascorbic acid and citric acid were added to the nutrient media once it had cooled to a temperature suitable to avoid degradation of these substances.

2.4. Regeneration

Two different methods were used for regeneration of explants. In the first treatment; due to the high browning rate in 1.0–3.0 cm long explants, 6.0–7.0 cm long axillary buds were isolated from walnut seedlings. BAP and IBA at determined concentrations were added to the MS medium and autoclaved. When the MS0 nutrient medium reached 35 °C, 100 mg/l ascorbic acid + 100 mg/l citric acid was added to the medium after filter sterilization. The medium was poured into GA_7 magenta containers and allowed to solidify. The study was set up according to a random plots design with 3 replicates, 5 explants in each GA_7 magenta.

In the second treatment; the explants were sprayed with fungicide every 1 week for 3 weeks. Explants 6-7 cm tall with at least 2 buds were used. In order to increase the efficiency of sterilization, 5 ml/l augmentin was added to the MS medium after the medium reached the appropriate temperature. Explants were kept in MS nutrient medium containing 10 mg/l BAP + 100 mg/l ascorbic acid + 100 mg/l citric acid and augmentin for a week. Then, it was transferred to the 2nd medium containing 0.5 mg/l BAP+1.0 mg/l IBA+100 mg/l ascorbic acid+100 mg/l citric acid and augmentin and subcultured every days. In the other application, which was considered as the control group, explants were cultured directly in MS nutrient medium containing 0.5 mg/l BAP+1.0 mg/l IBA+100 mg/l ascorbic acid+100 mg/l citric acid and augmentin and subcultured every two days (Table 1).

2.5. Rooting

Shoots that reached a length of approximately 4-5 cm were placed in MS rooting medium containing 4 mg/l IBA. They were kept in this environment for a week and subcultured every two days. Microshoots removed from the culture medium were deagared under running tap water and treated with 4 mg/l liquid IBA solution for 10 minutes (Figure 1f). They were planted in pots containing an oven-sterilized peat: perlite (1:1) mixture. After the microshoots were planted in the pots, the pots were covered with bags. The obtained data were subjected to variance analysis in the MINITAB 12.0 package program.

Table 1. Regeneration treatments applied to walnut explants

Treatment	Explants	Pre-treatment	Growth Regulators	Ascorbic acid, citric acid, Augmentin®	Subculture
T1	1 bud	None	0.5, 1.0, 1.5 mg/l BAP + 0.5, 1.0 mg/l IBA	100 mg/l ascorbic acid+ 100 mg/l citric acid	No subculture
T2	2 buds	Explants were sprayed with fungicide once every week for 3 weeks and one week pretreatment in MS containing 10 mg/l BAP	After pretreatment, explants were placed in MS nutrient medium containing 0.5 mg/L BAP + 1.0 mg/L IBA	100 mg/l ascorbic acid+ 100 mg/l citric acid+ 5 ml/l Augmentin®	subcultured every 2 days
Control	2 buds	None	0.5 mg/l BAP + 1.0 mg/l IBA	100 mg/l ascorbic acid+ 100 mg/l citric acid+ 5 ml/l Augmentin®	Subcultured every 2 days

3. Results and Discussion

It was determined that the contamination rate in the 20% sodium hypochlorite application for surface sterilization in Kaman 1 walnut variety and Kaman 5 walnut genotype was higher than the 40% and 60% sodium hypochlorite applications. No significant difference was detected between the sterilization applications in terms of the browning rate. As a result of our studies, the desired results were not obtained from sodium hypochlorite solution for sterilization, and in the following stages, the sterilization protocol was changed and the sterilization process was carried out with a solution prepared with 0.2% $HgCl_2$. The results of subculturing at 3, 24, 48 hour intervals to prevent browning are given in Table 2.

In order to prevent browning in the culture medium, the effects of different antioxidant applications were also examined. For this purpose, ascorbic acid and citric acid were added to the culture medium separately and together. The results of ascorbic acid and citric acid application to prevent browning are given in Table 2. The difference between the browning rates obtained after all three antioxidant applications in Kaman 1 walnut variety and Kaman 5 walnut genotype was found to be statistically insignificant. The effects of different amounts of growth regulators on explant browning, number of shoots per explant, shoot length and number of leaves in Kaman 1 walnut variety and Kaman 5 walnut genotype were determined the results are given in Table 3.

Table 2. Effects of subculturing application and different antioxidant treatments on the browning rate (%) in Kaman 1 walnut variety and Kaman 5 walnut genotype

	Subculturing Application (hour)			Antioxidant Treatments (mg/l)			
Varieties	3	24	48	Control	100 AA	100 SA	100 AA+100 SA
Kaman 1NS	66.7	100.0	60.0	73.3	66.7	66.7	60.0
Kaman 5NS	86.7	93.3	86.7	100.0	73.3	80.0	66.7

The difference between the all application averages was found to be nonsignificant (NS).

Table 3. Effects of different amounts of growth regulators on explant browning, number of shoots per explant, shoot length and number of leaves in Kaman 1 walnut variety and Kaman 5 walnut genotype

Treatments		Browning Rate (%)		Number of shoots per explant		Shoot length (cm)		Number of leaves	
BAP	IBA	K1*	K5 NS	K1*	K5 NS	K1NS	K5NS	K1NS	K5NS
0.5	0.5	33.3c	60.0	2.0ab	0.67	1.7	1.1	3.7	0.0
0.5	1.0	00.0d	73.3	2.67a	0.0	1.7	0.0	3.9	0.0
1.0	0.5	93.3a	73.3	1.0bc	0.0	1.8	0.0	5.3	0.0
1.0	1.0	80.0a	93.3	0.3c	0.0	1.8	0.0	5.0	0.0
1.5	0.5	66.7ab	73.3	0.3c	0.0	1.5	0.0	3.0	0.0
1.5	1.0	53.3bc	100.0	1.0bc	0.0	1.8	0.0	3.0	0.0

*The differences between the means shown with different letters in the same column were found to be nonsignificant ($P<0.05$). NS Nonsignificant.

In Kaman 1 variety, considerable variation in browning rates was observed depending on the concentration of growth regulators in the culture medium. The browning rate reached high levels of 93.3%, 80.0% and %66.7 in media containing 1.0 mg/l BAP + 0.5 mg/l IBA, 1.0 mg/l BAP + 1.0 mg/l IBA and 1.5 mg/l BAP + 0.5 mg/l IBA respectively. In contrast, no browning was observed in the medium supplemented with 0.5 mg/l BAP + 1.0 mg/l IBA, while a browning rate of 33.3% was recorded in the medium containing 0.5 mg/l BAP + 0.5 mg/l IBA.

The highest shoot regeneration, with an average of 2.7 shoots per explant, was achieved on MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l IBA. This was followed by the medium containing 0.5 mg/l BAP and 0.5 mg/l IBA, which produced an average of 2.00 shoots per explant. Shoot length varied between 1.5 cm and 1.8 cm across all media, and no statistically significant differences were observed among treatments in terms of shoot elongation (Figure 1e)

In Kaman 5 genotype, 60.0-100.0% browning was observed across all treatments. The difference between the applications in terms of browning rates was not found to be statistically significant. Shoot development was observed only in the medium containing 0.5 mg/l BAP + 0.5 mg/l IBA. The number of shoots per explant was determined as 0.67 and the shoot length was 1.1 cm. No shoots were obtained in any of the other applications. The effect of 10 mg/l BAP and 100 mg/l AA+100 mg/l SA pretreatment shoot regeneration in Kaman 1 walnut variety and Kaman 5 walnut genotype was determined and the results obtained are given in Table 4.

Table 4. Effect of pretreatment on shoot regeneration in Kaman 1 walnut variety and Kaman 5 walnut genotype

Treatment	Browning rate (%)^{NS}		Number of shoots per explant^{NS}	
	Kaman 1	Kaman 5	Kaman 1	Kaman 5
10 mg/l BAP	43.3	53.3	1.0	0.0
100 mg/l AA+100 mg/l SA	46.7	53.3	0.0	0.0

The difference between the application averages was found to be nonsignificant.

As a result of all pretreatments, only one shoot was obtained with 10 mg/l BAP treatment in Kaman 1 walnut variety. No significant difference was observed in terms of browning rate observed in the culture medium. In all treatments, when the shoots of Kaman 1 walnut variety and Kaman 5 walnut genotype reached 4-5 cm in length, signs of necrosis and chlorosis were observed in the form of yellowing of the leaves and browning of the leaf tips and edges. Later, it caused the leaves to fall off and adventitious root formation wasn't observed. In previous studies on the propagation of walnuts by tissue culture, it was reported that explants were treated with sodium hypochlorite at rates ranging from 0.5% to 20.0% and for periods ranging from 5 to 30 minutes, for surface sterilization purposes (Zamir et al., 2004).

Rios-Leal et al. (2007) reported that contamination was an important problem in explants taken from walnut trees and that oxidation occurring in the tissues during the surface sterilization stage caused low shoot formation. In our study, high levels of contamination and browning were observed in the culture medium during surface sterilization with sodium hypochlorite, and shoot regeneration was negatively affected. It has been observed that mercuric chloride is more effective for sterilization of explants. Many researchers reported that they encountered browning problems in the propagation of walnuts by tissue culture and that frequent subculturing could be effective in reducing this problem (Leslie and McGranahan, 1992). In our study, explants were subcultured for three different periods at 3, 24 and 48 hour intervals. However, subculturing was not effective in preventing browning. This situation shows that subculturing alone is not sufficient to solve the browning problem in the propagation of these varieties by tissue culture. Çördük and Akı (2011), also reported that antioxidants should be added to the medium to prevent browning and that adding 100 mg/l AA and 50 mg/l SA to the MS0 medium was an effective method to prevent browning. Tekinsoy and Gülşen (1993) stated that browning decreased in nutrient media containing 150 mg/l SA + 100 mg/l AA, but no successful development was achieved. Myrselaj et al. (2020) reported that the browning rate is a parameter that is highly affected by the ascorbic acid concentration and exposure time, and that a low browning rate is achieved if 200 mg/l ascorbic acid solution is used for 15 or 20 minutes. In our study, when 100 mg/l AA+100 mg/l SA was added to the MS0 nutrient medium, browning was observed at a rate of 60% in Kaman 1 variety and 66.7% in the Kaman 5 walnut genotype. Despite the high rate of browning, a lower rate of browning was observed compared to the control group. It is stated that one of the factors affecting the browning rate is the sterilization of explants for a long time and with high doses of chemicals (Ellialtıoğlu, 1999). In our study, it was determined that sodium hypochlorite was not effective in preventing contamination in surface sterilization and caused high rates of darkening. In our study, $HgCl_2$ was used as an alternative sterilization agent. Another factor affecting success in tissue culture studies is determining the appropriate growth regulator combination and concentration for each plant species and variety. Vahdati et al. (2009) stated that the success of tissue culture depends on the chemical composition of the culture medium and that micropropagation of walnuts is partially limited due to the lack of a suitable culture medium. In our study, the effects of BAP and IBA at different doses and concentrations on walnut shoot regeneration were examined. It was determined that the average number of shoots per explant was 2.67 and the shoot length was 1.7 cm in the medium containing 0.5 mg/l BAP + 0.1 mg/l IBA in Kaman 1 variety. A high level of browning was observed in explants of the Kaman 5 walnut genotype in almost all environments. In the medium containing 0.5 mg/l BAP+0.5 mg/l IBA, the average number of shoots per explant was determined as 0.67 and the shoot length was 1.1 cm.

Many researchers working on the propagation of walnut by tissue culture have stated that the best cytokinin concentration at the shoot propagation stage is 1 mg/l BA (Penuela et al. 1988; Revilla et al. 1989; Rodriquez et al. 1993; Saadat and Hennerty, 2002; Türen ve ark., 2024).

Gruselle et al. (1987), investigated the effects of different types and concentrations of cytokinins on shoot formation in walnut tissue culture and reported that the best result was obtained with 1 mg/L BAP.

In this study, the Güleryüz, 1982 best results were obtained from the medium containing 0.5 mg/l BAP. Auxins promote root formation, while cytokinins promote shoot formation. They contribute to organ formation and development in tissue culture environments.

Tuan et al. (2016) reported in their study that the best combination of shoot elongation and production of new axillary shoots was achieved in Rugini and DKW medium with 4.4 μM BA and 0.2 μM IBA, and the highest rooting percentages were obtained in DKW medium containing 12 μM IBA. For this purpose our study, the effect of preapplication with 10 mg/l BAP on increasing the number of shoots was examined. As a result of the study, no positive effect of pretreatment application on Kaman 5 genotype was observed; in Kaman 1 variety, one piece shoot formation per explant was observed. It was determined that there were significant differences between the varieties used in our study. Other researchers have also reported that significant differences occur between walnut varieties in tissue culture propagation (Navatel and Bourrain 2001; Vahdati et al. 2004). One of the most important problems encountered in the propagation of walnuts by tissue culture is the difficulty of rooting in the microshoots obtained. Among the shoots obtained from the axillary buds of Vlach, Chandler, Yalova-1 walnut varieties, only the Yalova-1 walnut variety was able to root in MS nutrient medium containing 4 mg/l IBA (Yıldırım, 2018). Kepenek and Kolağası (2016) reported that the best rooting environment for microshoots obtained from walnuts is nutrient media containing 5 mg/l IBA. Dirlik et al. (2022) reported that the most effective sterilization method in *in vitro* micropropagation from Paradox' (*Juglans regia*) node explants is 5 min 70% EtOH + 5 min 0.2% $HgCl_2$ (containing Tween-20) treatment. They reported that the highest number of shoots per explant (1.0 shoots/explant) was achieved in DKW medium containing 4mg/l BAP + 0.001 mg/l IBA + 200 casein hydrolyzate + 50 adenine hemisulphate. They stated that the best root induction was obtained in DKW medium containing 30 g/l sucrose and 4 mg/l IBA. In our study, 4 mg/l IBA was applied for rooting microshoots, but rooting could not be achieved.

4. Conclusion

In our study, the possibilities of propagation of Kaman 1 walnut variety and Kaman 5 walnut genotype by tissue culture were investigated. Procuring the plants used as explant sources from the external environment caused high contamination in the culture environment. Since sodium hypochlorite treatment was not sufficient for surface sterilization, $HgCl_2$ was used in surface sterilization and successful results were obtained. Although positive results were not obtained from subculturing practices to prevent browning, results were obtained that ascorbic acid and citric acid applications could positively affect the success. It was determined that the best results in Kaman 1 walnut variety in terms of shoot development were obtained from the medium containing 0.5 mg/l BAP + 1.0 mg/l IBA. In Kaman 5 walnut genotype, a low rate of shoots was obtained only in one environment, while no shoot development was observed in other environments. It shows that there is a significant difference between the ability of these varieties to grow in tissue culture. In order to increase shoot development, pretreatment with 10 mg/l BAP was applied, but positive results were obtained only in Kaman 1 walnut variety. It was concluded that both methods used for rooting were not successful in Kaman 1 walnut variety and Kaman 5 walnut genotype. As a result, it has been shown that the Kaman 1 walnut variety has the potential to be propagated by tissue culture, but it has been concluded that more detailed studies need to be carried out to determine a protocol that can be applied in a practical sense.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.Ş.	S.S.Y.	K.Y.
C	30	40	30
D	30	40	30
S	20	40	40
DCP	40	40	20
DAI	50	30	20
L	50	30	20
W	100		
CR	20	60	20
SR	50	30	20
PM	40	50	10
FA	40	20	40

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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IMPACT OF SUSTAINABLE AGRICULTURAL PRACTICES COMPARED TO CONVENTIONAL AGRICULTURE ON THE BASIN AND ECOSYSTEM OF LAKE EGIRDIR

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Abstract: This project is based on 25 years of research and ongoing field studies conducted in the Lake Egirdir basin. Lake Egirdir, the second-largest freshwater lake in Turkiye, has been under increasing threat of ecological collapse in recent years due to a combination of challenges in maintaining the balance between conservation and utilization, as well as the growing impact of climate change. The declining water level of the lake has led to a significant reduction in water volume, which in turn has increased the concentration and ecological impact of pollutants in both the water column and bottom sediments. This process has severely compromised the ecological integrity of the lake. In order to ensure the recovery and sustainability of the lake, it is first and foremost, necessary to maintain the critical minimum water level and prevent further decline. In parallel, a transition from conventional to sustainable agricultural practices in the surrounding areas is considered essential not only for the restoration of the lake's internal ecosystem but also for the overall ecological resilience and sustainability of the entire basin.

Keywords: Ecological resilience, Lake ecology, Sustainable basin management

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1. Introduction

Agricultural pollution can be defined as the sum of activities that negatively affect the ecosystem and, as part of it, humans and their economic interests through the biotic and abiotic by-products resulting from various agricultural practices (Norman et al., 1997). In other words, agricultural wastes (both liquid and solid) originating from agricultural activities include surface runoff from pesticides, fertilizers, and production areas, erosion and dust resulting from tillage, animal manure and carcasses, crop residues, and all related waste materials (GEMET, 2025). Agricultural pollution may be a point source or a non-point source, depending on the type of origin. While point sources are relatively easy to control, non-point sources are more difficult, costly and continuous and require long-term and integrated management strategies. For this reason, the characteristics of the source must be carefully considered during the planning process. Abiotic sources of pollution are, as the term implies, of non-biological origin. These include pesticides, commercial fertilizers and by-products containing heavy metals. Biotic sources, on the other hand, refer to biologically derived (living-origin) by-products such as animal manure, biologically derived pesticides, other waste products originating from animal activities, and biological residues resulting from land management practices. To summaries, the primary pollutants in the aquatic environment from agricultural activities - whether biotic or abiotic – are nitrogen and phosphorus, the main nutrients for plant growth, together with pesticides used in agriculture, sediment transport caused by erosion, and fecal bacteria and viruses from animal waste.

One of the most current and important issues is the management of post-harvest stubble from cultivated lands. As in many countries, this issue is particularly important in rainfed rural areas in Turkiye. A study examining soils obtained from long-term field trials of stubble retention and removal, no-till, and conventional tillage showed that leaving or retaining stubble in the soil increased the availability of carbon sources and led to a higher abundance of genes favorable for metabolic activity and plant-pathogen interactions, but tillage practices did not affect the structure or diversity of the soil prokaryote community (Xu et al., 2025). The same study also reported that stubble addition or retention resulted in a significant increase in Proteobacteria but a decrease in Chloroflexi compared to the no-till condition (Xu et al., 2025). As a result, soil biota can constantly face processes such as reassigning dominant species based on carbon source availability. Numerous other studies have shown that leaving stubble in the soil has cumulative effects on improving soil ecology, promoting sustainable agricultural practices, and increasing plant yield (Ninkuu et al., 2025). One of the issues negatively impacting sustainable agriculture is wastewater from settlements (septic tanks, unregulated landfills, etc.), which requires separate consideration. It's important to note that wastewater from settlements, whether localized or distributed, contributes not only to nitrogen and phosphorus loads but also to the presence of hormone-disrupting preparations.

Another issue is that the use of surface irrigation techniques, known as basin, border, and furrow irrigation, instead of modern pressurized irrigation techniques, can lead to overuse of water, leading to depletion, enrichment of groundwater nitrate levels, erosion of valuable surface soil, and contamination by being carried to other surface water bodies.

As outlined above, agricultural activities can lead to a variety of complex and difficult to manage problems. In this context, if all agricultural practices are carried out with sustainable farming techniques, significant benefits can be realized for both nature and the people who depend most on these resources. Sustainable agriculture is defined by the American Society of Agronomy and adopted by the United States Government as follows: Sustainable agriculture is defined as a long-term agricultural approach that conserves and enhances the ecological integrity of natural resources, ensures stable production of food and fiber to meet human needs, the supports economic viability of producers, and fosters socio-environmental resilience in rural landscapes (Norman et al., 1997). Another concise definition is: agricultural production that is conducted with environmental responsibility and ensures that ecosystem functions are preserved and that future generations have access to secure food supply and a healthy quality of life (Robertson, 2015). As these definitions suggest, the core principle of sustainability is to maintain a long-term balance between conservation and utilization. Otherwise, acute problems may cause irreversible damage to both humans and the environment.

The main goal of sustainability is to protect the Earth's biodiversity and to safeguard the ecosystem services that functioning ecosystems provide for humanity. These are recognized as two fundamental goals of natural resource conservation (UMASS, 2025). The same principles also apply to sustainable agriculture. The sustainable agriculture ecosystem is associated with various terms such as agroecology, alternative agriculture, organic (biological, ecological) farming, low-input agriculture, integrated production, and regenerative agriculture (Aksoy and Yasar, 1994). The most appropriate form of sustainable agriculture depends on the specific characteristics of the area.

Sustainable agriculture is based on three main pillars (NSAC, 2025):

1. Environmental health
2. Economic profitability
3. Social and economic equity

The most important sub-components of each pillar are presented below.

1) Environmental health
i. Promotion of biodiversity
ii. Reduced use of agricultural inputs
iii. Protection of habitats
iv. Conservation of natural resources
v. Reducing the consumption of fossil fuels and chemicals
2) Economic profitability
i. Ensuring a living income for farmers and agricultural workers
ii. Emphasize direct marketing, cooperatives, alternative products, and local sourcing
iii. Supporting agricultural stakeholders through various incentive mechanisms where necessary
3) Social and economic equity
i. Continuous and effective knowledge sharing among communities
ii. Ensuring food security at the community level
iii. Reducing inequalities between communities by improving quality of life

When evaluating the relationship between the environment and energy, experts emphasize that the global agricultural industry must reduce its dependence on fossil fuels to meet the growing global demand for food (RNZ, 2025). This issue is directly related to sustainability and global climate change. Currently, the global food supply chain consumes nearly one-third of global energy and is responsible for more than 20% of global greenhouse gas emissions. The FAO estimates that by 2050, the world will need to produce 75% more food than today due to population growth and increased protein demand (RNZ, 2025). Whilst this may seem paradoxical at first glance, the main points listed above can be addressed through comprehensive and strategic planning first at an international level, and then by each country individually. Previous studies have shown that conventional agricultural methods have negative environmental and socio-economic side effects. These include loss of biodiversity, habitat destruction, deforestation, pollution of water, air and soil, salinization, desertification, depletion of water resources, loss of agricultural land, declining soil fertility, reduced nutritional value of agricultural products and decline in economic, social and cultural values (Hanson and Hendrickson, 2009). Many of these adverse effects are directly linked to the use of agrochemicals, which can increase agricultural productivity if used correctly. However, due to limited knowledge and lack of user training, the incorrect or excessive use of these chemicals can lead to significant environmental harm rather than the intended benefits. For instance, nitrogen, phosphorus, and potassium are essential for the production of crops used for food, feed, fiber, and biofuels. If used excessively, these nutrients can be lost to the environment through volatilization, leaching into groundwater, emissions into the atmosphere, or surface runoff. These nutrient losses can be minimized through the application of Best Management Practices

(BMPs), which improve nutrient availability to plants, enhance nutrient uptake efficiency, and better match nutrient application to agricultural needs (USDA, 2012). On the other hand, drought management is a serious challenge facing countries in the Mediterranean basin. According to many climates modeling studies, the impact of drought will become more pronounced as the mid-century approaches and will intensify further towards the end of the century. In this geography, crop production based on water, not plant, is considered inevitable. For the sustainability of agricultural production, transitions from irrigated to dryland farming in sensitive areas, as well as shifts to less water-intensive methods within irrigated production, will be inevitable.

This study is based on field observations conducted over the last 25 years and is complemented by recent research in Lake Egirdir. It also provides a comparative assessment of the potential benefits of sustainable agricultural practices over conventional methods, focusing on how these practices can contribute to the improvement of Lake Egirdir and its surrounding basin.

2. Materials and Methods

2.1. Study Area

Lake Egirdir is located in the Lakes District of Turkiye lies between 35°37′41″- 38°16′55″ north latitude and 30°44′39″ – 30°57′43″ east longitude (Figure 1).

The lake covers an area of 487.8 km^2 and has a catchment area of 3.309 km^2. It is of tectonic origin. The average depth of the lake is 6.5 meters, with a maximum depth of 15 meters near the settlement of Barla. At its maximum water level of 919.00 m, the lake has a volume of around 4.000 hm^3. The minimum operational level is 914.62 m.

This study examines the current agricultural structure in the Lake Egirdir basin. It evaluates how current agricultural production patterns and management practices could affect practices might shape the degradation of the basin in the future if they remain unchanged. Conversely, the study also assesses the long-term economic, social, and environmental benefits that can be achieved by transitioning to sustainable agricultural practices across the basin.

Figure 1. Lake Egirdir overview.

3. Results an Discussion

When examining the impact of climate change on the agricultural sector in the across major biogeographical regions of Europe, it is found that the Lake Egirdir basin is exposed to several significant risks: sharp increase in extreme temperatures, decrease in precipitation, heightened drought, loss of biodiversity, increased water demand in agriculture, lower crop yields, and increasing risks in livestock farming (EEA, 2019). Therefore, the effects of climate change are particularly critical in areas with intensive agricultural activity, making the Lake Egirdir basin one of the most vulnerable regions, especially due to high water withdrawal. The agricultural land around Lake Egirdir covers around 96.000 hectares. In terms of economic yield, fruit production is the most prominent sector, particularly apple cultivation. With the expansion of the area under cultivation areas, the Egirdir Basin now accounts for approximately 25% of Turkiye's total apple production. Other economically important crops include cherry, apricot, and rose. Additionally, dry farming contributes to the local rural economy, and lavender has recently been introduced as an alternative crop.

The land use map (Figure 2) shows that cherry farming dominates the Hoyran sub-basin, particularly in the Uluborlu and Senirkent districts. Apple cultivation is also significant in this sub-basin. The Yalvac-Gelendost sub-basin is primarily focused on apple production, and the remaining sub-basins also feature apple as the dominant crop.

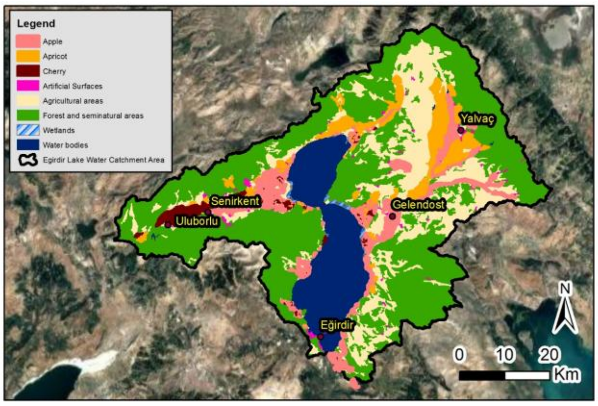

Figure 2. Agricultural production pattern in the Lake Egirdir basin.

Despite the increasing use of pressurized irrigation systems in the basin, their general distribution is still not optimal level. Due to climate change and excessive water withdrawal in recent years, the lake surface shrinkage has led to a shift to well-based irrigation, especially in areas affected by receding shorelines. This practice further contributes to the decline in the lake's water level. According to preliminary data from the General Directorate of State Hydraulic Works (DSI), the amount of water entering the lake due to climate change has decreased annually, dropping to approximately 370 hm^3

annually based on the last five-year average. However, the annual loss of water from the lake's surface is 470 hm³ due to evaporation alone. Combined with other uses, the annual water outflow from the lake during this period has reached approximately 590 hm³. Consequently, the balance between inflow and outflow has been disrupted. Since there isn't much that can be done to address evaporation, it has become mandatory for all irrigation systems to be under the supervision of the DSI. Control of the lake and groundwater is essential to protect this critical water source. It is crucial to reassess irrigation allocations in the Lake Egirdir sub-basins and revise them in line with climate change projections. Key sustainable measures include:

- Identification and licensing of illegal wells
- Installation of meters on all wells and strict monitoring
- Five-year review of water allocations for irrigation based on climate scenarios
- Fair pricing of allocated water

Without these measures, access to water could become increasingly difficult. Irrigation improves both crop productivity and resilience, and limited access to water can exacerbate problems, especially in areas that rely solely on rainfall (Carlisle et al., 2019). In regions such Lake Egirdir with intensive agriculture, the use of organic farming techniques within the 270-meter protection zone outside the "Green Belt Zone" is considered essential. This area is particularly sensitive to diffuse pollution. A previous study calculated the pollutant loads that could reach the lake from all agricultural areas. The calculations found that the nitrogen and phosphorus loads reaching the lake from cultivated agricultural areas were 461.6 and 46.1 tons/year, respectively (Gunes, 2008). Studies show that organic practices significantly reduce nitrate leaching (Mondelaers et al., 2009; Shepherd et al., 2003). If conventional techniques are continued, a degradation of soil functions, a loss of ecosystem services and lower long-term productivity are to be expected (Sharma et al., 2024). Outside the buffer zone, promoting Good Agricultural Practices (GAP) is also critical for the management of lakes and basin management. These two approaches organic agriculture and GAP are the main pillars of sustainable agriculture in the region. In the face of climate change, gradual changes in cropping patterns are needed. This not only helps conserve water but also reduces diffuse pollution from agriculture. For instance, studies show that apple farming requires around 25 pesticide applications per season, while cherry production requires only about 12. Even this difference in pesticide use yields significant environmental and economic benefits. Additionally, promoting dwarf fruit trees in apple orchards contributes positively to this transition.

Regardless of current or planned cropping patterns, urgent implementation of sustainable agricultural practices instead of conventional methods would likely lead to the following outcomes:

i. Efficient water use can alleviate, the critical issue of water scarcity, and in the long term, it may become easier to reach at least the minimum lake water level of 914.62 meters.

ii. Reduce input costs for farmers, thereby increasing profitability and reducing greenhouse gas emissions

iii. With an average lake depth of 3–3.5 m, less pollution can be expected in the drier months (September–November)

iv. Improved productivity of the lake's ecosystem

v. Reduced shoreline recession

vi. Improved livelihoods of communities economically dependent on the lake

vii. Fewer losses in tourism

In addition, the welfare of farmers can be improved through these gains, which strengthens social resilience. These outcomes can only be achieved through effective and continuous awareness-raising, incentives, and regulatory enforcement.

4. Conclusion

The severe water loss observed in Lake Egirdir in recent years has resulted in dual threats: Firstly, increased evaporation due to climate change, and secondly, excessive water consumption in agriculture. If these trends continue, they could lead to a significant restriction or eventual collapse of agricultural activities. Lake Eğirdir, with a maximum water volume of 4000 hm³, has lost approximately 60% of its maximum water volume due to dramatic evaporation due to climate change, coupled with other uses. It is estimated that the lake's water volume will drop to 1500 hm³ after the second half of 2025. Therefore, although it is long overdue, sustainable agricultural policies, the most important investment for the future, must be implemented immediately to ensure the continuity of agriculture in the region.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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UAV IMAGE-BASED ESTIMATION OF SURFACE ENERGY BALANCE COMPONENTS IN SORGHUM UNDER DIFFERENT IRRIGATION REGIMES

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Abstract: Precise knowledge of plot-scale surface energy partitioning is fundamental for agricultural water management, yet conventional ground or satellite techniques rarely resolve the heterogeneity for the plot scale areas. This study couples multispectral and thermal imagery acquired weekly–bi-weekly by a DJI Matrice-300 RTK/MicaSense-Altum platform with the physically based Two-Source Energy Balance (TSEB) model to quantify net radiation (Rn), soil heat flux (G), sensible heat flux (H) and latent heat flux (LE) in a randomized sorghum experiment comprising four irrigation levels (100, 70, 40 % ETc and rain-fed) in the Bafra Plain, Türkiye (May–October 2021). The model produced consistent seasonal trends: midday Rn peaked at 797 W m^{-2}, while G/Rn declined from ≈0.20 at emergence to <0.10 under full canopy. Strong irrigation-induced contrasts were detected; fully irrigated plots reached maximum LE of 692 W m^{-2} and H of 10 W m^{-2}, whereas rain-fed plots dropped to LE of 215 W m^{-2} and exceeded H of 450 W m^{-2} during peak stress. Flux magnitudes and partitioning agreed with published eddy covariance and lysimeter studies, indicating that UAV-driven TSEB reliably bridges the scale gap between point sensors and satellites. The approach offers significant potential for real-time irrigation scheduling and water resource optimization, with applications extending to diverse agricultural systems and climate conditions.

Keywords: Unmanned aerial vehicle, Two-source energy balance, Surface energy flux, Sorghum

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1. Introduction

Surface energy balance (SEB) is a crucial regulatory system governing energy exchange between the Earth's surface and the atmosphere, significantly impacting several environmental processes. The accurate quantification of its components—net radiation (Rn), soil heat flux (G), sensible heat flux (H), and latent heat flux (LE)—is important for applications ranging from agricultural water management to climate change modeling (Allen et al., 2007; Bastiaanssen et al., 1998). Rn, serving as the primary energy input, represents the equilibrium between incoming and outgoing radiation. This available energy is subsequently partitioned into G, the energy conducted into the soil; H, which heats the overlying air; and LE, the energy consumed during evapotranspiration (ET). Precisely estimating these components, particularly LE, is essential for diverse agriculture, hydrology, and climate studies applications. Despite its importance, precisely calculating SEB components at high spatial resolutions can be challenging due to surface heterogeneity, prompting the utilization of remote sensing approaches to assess the SEB across continuous geographical domains (Allen et al., 2011; Anderson et al., 2012).

Historically, traditional methods of measuring SEB components have relied on ground-based instruments, which, while providing accurate point measurements, are naturally limited in their spatial representation, especially in heterogeneous landscapes (Bastiaanssen et al., 2005). The advent of remote sensing offered an effective solution to this spatial limitation, with technology evolving from ground-based platforms to satellite and, more recently, unmanned aerial vehicle (UAV) platforms. Satellite remote sensing, utilizing thermal and multispectral sensors on platforms such as Landsat and MODIS, enabled regional and global SEB estimation (Novák and Křížová, 2020). This technological advancement enabled the development of sophisticated models for translating satellite data into energy flux estimates. Notable examples include one-source models like SEBAL (Surface Energy Balance Algorithm for Land), developed by Bastiaanssen et al., (1998), and METRIC (Mapping Evapotranspiration at high Resolution with Internalized Calibration), developed by Allen et al. (2007), both of which conceptualize the surface as a single entity. Concurrently, two-source models such as TSEB (Two-Source Energy Balance), pioneered by Norman et al., (1995), were designed to explicitly partition fluxes between soil and canopy, offering a more physically realistic approach for heterogeneous surfaces like row crops. Additionally, simplified approaches such as SSEBop (Operational Simplified Surface Energy Balance), developed by Senay et al., (2013), were created for large-scale operational monitoring.

The inherent limitations of satellite-based methodologies have illustrated a critical research gap in monitoring SEB at scales relevant to agricultural management. Satellites are frequently constrained by coarse spatial resolution and long revisit intervals, making monitoring crops at the farm-plot level challenging. UAVs have emerged as a transformative technology to bridge this gap, offering distinct advantages in operational flexibility and the capability to acquire data at ultra-high spatial and temporal resolutions. These platforms can have advanced, lightweight sensors that simultaneously capture radiometrically calibrated thermal imagery, high-resolution multispectral data across key vegetative bands (Tunca et al., 2018). This synergy of cutting-edge platforms and sensors facilitates detailed characterization of spatial variability within individual fields. Nevertheless, a significant practical challenge persists, as the robust estimation of SEB components using UAV-based imagery.

This study aims to estimate SEB components using UAV-based imagery using the physically-based TSEB model, with particular emphasis on the model's potential and limitations under field experiment conditions.

2. Materials and Methods

2.1. Study Area

The study was conducted during the 2021 sorghum growing season (May to October) in the Bafra district of Samsun province, Türkiye. The Bafra plain is a significant agricultural delta located on the Black Sea coast. The region is characterized by a semi-humid subtropical climate with warm, humid summers and mild, wet winters (Cemek et al., 2022; Yıldırım et al., 2019). Throughout the growing summer season, average temperatures remain approximately 22.4°C, coupled with increased relative humidity frequently surpassing 70-75%. Although the region experiences greater yearly precipitation than Türkiye's interior, arid periods during the peak growing months of July and August are typical, making supplemental irrigation essential for maximizing the yield of summer crops such as sorghum.

The experiment was laid out in a randomized complete block design with four irrigation treatments and three replications, resulting in a total of 12 experimental plots (Figure 1). Each plot had dimensions of 10 m x 6 m. The crop grown was grain sorghum (*Sorghum bicolor L.*). The four irrigation treatments were:

- S1 (Full Irrigation): Plots were irrigated to 100% of crop water requirement, serving as the control (Plots 1, 7, 10).
- S2 (Deficit Irrigation): Plots received 70% of the water applied to S1 (Plots 3, 8, 9).
- S3 (Deficit Irrigation): Plots received 40% of the water applied to S1 (Plots 4, 6, 11).
- S4 (Rainfed): Plots received no irrigation and relied solely on seasonal rainfall (Plots 2, 5, 12).

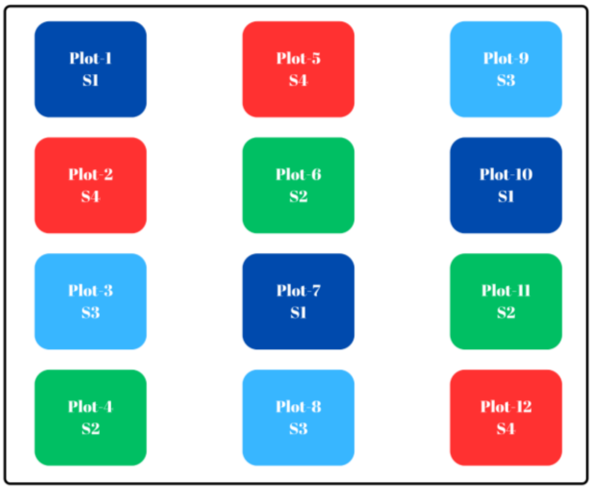

Figure 1. Experimental design. S1, S2, S3, and S4 represent irrigation treatments.

2.2. UAV Data Acquisition

High-resolution aerial imagery was acquired using a DJI Matrice 300 RTK (M300). The UAV was equipped with a MicaSense Altum sensor, a sophisticated payload that captures synchronized multispectral and thermal data. The sensor has five discrete bands including Blue (475 nm center, 32 nm bandwidth), Green (560 nm center, 27 nm bandwidth), Red (668 nm center, 14 nm bandwidth), Red Edge (717 nm center, 12 nm bandwidth), and Near-Infrared (NIR) (842 nm center, 57 nm bandwidth). Also have a radiometrically calibrated FLIR LWIR thermal camera, sensitive in the 7.5-13.5 μm spectrum. UAV flights were conducted on a weekly to bi-weekly basis throughout the growing season. All flights were performed near solar noon (between 12:00 and 14:00 local time) to minimize shadows. Flight missions were planned and executed autonomously at an altitude of 40 m above ground level. This resulted in a ground sampling distance (GSD) of approximately 2 cm. A forward and side overlap of 85% was maintained to ensure high-quality photogrammetric reconstruction. A Downwelling Light Sensor (DLS 2) mounted on the UAV and a Calibrated Reflectance Panel (CRP) imaged on the ground before and after each flight were used for precise radiometric correction.

2.3. Image Processing

The acquired high resolution UAV imagery was processed using Agisoft Metashape Professional photogrammetry software. The workflow included: (I) Image Alignment and Georeferencing, (II) radiometric correction, (III) orthomosaic and Digital Surface Model (DSM) generation. In the image alignment and georeferencing step, images were aligned using their EXIF metadata. The GCPs were used to obtain high precision georeferencing and ensuring minimal geometric errors in the final products. Then, the DLS 2 and CRP data were used to convert the raw digital numbers of the multispectral images into absolute surface reflectance values, correcting for illumination changes during the flight. Finally, dense point clouds were generated, from which high-resolution orthomosaic were created for each band. DSM, representing the top of the crop canopy, was also generated for each flight date separately.

2.4.TSEB Model Implementation

TSEB model was implemented to estimate the surface energy fluxes. The TSEB model partitions the energy balance between the soil and canopy components and calculates the LE as the residual of the surface energy balance equation (equation 1):

$$LE = Rn - G - H \tag{1}$$

The following input parameters, derived from the processed UAV data and on-site meteorological measurements were used to calculate TSEB based surface energy balance components: Radiometric Surface Temperature (Ts) derived directly from the Altum's thermal orthomosaic. Rn is calculated using incoming solar radiation data from an on-site weather station, surface albedo derived from the multispectral orthomosaic, and Ts. G is estimated as a fraction of Rn. The G/Rn ratio was dynamically adjusted based on the fractional vegetation cover (Fc). Fc was calculated from the high-resolution multispectral orthomosaic by applying the Normalized Difference Vegetation Index (NDVI) and classifying the pixels into vegetation and soil categories. Canopy Height (Hc) was determined by subtracting a Digital Terrain Model (DTM) of the bare ground (generated from the first flight date) from the DSM of each subsequent flight date. Used meteorological data-Air temperature, wind speed, relative humidity, and incoming solar radiation- were obtained from an automated weather station located adjacent to the experimental plots.

The model was implemented by first calculating Rn and G. Then, an iterative process was used to solve for H based on the temperature gradients between the surface, canopy, and air, and the calculated aerodynamic and canopy resistances. Finally, LE was calculated as the residual term. A key assumption in the TSEB implementation is the use of the Priestley-Taylor formulation to provide an initial estimate of canopy transpiration, which guides the partitioning of H between the soil and canopy. Calculations were perfomed using Python 3.11 and pyTSEB library.

3. Results

The analysis of UAV-derived imagery using the TSEB model yielded detailed spatial and temporal information on the surface energy fluxes across the four different irrigation treatments. The results demonstrate the model's high sensitivity to varying levels of water availability.

3.1. Surface Energy Balance Component Maps

For each UAV flight date, a series of maps was produced showing the spatial distribution of the main SEB components: Rn, G, H, and LE. The Rn maps were relatively uniform across the study area, with minor variations attributable to slight differences in surface albedo between treatments (Figure 2). In contrast, the maps for G, H, and LE revealed distinct spatial patterns that were strongly correlated with the experimental irrigation design (Figure 3, Figure 4 and Figure 5). The fully irrigated S1 plots (1, 7, 10) consistently appeared as "cool spots" on the H maps and "hot spots" on the LE maps, indicating high rates of evaporative cooling. Conversely, the rainfed S4 plots (2, 5, 12) were clearly delineated as high H and low LE, indicating significant water stress. The deficit-irrigated S2 and S3 treatments exhibited intermediate and transitional patterns, clearly stratifying between the two extremes. This spatial clustering directly reflects the influence of water availability on energy partitioning at the plot scale.

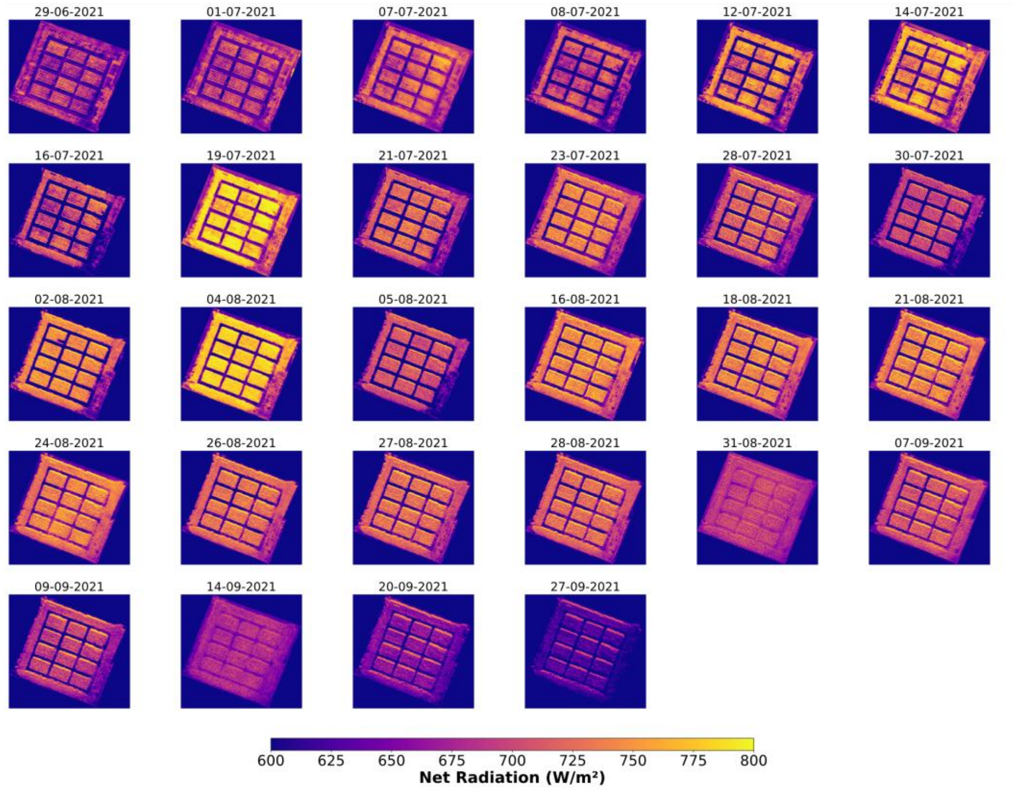

Figure 2. Sorghum Net Radiation Maps during the 2021 growing periods.

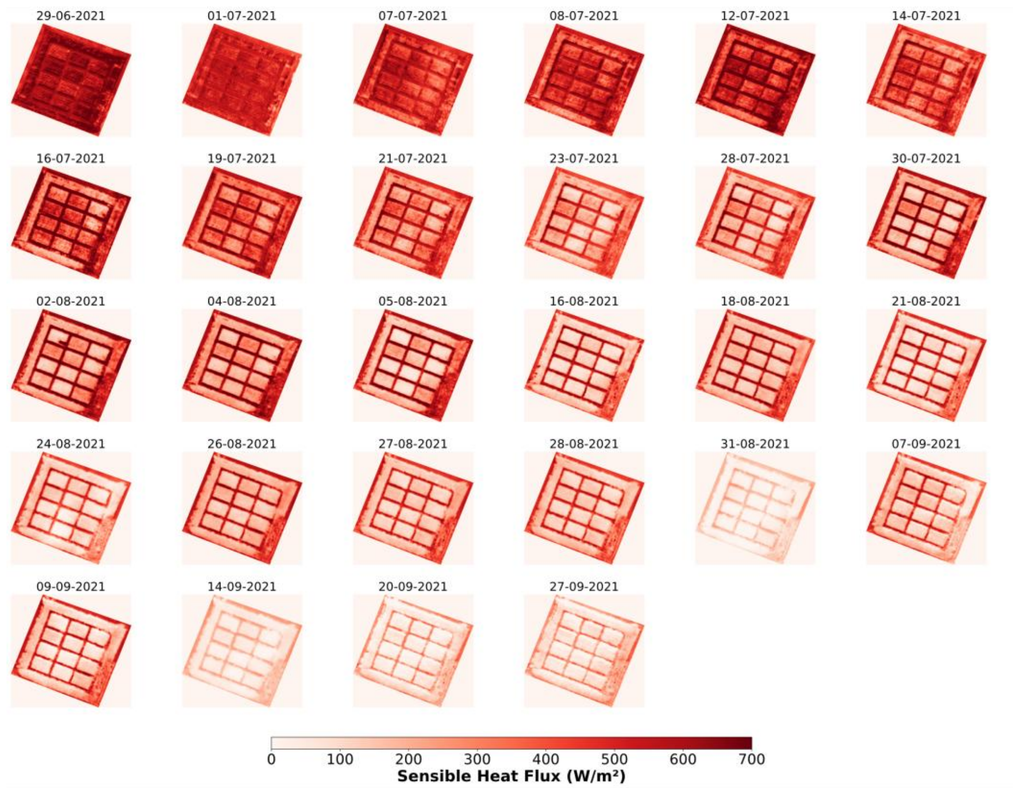

Figure 3. Sorghum Sensible Heat Flux Maps during the 2021 growing periods.

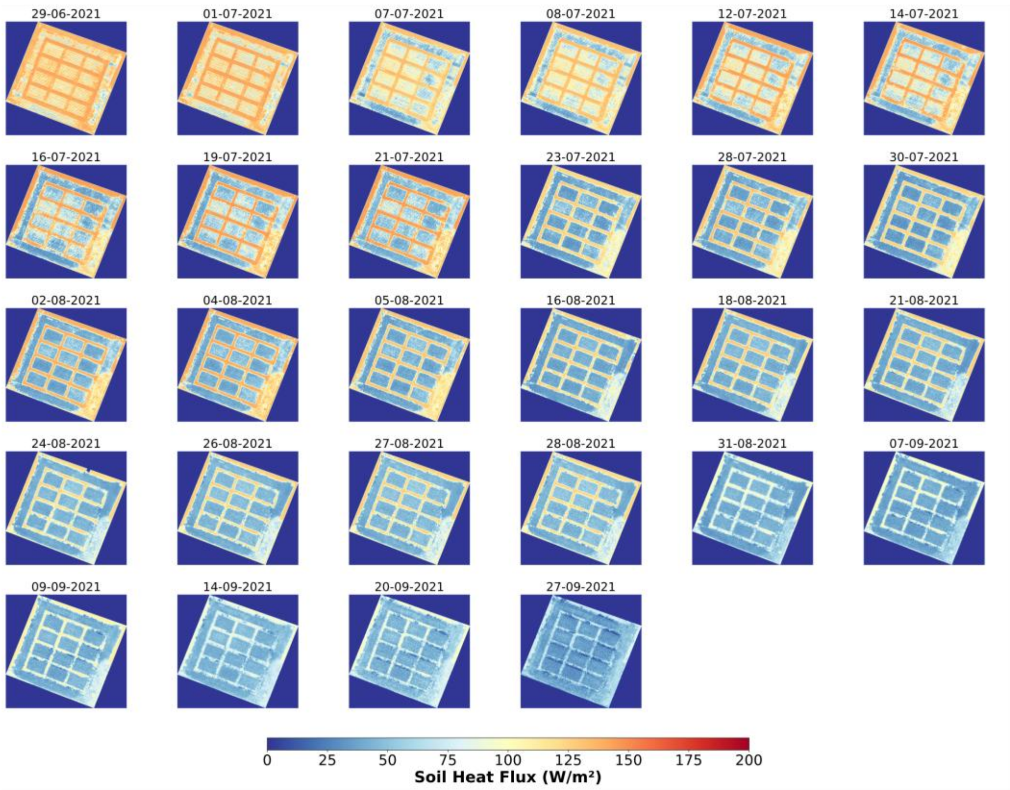

Figure 4. Sorghum Soil Heat Flux Maps during the 2021 growing periods.

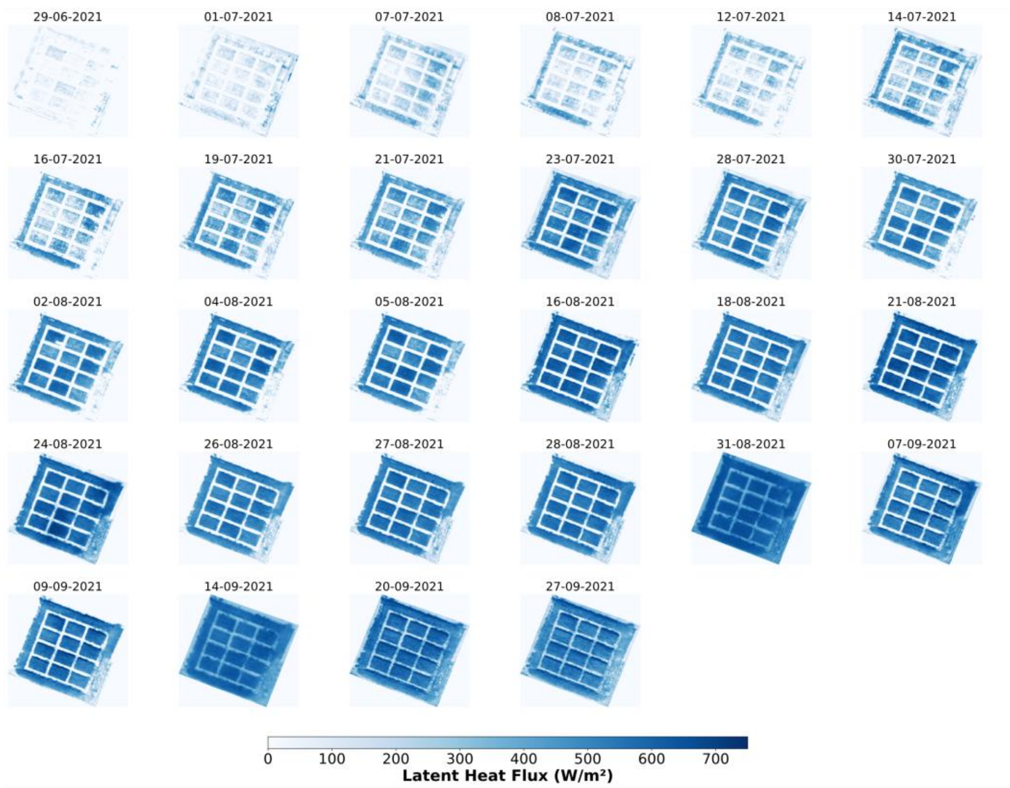

Figure 5. Sorghum Latent Heat Flux Maps during the 2021 growing periods.

3.2. Temporal Analysis of Energy Fluxes

The daily calculated surface energy balance components during the sorghum growing periods were given in Figure 6. Rn values were highest during the peak growing season of July and August, frequently ranging from 700 to 780 W/m^2 on clear days, with a maximum value of 797.2 W/m^2 observed on July 19. The partitioning of this energy was also found to be biophysically sound. The G correctly demonstrated its highest contribution early in the season, reaching values as high as 131 W/m^2 on June

10, which corresponded to a G/Rn ratio of approximately 20%. As the canopy developed and shaded the ground, this contribution diminished, dropping to values between 40 and 50 W/m² during August, representing less than 10% of Rn at full canopy cover.

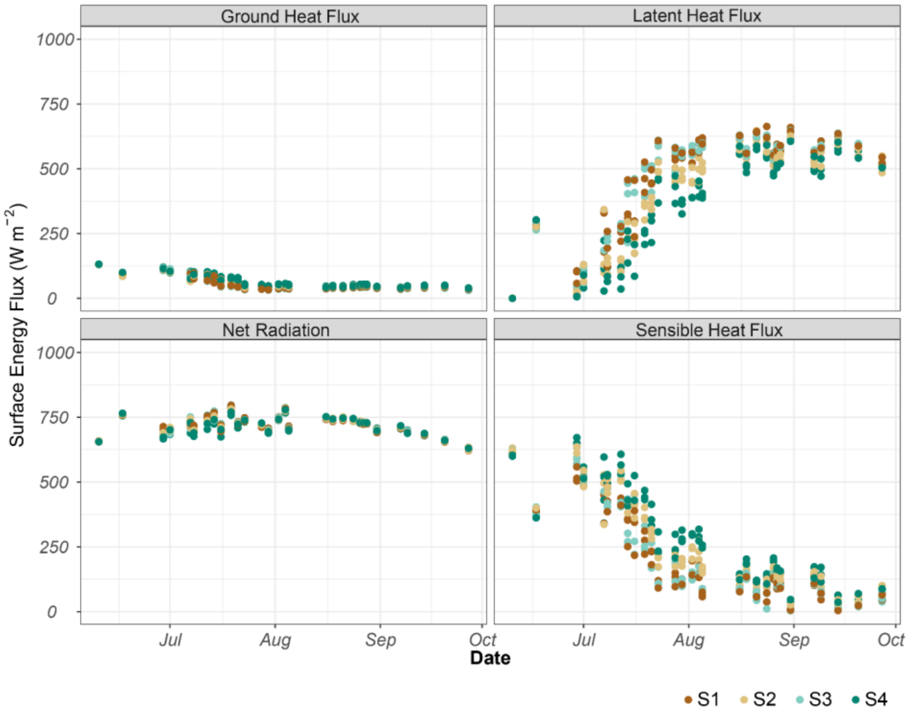

Figure 6 The variation of estimated surface energy balance components using TSEB model and UAV images.

Most importantly, the model clearly captured the various plot LE values based on water availability. The non-stressed S1 treatment exhibited a significant seasonal rise, reaching a maximum LE value of 692.2 W/m² on August 24. The rainfed S4 treatment displayed significant stress; for instance, on July 19, when S1 panels transpired at approximately 500 W/m², S4 plots did not exceed 215 W/m². Following the late-August peak, LE values for all treatments began a steady decline into September as the crop entered senescence, with S1 values dropping to a range of 540-550 W/m² by the end of the month.

This divergence in LE was mirrored by the H. On peak stress days in mid-July, H values in the S4 plots frequently exceeded 450 W/m² (e.g., 493.3 W/m² for Plot 2 on July 14). Conversely, during periods of maximum transpiration in late August, the well-watered S1 plots maintained extremely low H values, often below 100 W/m² and reaching as low as 10.4 W/m² on August 24, confirming that nearly all available energy was being partitioned into evaporative cooling.

4. Discussion

4.1. Impact of Irrigation Regimes on Energy Partitioning

The results of this study clearly demonstrate that the spatial patterns observed in the SEB component maps are a direct outcome of the differential irrigation treatments. The distinct partitioning of energy between LE and H is a quantifiable indicator of the sorghum crop's physiological response to water availability. In the S1 plots, readily available water allowed unrestricted transpiration, maximizing LE and minimizing H, which is conducive to optimal growth. In the S3 and S4 plots, water limitation triggered stomatal closure to conserve moisture, which reducing LE and caused rising in canopy temperatures, thereby increasing the convective heat loss to the atmosphere (Tunca and Köksal, 2024).

4.2. Comparison with Previous Studies

The observed flux magnitudes and energy partitioning in this study are not only internally consistent but also align well with findings from previous research. The peak LE values of over 600 W/m² in the non-stressed S1 plots are in excellent agreement with lysimeter-based measurements for irrigated sorghum reported by Howell et al., (2007). Also, these values are highly consistent with values reported by Gao et al. (2023) and Nassar et al. (2021). The G/Rn ratio's decline with increasing vegetation cover follows the classic relationship documented by Kustas and Daughtry (1990) and Norman et al., (1995). Furthermore, the strong inverse relationship between LE and H, and the use of elevated surface temperature to diagnose stress, is the foundational principle of the Crop Water Stress Index (CWSI) developed by Jackson et al., (1981). The ability of our UAV-TSEB approach to replicate these established relationships and flux magnitudes provides strong confidence in the validity of the results, even without direct ground-based flux validation.

4.3. Model Uncertainties and Study Limitations

The primary limitation of this study is the lack of concurrent ground validation data from instruments like an eddy covariance tower. Therefore, the model evaluation relies on the inter-comparison of treatments and consistency with literature values rather than a direct assessment of absolute accuracy.

The use of a single meteorological station assumes uniform atmospheric conditions across the field, which may not fully capture micro-climatic variations. While high-quality sensors were used, any uncorrected drift or bias in the thermal camera's calibration would directly impact the estimation of H and LE. Finally, chosen software for photogrammetric processing, such as the method for creating the digital surface model, can introduce minor uncertainties in orthomosaic image.

4.4. Implications for Future Research

The most critical next step is to conduct a similar experiment with a ground-based eddy covariance system to perform a direct validation of the UAV-TSEB flux estimates. This would allow for a robust assessment of the model's absolute accuracy and potential calibration. Future work should extend this monitoring over multiple growing seasons to assess the impact of inter-annual climate variability on the crop's water use patterns. The most significant implication is the integration of these seasonal ET maps with final grain yield data. This would enable the calculation of crop water productivity at a sub-field scale, providing invaluable data for both agronomic and economic analyses of irrigation strategies. This methodology has direct applications in precision agriculture, providing the data necessary to implement VRI systems that apply water according to localized crop needs. On a larger scale, this approach can be used by water managers to better monitor and manage water resources within an entire irrigation district, contributing to more sustainable water use in agriculture.

5.Conclusion

This study successfully demonstrated that coupling high-resolution UAV imagery with the physically based TSEB model provides a robust method for quantifying surface energy balance components across different irrigation regimes in a sorghum field. The model accurately captured the biophysical responses to water availability, clearly distinguishing the high latent heat flux in fully irrigated plots from the high sensible heat flux in rain-fed plots. The strong agreement of flux magnitudes and partitioning patterns with established literature values provides high confidence in the approach's validity for diagnosing crop water stress at the sub-field scale. By effectively bridging the gap between point-based ground sensors and coarse-resolution satellites, this methodology offers a direct pathway for generating high-quality evapotranspiration maps essential for precision agriculture applications, such as variable rate irrigation. The critical next step involves direct validation with ground-based flux measurements, which will further refine the model and generalize its application for sustainable agricultural water management.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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SOIL EROSION FACTOR IN DETERMINING EROSION SUSCEPTIBILITY IN AGRICULTURAL AND PASTURE LANDS

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Abstract: Erosion is a process that deviates from its natural course due to human intervention. Erosion has many causes and continues to intensify due to mistakes made in agricultural areas and improper use of pastures. In order to understand the level of soil erosion, it is essential to understand the physical and chemical properties of the soil. This study aimed to determine the value of the K factor, also known as the soil erosion index in Alaçam district. The study includes collecting and analyzing soil samples, descriptive statistics and interpretation of the results, calculation of the K factor, and mapping of the K index by appropriate geostatistical method. SPSS 17.0 program was used for the statistics. In determining the geostatistical methods, the method with the lowest mean square error was preferred, and the ArcGIS 10.2.2 program was used during these processes. As a result of the research, it was observed that the K value was lower in agricultural areas. This situation is related to the amount of organic matter and permeability values. The fact that the erodibility is higher in agricultural areas with low slopes than in pasture lands with higher slopes indicates that agricultural activities are carried out to increase erosion. Although erosion is controllable throughout the district, agricultural practices and activities related to pasture animal husbandry should be meticulously monitored.

Keywords: Erosion, K factor, Agriculture land, Pasture

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1. Introduction

Erosion, which we can define as the wearing and moving of soil by external forces, is also a fundamental process in geomorphology. While the natural erosion process does not usually pose a problem, this process emerges as a natural environment problem due to the increase of human intervention. Many factors cause erosion. The main factors are soil becoming infertile over time due to incorrect agricultural practices, and pastures gradually deteriorating due to incorrect animal husbandry techniques. Various erosional forms, such as gullies that occasionally observed to time in the field, remind the importance of protecting limited natural resources. Soils with differing physical and chemical properties may exhibit varying properties to erosion under the same climatic conditions (Yakupoglu and Demirci 2013). The best way to understand the erosion properties of soils is to reveal their physical and chemical properties. Therefore, the accurate analysis of soil properties is essential for correct and effective erosion control. Ekberli and Dengiz (2017) emphasized that comprehensive soil physical and chemical data are necessary for developing techniques to protect, predict, and enhance soil fertility. The soil K factor is fundamentally important in soil erosion measurement models such as the Universal Soil Loss Equation (USLE) and the revised RUSLE.

In recent years, studies using these models have gained importance (Değerliyurt 2013; Ege 2019; Fiçici and Soykan 2022; Durak and Cürebal 2025; Fahd et al. 2025; Ocak and Bahadır 2025) and have become widely applied by scientists. Since this factor is directly related to the properties of the soil regarding erosion and sediment transport, it is also used as the soil erosion index (Demir et al. 2022). Soil analyses must be performed to determine the K factor, which is complex and slow. For this reason, (İrvem and Tülücü 2004; Cebel et al. 2013) conducted studies to determine the K values of large soil groups throughout the country. Researchers have accepted and used the values obtained in these studies (Mutlu and Soykan 2018; Özdemir and Tatar Dönmez 2016; Aktaş et al. 2024). This research was carried out to determine the K values for agricultural and pasture lands. According to the results obtained, the district's land use in terms of agriculture and animal husbandry was evaluated.

2. Materials and Methods

2.1. Research Area Location

Alaçam, located on the western coast of Samsun province, is a coastal district covering approximately 630 km² (Figure 1). The Black sea climate, which gradually loses its influence toward the inland areas, prevails in the region. The district is rich in terms of vegetation cover. The district lands, which contain broad-leaved, mixed, and coniferous forests, which gradually diminish toward the south. The highest area in the south is the Kızlan Plateau, which consists mostly of meadows. The most common soils in the study area are alluvial soils. These soils are classified as fluviosols according to the soil classification system. It is also found in acidic, clay-rich, infertile allisols soils.

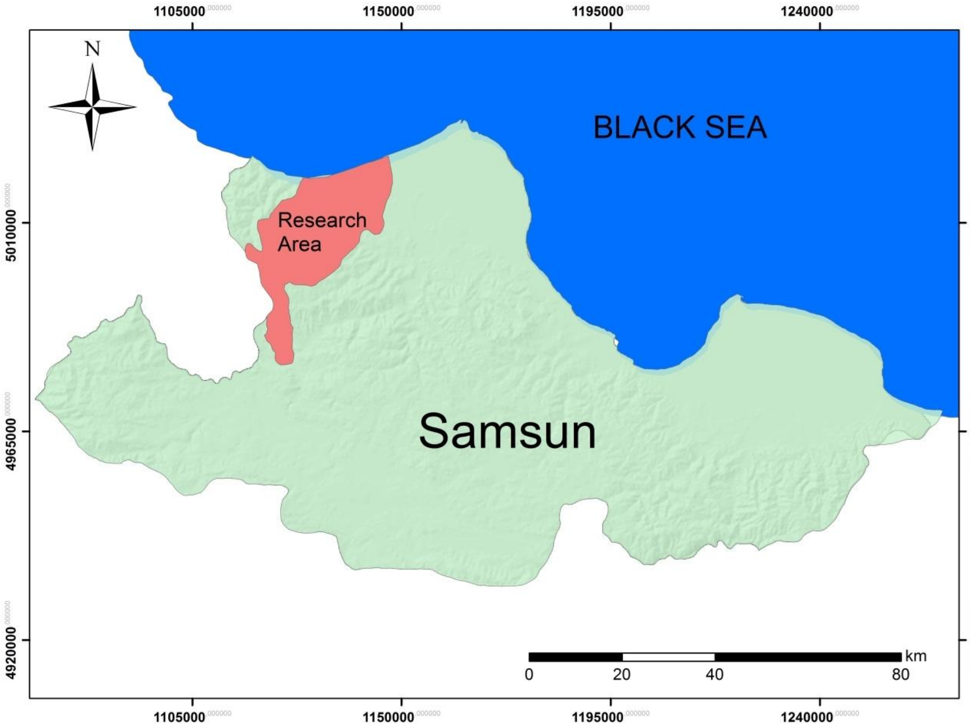

Figure 1. Research area district boundaries.

2.2. Method

Soil samples were collected from 32 different locations, comprising 16 pasturelands, and 16 agricultural fields with different topographic features in the research area (Figure 2). Following the sampling process, the soils were prepared for analysis in the laboratory, and various analyses were carried out on the soils. Texture was determined using the Bouyoucos hydrometer method (Bouyoucos, 1951); hydraulic conductivity was determined using saturated soil samples (Blake and Hartge, 1986); organic matter content was determined using the Walkley-Black method modified by Tüzüner (1990); and structural stability index was determined using the hydrometer method (Tüzüner, 1990). The results obtained were subjected to geostatistical analyses using the ArcGIS 10.2.2 program. The SPSS 17.0 program was used to perform descriptive statistics.

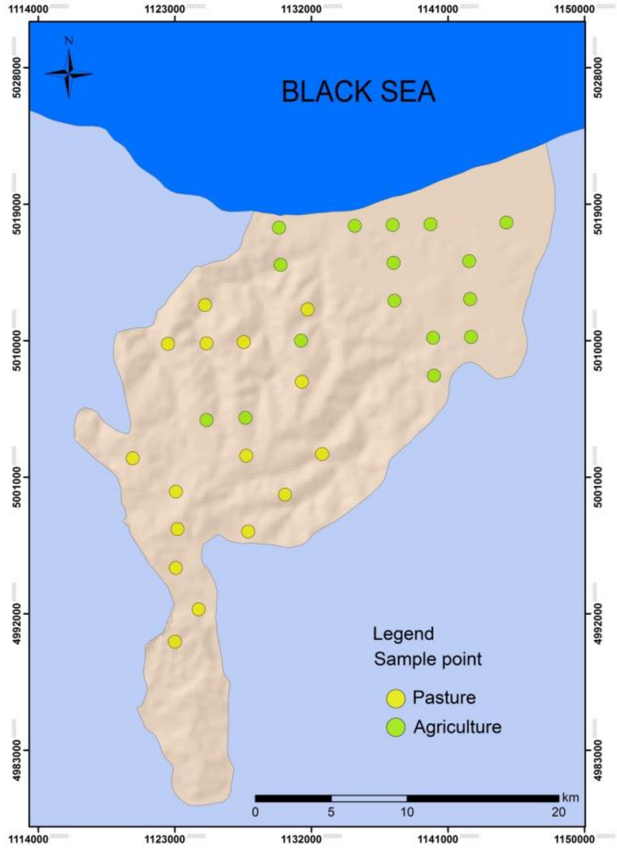

Figure 2. Sample point.

2.3. The soil erodibility factor (K Factor)

The K factor, which represents the erodibility of the soil, integrates various soil properties to indicate the soil's resistance to erosion by external forces. The rate of this resistance is determined according to the index value obtained on a 9% slope and 22.1 meters high land (Wischmeier and Smith, 1978). The equation 1 used to determine the K value is as follows:

$$K = \frac{1}{100}\left\{2.1\times10^{-4}\times(12-OM)\times[SI\times(SA+SI)]^{1.14}+2.5\times(PE-3)+3.25\times(ST-2)\right\} \tag{1}$$

In the formula, OM refers to the percentage of organic matter, SI represents the percentage of silt, SA indicates the percentage of sand, PE denotes the hydraulic conductivity, and ST corresponds to the structure stability index. The erodibility class valuesof the method are given in Table 1.

Table 1. Erodibility (K) classes and values (Wishmeier and Smith 1978)

Definition	Classes	Value
Very Little Erodobility	1	0.00-0.05
Low Erodobility	2	0.05-0.10
Moderate Erodobility	3	0.10-0.20
High Erodobility	4	0.20-0.40
Very High Erodobility	5	0.40-0.60

2.4. Descriptive and Geostatistical Analyses

Descriptive statistics were performed on 32 samples from agricultural and pasture lands in the research area. In addition to these statistics, the normal distribution of soil properties were checked with the Kolmogorov-Smirnov (K-S) test. Differences between land use types were assessed using multiple comparison tests to evaluate variations in soil properties and the corresponding differences in the K factor.

The interpolation method was used to determine the area distribution of the K factor. The root mean square error method (RMSE) was used to determine the appropriate method.

The method yielding the lowest RMSE value, as presented in Table 2, was selected as the most reliable interpolation technique. The most appropriate method was determined as Radial Basis Functions (RBF) Completely Regularized Spline (Table 2).

Table 2. RMSE values table for K value interpolation

Geostatistical Models			K factor
Inverse Distance Weighting (IDW)		1	0.01575
		2	0.01559
		3	0.01561
Radial Basis Functions (RBF)		Completely Regularized Spline	0.01554
		Thin Plate Spline	0.01747
Kriging	Ordinary Kriging	Spherical	0.01565
		Exponential	0.01576
		Gaussian	0.01557
	Simple Kriging	Spherical	0.01605
		Exponential	0.01616
		Gaussian	0.01623
	Universal Kriging	Spherical	0.01565
		Exponential	0.01576
		Gaussian	0.01557

3. Results

3.1. Topographic Features

Alaçam, a small coastal district of Samsun Province located along the Black Sea, has an average elevation of 15 meters and covers an area of approximately 630 km^2. It borders the Bafra district to the west, situated on the left bank of the Bafra Plain (Figure 3). The district has approximately 17 km of coastline and is an important recreation area with the Geyikkoşan forest area. It is also an important festival area; the fair and wrestling festivals are held annually. Although the lands in the south are limited, the Kızlan plateau at 1600 m and Dütmen Mountain are also tourism areas of the district. While the slope values show near-flat values on the coast, it is seen that the valleys consist of deeply cut, high-sloping slopes as you go up (Figure 4). On the other hand, as seen in the Canik Mountains, near-flat lands on erosion surfaces at high altitudes.

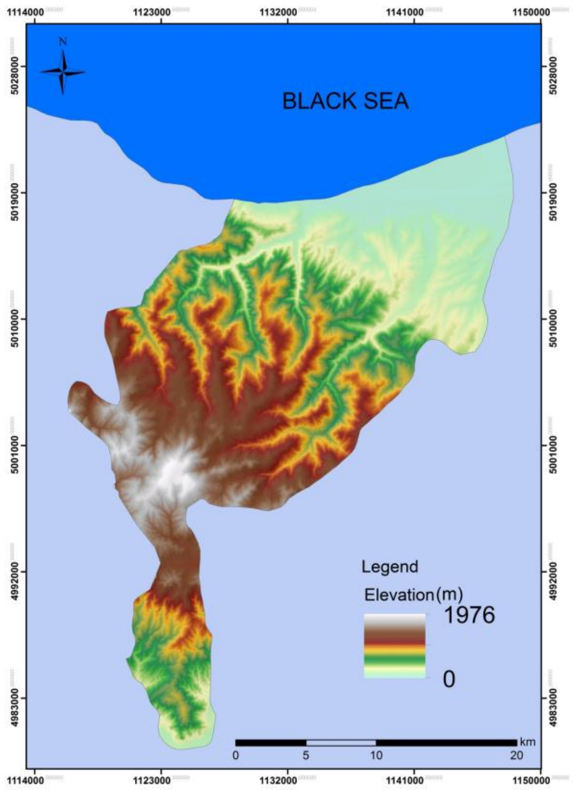

Figure 3. Elevation map.

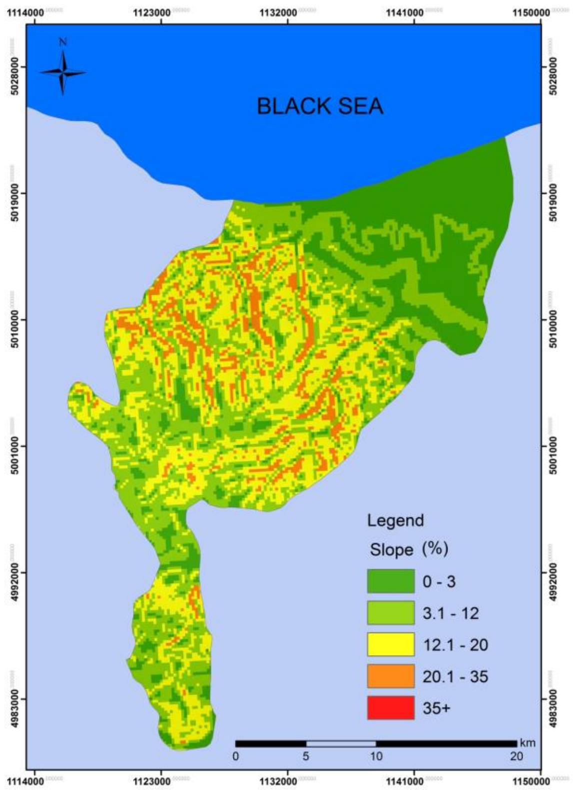

Figure 4. Slope map.

3.2. Land use and land cover

Alluviums are at the level of the plain in the research area; these are areas with existing land uses such as paddy fields, irrigated and non-irrigated agricultural, and mixed lands (Figure 5). In the district where settlements are located in the coastal area, pasture lands and forest lands begin to appear with the increase in elevation. A significant part of the pastures is located within forest lands. These lands are presumed to be old agricultural areas, and unproductive areas have been converted into pastures.

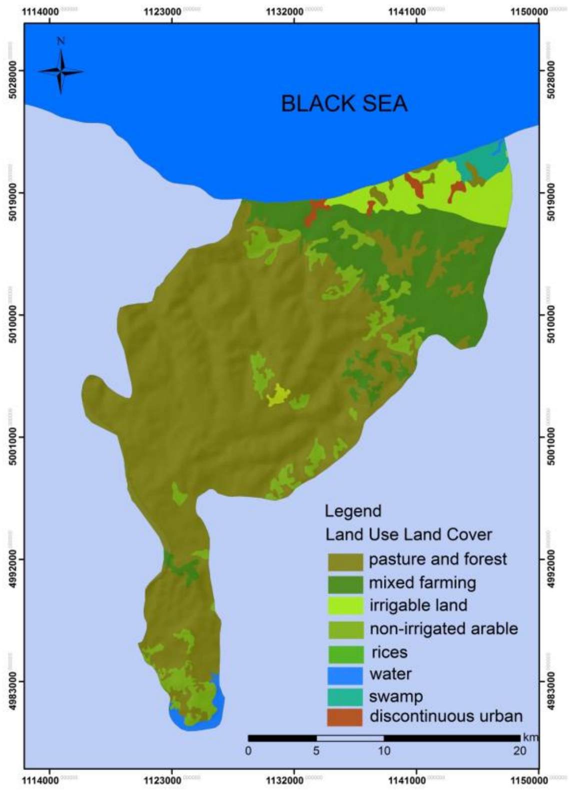

Figure 5. Land use map (Corine 2018).

3.3. Basic Descriptive Statistics

The descriptive statistics of soil properties for pasture and agricultural areas are given in Tables 3 and 4. According to the descriptive statistics tables of soils, it was observed that the K value was homogeneous in agricultural areas and pasture lands, and the coefficient of variation of K value was distributed homogeneously in both land use types.

Table 3. Descriptive statistics of pasture areas

Feature	Minm.	Maks.	Mean	Standard deviation	Coefficient of variation (CV)	Skewness Coefficient	K-S
EC dS/cm	0.167	0.547	0.38	0.10	0.263	-0.219	0.976
pH	5.84	7.81	6.87	0.77	0.108	0.004	0.362
Kireç %	0.2	20.4	4.58	7.34	1.549	1.473	0.056
Phosphorus	0.2	67.41	12.56	16.92	1.304	2.379	0.060
Potassium	14	166	45.1	37.93	0.814	2.184	0.315
Sand	20.97	58.45	39.68	10.07	0.254	-0.448	0.331
Silt	22.10	40.87	30.50	4.78	0.157	0.368	0.933
Clay	19.45	41.84	29.81	6.15	0.206	0.571	0.519
Org. M	1.36	4.48	2.93	0.74	0.243	0.183	0.571
Hc cm/sa	0.6	1.98	0.85	0.42	0.480	1.308	0.511
K	0.093	0.131	0.0965	0.0157	0.157	0.053	0.593
HA gr/cm3	1.37	1.5	1.41	0.064	0.044	-0.442	0.455

According to descriptive statistics, it was observed that most variables in the samples were close to normal distribution, K value was homogeneous according to the coefficient of variation, and according to the K-S test result, it was found to be in conformed to normal distribution ($P>0.05$).

Table 4. Descriptive statistics of agricultural areas

Feature	Minm.	Maks.	Mean	Standard deviation	Coefficient of variation (CV)	Skewness Coefficient	K-S
EC dS/cm	0.249	0.611	0.4129	0.0912	0.2139	0.3632	0.786
pH	6.06	7.82	7.3338	0.5496	0.0726	-1.3021	0.090
Kireç %	0.2	33.9	7.2938	9.0052	1.1954	1.7092	0.391
Phosphorus	1	47.9	14.6562	14.3015	0.9448	1.2472	0.414
Potassium	22	102	54.00	23.8076	0.4269	0.4598	0.973
Sand	9.53	53.24	27.68	11.17	0.403	0.743	0.409
Silt	22.64	51.93	35.77	7.16	0.200	0.387	0.904
Clay	24.12	50.53	36.54	7.05	0.193	-0.161	0.423
Org. M	1.36	4.84	2.6612	0.9403	0.3421	0.7844	0.847
Hc cm/sa	0.175	3.239	0.6984	0.7334	1.0167	2.7891	0.143
K	0.0732	0.1468	0.1162	0.0193	0.1606	-0.7092	0.592
HA gr/cm3	1.27	1.53	1.3656	0.0678	0.0481	0.8965	0.481

In agricultural areas, pH, lime, and hydraulic conductivity values have a high coefficient of skewness, and the distribution is asymmetric, while other values show normal distribution.

Comparison of soil properties between agricultural and pasture lands, revealed that pH values did not differ significantly, and there are slightly higher averages in agricultural areas. It was observed that phosphorus and potassium amounts were higher in agricultural areas, and it was thought that this situation could be related to fertilizer application. The amount of organic matter was higher in pastures. Its that the hydraulic conductivity was higher in pastures, which shows that pasture lands may be more resistant to erosion and that surface runoff will be less. It is seen that the average K values are low in both land uses. K values are seen to be higher than the average in agricultural areas. This shows that the sensitivity to erosion is higher in agricultural areas. This situation is also related to the continuous processing of agricultural areas.

3.4. Distribution of the K factor

The accuracy of the K factor in the Alaçam district soils was evaluated with different geostatistical methods, and the most appropriate distribution was determined. During the geostatistical modeling of the K factor, the most accurate results were obtained using the Radial Basis Function (RBF) interpolation method, specifically the Completely Regularized Spline model, which was found to be the most suitable approach based on error metrics. According to the map obtained with this method, it was determined that the K value in the district soils were generally low to moderate (Figure 6). Although spatial variations in K values were observed between pasture and agricultural lands, the overall differences between these land use types were not pronounced (Figure 7).

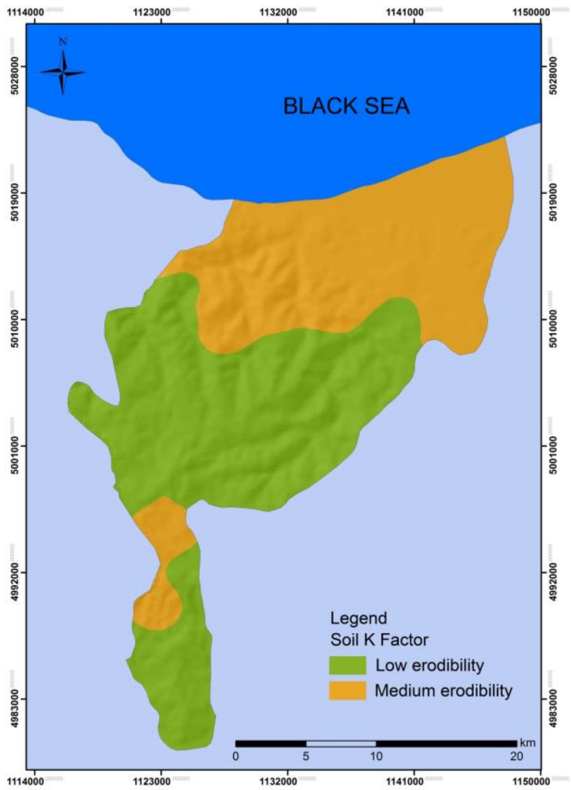

Figure 6. K factor map.

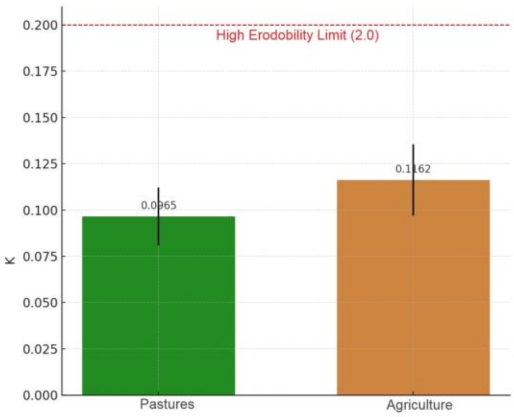

Figure 7. K value in agricultural and pasture areas.

4. Discussion and Conclusion

It is considered that K values are generally higher in agricultural areas compared to pastures, and the main reason may be the low organic matter and low permeability. İmamoğlu et al. (2016) found a similar result in their study in Çorum Alaca and determined a positive significant relationship between organic matter and K value. Özdemir et al. (2015) found that the effect of organic amendments on soil loss varies depending on texture and is more effective in soils under low rainfall intensity. Dengiz and İmamoğlu, (2016) in their study in Samsun province, they determined that the K factor was in similar ranges in agricultural pasture and forest lands throughout the province, however the erosion index values were higher in pasture areas. Low permeability facilitates surface runoff, and in such cases, slope becomes a critical factor. As a result, in agricultural areas with high slope values, surface flow is high, and the amount of erosion is higher. Although the K values obtained in both land uses showed there were no lands in high and very high erosion classes, and this showed that erosion was at a manageable level. Therefore, controlling animal husbandry in pasture areas and preventing the conversion of steep, high-altitude lands into agricultural fields are considered essential conservation measures to mitigate erosion risk.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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EFFECTS OF DIETARY BETAINE SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS TRAITS, MEAT QUALITY, AND FATTY ACID COMPOSITION IN BROILER CHICKENS

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Abstract: Betaine, a natural methyl donor and osmolyte, influences broiler growth performance, protein and lipid metabolism, and meat quality. This study was conducted to investigate the effects of dietary betaine supplementation on the growth performance, carcass traits, meat quality, and fatty acid composition of the breast and leg muscles of broiler chickens. A total of 120 one-day-old male Cobb-500 broiler chicks were randomly allocated into 3 groups, each with 10 replicates of 4 chicks, for 42 days. The birds were fed a basal diet without betaine (control) and basal diets supplemented with 1 or 2 g/kg betaine. Dietary betaine increased body weight, average daily gain, and average daily feed intake compared with the control group throughout the trial (P<0.05). The feed conversion ratio was improved in both betaine-supplemented groups during the finisher and overall phases, especially in the 1 g/kg betaine group (P<0.05). Betaine supplementation significantly increased the carcass and leg muscle yields (P<0.05) but did not affect the breast muscle yield (P>0.05). Meat quality indicators, including squeezable water loss in the breast muscle, shear force, and cooking loss in both muscle types, were improved by supplementation with 1 g/kg betaine (P<0.05). The ultimate pH and L*, a*, and b* values of the breast muscle at 45 min were significantly lower in the 2 g/kg betaine-supplemented group than in the control group (P<0.05). However, the L* values in the breast and leg muscles and the b* value in the breast muscle at 24 h were the highest in the 1 g/kg betaine group (P<0.05). The crude fat content in the breast and leg muscles was elevated in the 1 g/kg betaine group (P<0.05), whereas betaine levels had no effect on the crude protein contents in either muscle type (P>0.05). Furthermore, dietary betaine supplementation significantly modulated the fatty acid composition of the breast and leg muscles (P<0.05). In the breast muscle, the 2 g/kg betaine group exhibited the greatest Σn-3 content and the most favorable Σn-6/Σn-3 ratio, while the 1 g/kg betaine group had the highest monounsaturated fatty acid (MUFA) content (P<0.05). In the leg muscle, the 1 g/kg betaine group also had elevated MUFA content and an increased Σn-6/Σn-3 ratio (P<0.05). Moreover, saturated fatty acid levels were reduced at both betaine levels (P<0.05), whereas polyunsaturated fatty acid levels showed no significant change (P>0.05). In conclusion, 1 g/kg dietary betaine was the most effective level for enhancing broiler growth efficiency, carcass traits, and meat lipid profile. Therefore, betaine may be used as a functional feed additive to improve broiler performance and meat quality.

Keywords: Betaine, Broilers, Growth performance, Meat quality, Fatty acid profile

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1. Introduction

Poultry meat is a major source of animal protein globally, and enhancing its production efficiency and nutritional quality remains a key objective in modern broiler production. The increasing global demand for poultry meat has significantly influenced worldwide production and consumption patterns. Poultry meat is expected to account for 41% of the protein consumed from all meat sources in 2032, followed by pig, bovine, and ovine meat (OECD/FAO, 2023). The poultry sector, particularly intensive broiler chicken production, has experienced remarkable advancements through novel breed development and nutritionally balanced diet formulation. As major contributors to global food security, broiler chickens have substantial economic and nutritional value (FAO, 2024). Global poultry meat production reached approximately 148 million tons (carcass weight) in 2024, with broiler chickens accounting for around 104 million tons (USDA, 2025). Therefore, the sector continuously faces pressure to enhance growth efficiency, carcass quality, and meat traits, placing nutrition at the primary focus of production strategies. In recent years, dietary supplementation strategies have increasingly targeted natural compounds such as betaine, which is recognized for its potential to improve broiler performance, carcass characteristics, and meat quality (Yang et al., 2022; Suliman et al., 2023). Betaine, or trimethylglycine, is a naturally occurring methyl derivative of glycine that is commonly found in sugar beet and other plants. It is also endogenously synthesized through choline metabolism or consumed exogenously through dietary intake (Arumugam et al., 2021). Betaine is available in several forms, including anhydrous, monohydrate, and hydrochloride, all of which have been reported to benefit broiler production (Eklund et al., 2005). Anhydrous betaine is a pure, water-free form often derived from natural sources. Betaine hydrochloride, a synthetic form of betaine, is commonly used because of its stability and cost-effectiveness (Chen et al., 2022). Natural betaine, which is extracted from plants, especially sugar beets, is sometimes labeled as “n-betaine” (Awad et al., 2022). The European Food Safety Authority has approved all forms of betaine for use in animal feed. Specifically, betaine anhydrous (97%, 96%, or 91%) is considered safe for the target species at a maximum supplementation level of 2000 mg/kg of complete feed, posing no safety concerns for consumers (EFSA FEEDAP Panel, 2018).

Betaine absorption in broilers occurs primarily in the small intestine through sodium-dependent and -independent transport mechanisms (Kettunen et al., 2001; Metzler-Zebeli et al., 2009). After absorption, betaine is predominantly metabolized in the liver by betaine-homocysteine methyltransferase (BHMT), which facilitates transmethylation by donating methyl groups to convert homocysteine into methionine, thereby influencing protein synthesis and lipid metabolism (Ratriyanto et al., 2009; Obeid, 2013). The mode of action of betaine includes improving growth performance (Chen et al., 2018), enhancing carcass characteristics (Nutautaitė et al., 2020), methyl donor activity (Wang et al., 2025), osmolytic activity (Alahgholi et al., 2014), and counteracting stress (Wen et al., 2019; 2021) in broiler chickens. Recent studies on broiler nutrition have consistently reported improved average daily gain (ADG), feed intake, and feed conversion ratio (FCR) with betaine supplementation. Yang et al. (2022) found that dietary betaine supplementation (500–1000 mg/kg) significantly increased ADG and improved FCR in slow-growing broilers. Similarly, Chen et al. (2018) reported that dietary betaine supplementation increased body weight gain (BWG) and improved FCR in broilers. Eldamrawy et al. (2023) also reported that dietary betaine supplementation (1 and 2 g/kg) significantly increased BWG and feed intake and improved FCR in broiler chickens. In addition, recent evidence has highlighted the role of betaine in increasing muscle yield, reducing fat deposition, and improving meat quality. Chen et al. (2022) demonstrated that dietary betaine significantly increased breast muscle yield, enhanced antioxidant capacity, and reduced drip loss in broilers. Liu et al. (2019) showed that betaine supplementation improved carcass traits and breast muscle yield, indicating reduced fat deposition and improved growth efficiency under heat stress conditions. Betaine supplementation has also been shown to beneficially alter the fatty acid profile of broiler chicken meat by decreasing saturated and increasing unsaturated fatty acid levels (Yang et al., 2022). The underlying mechanisms include the activation of lipid oxidation pathways (PPAR/AMPK) and stimulation of protein synthesis via mTOR signaling pathways, thereby promoting lean muscle development and reducing adiposity (Chen et al., 2018; Du et al., 2018). Despite previous research, a knowledge gap remains regarding the optimal dietary betaine levels needed to achieve balanced improvements in broiler growth performance, carcass characteristics, meat quality, and lipid metabolism. To address this gap, the present study investigated the effects of different dietary betaine supplementation levels on growth performance, carcasstraits, meat quality parameters, and fatty acid profiles in broiler chickens.

2. Materials and Methods

2.1. Experimental Design, Management, and Feeding

A total of 120 one-day-old male Cobb 500 broiler chicks were obtained from a local hatchery. Birds were randomly assigned to 3 dietary treatment groups, each consisting of 40 chicks. Each treatment group was subdivided into 10 replicates, with 4 chicks per replicate (0.1 m^2 per bird). Dietary treatments included a control basal diet without betaine (0 g/kg) and basal diets supplemented with 1 and 2 g/kg betaine. Anhydrous betaine (trimethylglycine; purity ≥ 96%) was supplied from a commercial supplier in Türkiye. The chicks were reared in floor pens covered with wood shavings, each equipped with a pan feeder and nipple drinker. All birds had free access to water and were fed *ad libitum*. The lighting program was 21L:3D throughout the experimental period. The initial temperature of the broiler house was maintained at 32–34°C during the first 5 days and was gradually reduced to a minimum of 22–24°C for the remainder of the experiment. The relative humidity was maintained at 55–60%. The experimental period lasted for 42 days. The birds were fed a three-phase feeding program consisting of starter (d0–14), grower (d15–28), and finisher (d29–42) diets. All diets were formulated in mash form at the feed production facility of the Department of Animal Science, Faculty of Agriculture, Dicle University, based on the NRC (1994) nutrient guidelines for male broilers. The proximate composition of the primary feed ingredients was analyzed prior to diet formulation according to the AOAC (2000) methods. Dietary sugar and starch contents were determined using the Luff-Schoorl method and the two-step procedure outlined in the TS 12232 and TS ISO 6493 standards (TSE, 1997; 2004). The ingredient composition, nutrient content, and metabolizable energy levels of the experimental diets are presented in Table 1.

Table 1. Ingredients (g/kg) and nutrient composition (%, as fed basis) of the experimental starter, grower, and finisher broiler diets.

Period	Starter (d0-14)			Grower (d15-28)			Finisher (d29-42)		
Betaine levels (g/kg)	0	1	2	0	1	2	0	1	2
Ingredients									
Corn	553.00	553.00	553.00	553.00	553.00	553.00	553.00	553.00	553.00
Soybean meal (46% CP)	380.00	380.00	380.00	380.00	380.00	380.00	380.00	380.00	380.00
Limestone	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Dicalcium phosphate	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Sunflower oil (8800 Kcal/kg)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
DL-Methionine	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
L-Lysine	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin-Mineral Premix[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
NaCl	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Betaine	-	1.00	2.00	-	1.00	2.00	-	1.00	2.00
Chemical composition analyzed, %									
Dry matter	90.10	90.05	89.80	89.85	90.00	89.90	90.10	90.00	90.20
Crude protein	23.05	23.00	22.95	21.50	21.45	21.40	20.10	20.05	20.00
Ether extract	5.73	5.72	5.75	6.75	6.70	6.75	8.37	8.30	8.36
Crude fiber	2.70	2.75	2.70	2.61	2.60	2.64	2.54	2.50	2.53
Crude ash	6.45	6.35	6.42	5.74	5.81	5.80	5.61	5.66	5.71
Starch	38.23	38.15	38.20	39.97	40.02	39.86	40.27	40.22	40.25
Sugar	4.17	4.20	4.22	3.95	3.95	4.01	3.80	3.86	3.92
Metabolizable energy (ME poultry), MJ/kg	12.46	12.44	12.45	12.83	12.82	12.81	13.20	13.18	13.19
Calculated values, %									
Lysine	1.43	1.43	1.43	1.32	1.32	1.32	1.26	1.26	1.26
Methionine+cystine	1.07	1.07	1.07	0.97	0.97	0.97	0.94	0.94	0.94
Ca	0.93	0.93	0.93	0.79	0.79	0.79	0.79	0.79	0.79
Available P	0.46	0.46	0.46	0.38	0.38	0.38	0.38	0.38	0.38

[1]Vitamin and mineral premix provided per 2.5 kg of diet: vitamin A, 12000000 IU; vitamin D3, 1500000 IU; vitamin E, 40000 mg; vitamin K3, 5000 mg; vitamin B1, 3000 mg; vitamin B2, 7000 mg; vitamin B6, 5000 mg; vitamin B12, 30 mg; CAL-D pantothenate, 10000 mg; biotin, 75 mg; folic acid, 1000 mg; niacin amide, 4000 mg; choline chloride, 400000 mg; Mn sulfate, 80000 mg; Fe (II) sulfate, 60000 mg; Cu (II) sulfate, 5000 mg; Zn sulfate, 60000 mg; Ca iodide, 1000 mg; Na selenite, 150 mg; and $CaCO_3$ 1135000 mg.

2.2. Performance Measurements

The body weight (BW) of individual birds and feed residues were recorded on days 0, 14, 28, and 42. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR, adjusted for mortality and calculated as total feed intake divided by total gain including weight of lost birds) were calculated for the starter, grower, finisher, and overall experimental periods, respectively. Mortality was monitored daily, and the mortality rate was determined by expressing the number of deaths as a percentage of the total number of birds in each treatment group.

2.3. Carcass Traits, Organ Weights, and Sampling

At the end of the experimental period (d42), one bird per replicate (10 birds per treatment) was selected based on BW within ±5% of the group mean. The selected birds were fasted for 12 h with free access to water before slaughter. Each bird was weighed and then slaughtered by severing the left carotid artery, followed by decapitation and bleeding for approximately 2 min in accordance with standard humane procedures. The birds were scalded (60°C for 120 s), defeathered, and eviscerated immediately after slaughter. Carcass yield was calculated as a percentage of the BW. The heart, spleen, liver, proventriculus, gizzard, small intestine, cecum, large intestine, and abdominal fat pad were carefully removed and weighed, and their relative weights were expressed as percentages of BW.

The breast and leg muscles were dissected and weighed to calculate their relative weights as a percentage of BW.

All parts were processed with intact skin and bone after being cut up. Samples of breast (*Pectoralis major*) and leg (thigh and drumstick) meat were collected for quality analyses. All samples were vacuum-packed and stored at 4°C for 24 h postmortem before instrumental meat quality measurements were performed. Additional samples were stored at −20°C for subsequent chemical analyses.

2.4. Meat Quality

2.4.1. Instrumental Analysis of Meat Quality

The pH values of the muscles were measured at 45 min postmortem (initial pH) and 24 h postmortem (ultimate pH) using a portable pH meter (Model HI-99163, Hanna Instruments, Woonsocket, RI, USA) equipped with a spear-tip electrode to evaluate meat quality parameters. Three measurements were obtained from the cranial and caudal regions of each muscle sample. Simultaneously with the pH measurements, color parameters were recorded using a MiniScan colorimeter (3nh Technology Co., Ltd., Shenzhen, China) to determine the CIE L* (lightness), a* (redness), and b* (yellowness) values, according to the Commission Internationale de l'Éclairage (1976) standards. Three readings were taken for each sample, and the instrument was calibrated against white and black tiles before the measurement. The squeezable water content was assessed using the press method described by Grau and Hamm (1953), with slight modifications. Approximately 1 g of minced breast and leg muscle was placed between filter papers and compressed under a weight of 1 kg for 5 min. After compression, the samples were reweighed, and the moisture loss percentage was calculated as an indicator of free water release. For the determination of drip loss, the right breast fillets and leg muscles were deboned, placed in individual polypropylene bags, and suspended at 4 ± 2°C for 24 h. Subsequently, the samples were surface-dried and reweighed according to Christensen (2003). The drip loss was calculated as follows: [(initial weight − final weight) / initial weight] × 100. The thawing loss was measured using the freeze-thaw method. The remaining samples were vacuum-sealed, stored at −20°C for 4 days, and subsequently thawed at 5°C for 24 h. After surface drying, the samples were reweighed, and thawing loss was calculated as follows: [(pre-thaw weight − post-thaw weight) / pre-thaw weight] × 100. The cooking loss was evaluated following the thawing loss procedure, according to Boccard et al. (1981). The thawed samples were cooked in a water bath maintained at 80°C for 30 min until the internal temperature reached approximately 75°C. After cooling and surface drying, the cooking loss was calculated using the following formula: [(pre-cooking weight − post-cooking weight) / pre-cooking weight] × 100. The squeezable water content, drip loss, thawing loss, and cooking loss were used as indicators of the water-holding capacity. A Texture Analyzer (Stable Micro Systems, Surrey, UK) fitted with a Warner-Bratzler shear probe was used for texture analysis. The cooked samples were cored into cylindrical pieces (1.25 cm diameter), and four cores per sample were analyzed. Measurements were performed perpendicular to the direction of the muscle fiber using a 100 kg load cell at a crosshead speed of 2 mm/s, as described by Boccard et al. (1981).

2.4.2. Proximate composition analysis

The dry matter (ISO 1442:1973), crude protein (ISO 937:1978), crude fat (ISO 1444:1973), and crude ash (ISO 936:2000) contents of the breast and leg meat samples were analyzed. All analyses were conducted in duplicate, and the results were expressed as g/100 g of fresh matter.

2.4.3. Fatty acid analysis

Lipids were extracted from breast and leg meat samples using a modified version of the chloroform/methanol (2:1, v/v) extraction method, as described by Folch et al. (1957). Following methylation with NaOH/MeOH and HCl/MeOH, fatty acid methyl esters (FAME) were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GC-MS system equipped with an RTB-5MS capillary column (60.0 m × 0.25 mm × 0.25 µm film thickness). The GC conditions were as follows: injector temperature, 250°C; injection mode, split (split ratio 60.0); carrier gas, helium at a flow rate of 0.73 mL/min; column temperature program, initial temperature 140°C (held for 0 min), ramping at 10.00°C/min to 200°C (held for 5.00 min), followed by an increase at 3.00°C/min to 220°C (held for 2.00 min), and then increased at 2.00°C/min to 240°C (held for 2.00 min). Individual fatty acids were identified by comparing their retention times with those of the corresponding standards (Sigma-Aldrich, St. Louis, MO, USA; Nu-Chek Prep., Waterville, MN, USA) and confirmed by mass spectral analysis. All analyses were performed in duplicate to ensure precision. The fatty acid composition was expressed as g/100 g of total FAME.

2.5 Statistical Analysis

All data were subjected to statistical analysis using SPSS software (Version 22.0; SPSS Inc., 2013). Prior to the analysis, the normality of the data distribution was assessed using the Shapiro-Wilk test. The treatment effects were evaluated using one-way analysis of variance (ANOVA). When significant differences were detected, the means were compared using Duncan's multiple range test. Statistical significance was defined as $P<0.05$ was considered as a trend. Orthogonal polynomial contrasts were applied to evaluate linear and quadratic responses to dietary treatment levels. Mortality rates were compared among treatment groups using the chi-square test. All results are expressed as mean ± standard error of the mean (SEM).

3. Results

3.1 Growth Performance

The effects of dietary betaine supplementation on the growth performance and mortality of broiler chickens are presented in Table 2. In the starter period, birds treated with 1 g/kg and 2 g/kg betaine showed higher

BW on d14 than the control group (P<0.05). Dietary betaine significantly increased ADG and ADFI, with this improvement being linearly related to increasing betaine inclusion levels (P_{lin} < 0.05). No significant differences were observed in FCR during this period (P>0.05). In the growing period, dietary betaine supplementation significantly improved BW, ADG, ADFI, and FCR (P<0.05), and betaine supplementation significantly enhanced BW on d28 (P<0.05). Birds treated with 1 g/kg betaine exhibited a lower FCR than the control group (1.42 *vs.* 1.50; P<0.05). Moreover, BW, ADG, and ADFI showed significant linear responses (P_{lin} < 0.05), whereas FCR exhibited a significant quadratic response (P_{quad} < 0.05) to dietary betaine levels. During the finisher period, dietary betaine supplementation significantly enhanced BW, ADG, ADFI, and FCR (P<0.05), demonstrating a linear response (P_{lin} < 0.05). The final BW and ADG were significantly higher in the betaine-supplemented groups (P<0.05), with this increase being linearly related to betaine levels (P_{lin} < 0.05). Additionally, dietary betaine significantly improved the overall FCR (1.82 *vs.* 1.76 and 1.77 for control *vs.* betaine groups; P<0.05). Dietary treatments did not affect mortality rates during the entire experimental period (P>0.05).

3.2. Carcass Traits and Organ Weights

The effects of dietary betaine supplementation on carcass characteristics and relative organ weights (% of BW) are given in Table 3. Carcass yield significantly and linearly increased with betaine supplementation, with the 1 g/kg (69.23%) and 2 g/kg (69.01%) betaine groups showing higher values than the control group (67.81%) (P<0.05). Similarly, the leg meat yield was significantly higher in the betaine-supplemented groups (21.78 *vs.* 22.70 and 23.01% for the control *vs.* betaine groups, respectively; P<0.05). However, the dietary treatments did not affect breast meat yield (P>0.05). Relative small intestinal weight showed the most pronounced response to dietary betaine supplementation. Birds treated with 2 g/kg betaine exhibited greater small intestine weight (3.40%) than those receiving 1 g/kg betaine (3.02%) and the control (2.47%) (P<0.05). In contrast, no significant differences were observed in the relative weights of the other organs, including the heart, spleen, liver, proventriculus, gizzard, abdominal fat, cecum, and large intestine (P>0.05).

Table 2. Effect of betaine supplementation on growth performance and mortality in broiler chickens.

Treatment[1]	Betaine levels (g/kg)			SEM[2]	P value		
Parameters	0	1	2		Main	Linear	Quadratic
Starter (d0-14)							
BW d0, g	37.01	36.80	36.16	0.289	0.470	0.248	0.724
BW d14, g	436.60^{b}	463.79^{a}	469.54^{a}	4.767	0.009	0.004	0.262
ADG, g/d	28.87^{b}	30.79^{a}	31.29^{a}	0.278	<0.001	<0.001	0.182
ADFI, g/d	35.16^{b}	37.19^{a}	37.89^{a}	0.385	0.009	0.003	0.388
FCR	1.23	1.22	1.22	0.004	0.391	0.236	0.492
Grower (d15–28)							
BW d28, g	1434.06^{b}	1500.28^{a}	1496.51^{a}	8.821	0.002	0.003	0.043
ADG, g/d	71.27^{b}	74.24^{a}	73.54^{a}	0.321	<0.001	0.002	0.003
ADFI, g/d	101.55^{b}	102.24^{ab}	103.73^{a}	0.363	0.040	0.013	0.595
FCR	1.42^{a}	1.38^{b}	1.41^{a}	0.006	0.013	0.466	0.004
Finisher (d22-42)							
BW d42, g	2356.28^{b}	2471.59^{a}	2476.94^{a}	22.24	0.041	0.025	0.231
ADG, g/d	66.02^{b}	69.46^{a}	69.71^{a}	0.365	<0.001	<0.001	0.020
ADFI, g/d	164.52^{b}	166.92^{a}	167.77^{a}	0.449	0.007	0.002	0.386
FCR	2.49^{a}	2.41^{b}	2.40^{b}	0.011	0.001	0.001	0.090
Total (d0-42)							
ADG, g/d	55.31^{b}	58.06^{a}	58.17^{a}	0.351	<0.001	<0.001	0.051
ADFI, g/d	100.47^{b}	102.19^{ab}	103.19^{a}	0.376	0.009	0.003	0.632
FCR	1.82^{a}	1.76^{b}	1.77^{b}	0.007	<0.001	0.002	0.006
Mortality, %	2.5	2.5	5	-	0.772	-	-

Means with different superscript letters within the same row are significantly different (P<0.05). BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

[1]Broilers were fed a basal diet supplemented with 0, 1, and 2 g/kg betaine.

[2]Standard error of the mean.

Table 3. Effect of dietary betaine supplementation on carcass traits and organ weights (g/100 g body weight) in broiler chickens.

Treatment[1]	Betaine levels (g/kg)				P value		
Parameters	0	1	2	SEM[2]	Main	Linear	Quadratic
Carcass yield	67.81[b]	69.23[a]	69.01[a]	0.214	0.012	0.018	0.060
Breast meat	33.92	34.65	34.56	0.173	0.171	0.130	0.262
Leg meat	21.78[b]	22.70[a]	23.01[a]	0.159	0.003	0.001	0.334
Heart	0.52	0.53	0.55	0.019	0.873	0.623	0.880
Spleen	0.12	0.09	0.10	0.008	0.584	0.581	0.385
Liver	1.93	1.75	1.80	0.050	0.337	0.299	0.295
Proventriculus	0.32	0.35	0.32	0.012	0.549	0.947	0.281
Gizzard	2.13	2.34	2.37	0.108	0.660	0.401	0.715
Abdominal fat	0.78	0.67	0.64	0.073	0.748	0.487	0.796
Small intestine	2.47[c]	3.02[b]	3.40[a]	0.107	<0.001	<0.001	0.556
Cecum	0.61	0.48	0.58	0.031	0.258	0.720	0.110
Large intestine	0.14	0.13	0.13	0.006	0.572	0.420	0.481

Means with different superscript letters within the same row are significantly different (P<0.05).
[1]Broilers were fed a basal diet supplemented with 0, 1, and 2 g/kg betaine.
[2]Standard error of the mean.

3.3. Meat Quality Parameters

The parameters of the instrumental meat quality are reported in Table 4. The initial pH remained unaffected in both muscle types (P>0.05). However, betaine supplementation significantly reduced the ultimate pH in the breast muscle (5.97 *vs.* 5.86 and 5.70 for control *vs.* betaine groups; P<0.05). Similarly, the ultimate pH of leg muscles showed a decreasing trend with betaine inclusion, and this trend was linearly related to betaine levels (P_{lin} < 0.05). Water-holding capacity varied among muscle types. Betaine supplementation significantly reduced (P<0.05) the squeezable water loss in breast meat (6.28% in control *vs.* 5.51% at 1 g/kg betaine group), showing both linear and quadratic effects. Conversely, squeezable water loss in the leg muscle was significantly increased following betaine supplementation (P<0.05). The highest thawing and drip losses were observed in the betaine-supplemented groups for both breast and leg muscles, whereas the control group showed marked reductions (P<0.05). Drip loss in the breast muscle was significantly increased with betaine supplementation (P<0.05), whereas that in the leg muscle remained unchanged (P>0.05). Cooking loss was significantly lower in the breast and leg muscles of birds receiving 1 g/kg betaine-supplemented diets than in those of the other experimental groups (P<0.05). Additionally, birds receiving 1 g/kg betaine exhibited the lowest shear force values in both breast (14.38 N) and leg (15.64 N) muscles (P<0.05), in a quadratic manner (P_{quad} < 0.05). The color parameters exhibited dose-dependent changes. At 45 min postmortem, the breast meat of birds receiving 2 g/kg betaine supplementation exhibited reduced L*, a*, and b* values compared with those of the other treatment groups (P<0.05). In contrast, the dietary treatments did not affect the color parameters of the leg meat (P>0.05). At 24 h postmortem, the highest L*, a*, and b* values in breast meat and the highest a* value in leg meat were found in the 1 g/kg betaine group. No changes were observed in the other color values of the leg meat at 24 h postmortem.

3.4. Proximate Composition

The proximate composition data showed that dietary betaine supplementation did not influence the dry matter and crude ash contents in either the breast or leg muscles (P>0.05; Table 5). Similarly, no significant differences were detected in the crude protein content across treatments (P>0.05). In contrast, crude fat content was significantly increased in both breast and leg muscles (P<0.05), with the highest values observed in the 1 g/kg betaine group (1.44% and 2.43%, respectively) compared with the control group (0.80% and 1.00%, respectively).

Table 4. Effect of dietary betaine supplementation on broiler breast and leg muscle quality parameters.

Treatment[1] Parameters	Muscle Type	Betaine levels (g/kg) 0	1	2	SEM[2]	P value Main	Linear	Quadratic
Initial pH	Breast	6.55	6.61	6.65	0.043	0.607	0.323	0.900
	Leg	6.61	6.68	6.71	0.040	0.560	0.299	0.792
Ultimate pH	Breast	5.97^{a}	5.86^{ab}	5.70^{b}	0.041	0.026	0.007	0.784
	Leg	6.05	5.90	5.79	0.048	0.075	0.024	0.856
Squeezable water loss (%)	Breast	6.28^{a}	5.51^{b}	5.81^{b}	0.082	<0.001	0.010	0.001
	Leg	2.22^{b}	2.44^{a}	2.63^{a}	0.046	0.001	<0.001	0.845
Thawing loss (%)	Breast	1.80^{b}	2.65^{a}	2.63^{a}	0.074	<0.001	<0.001	<0.001
	Leg	1.55^{c}	2.05^{b}	2.23^{a}	0.048	<0.001	<0.001	0.017
Drip loss (%)	Breast	0.71^{b}	0.95^{a}	0.91^{a}	0.038	0.013	0.022	0.055
	Leg	0.60	0.65	0.67	0.015	0.148	0.060	0.606
Cooking loss (%)	Breast	23.67^{a}	21.53^{b}	22.33^{ab}	0.315	0.013	0.050	0.020
	Leg	24.61^{a}	23.88^{b}	24.76^{a}	0.119	0.002	0.515	<0.001
Shear force (N)	Breast	15.31^{ab}	14.38^{b}	15.83^{a}	0.244	0.047	0.374	0.019
	Leg	17.54^{a}	15.64^{b}	18.51^{a}	0.396	0.009	0.287	0.004
Color $L^*_{45\ min}$	Breast	48.51^{a}	48.76^{a}	47.60^{b}	0.124	<0.001	0.001	0.003
	Leg	49.76	50.03	49.22	0.758	0.914	0.784	0.751
Color $a^*_{45\ min}$	Breast	9.37^{a}	9.29^{a}	8.36^{b}	0.116	<0.001	<0.001	0.050
	Leg	9.01	9.59	9.72	0.467	0.828	0.564	0.829
Color $b^*_{45\ min}$	Breast	23.53^{a}	23.20^{a}	18.34^{b}	0.481	<0.001	<0.001	0.006
	Leg	23.25	23.03	23.12	1.002	0.997	0.962	0.947
Color $L^*_{24\ h}$	Breast	43.52^{c}	48.85^{a}	46.21^{b}	0.696	0.002	0.034	0.002
	Leg	42.51^{b}	47.14^{a}	44.08^{ab}	0.736	0.024	0.322	0.010
Color $a^*_{24\ h}$	Breast	5.65^{a}	5.75^{a}	4.71^{b}	0.195	0.043	0.045	0.134
	Leg	5.39	4.49	4.77	0.191	0.147	0.184	0.140
Color $b^*_{24\ h}$	Breast	7.01^{b}	8.08^{a}	6.71^{b}	0.128	<0.001	0.237	<0.001
	Leg	5.38	5.13	5.40	0.236	0.885	0.968	0.627

Means with different superscript letters within the same row are significantly different (P<0.05). $L^*_{45\ min}$, muscle lightness value at 45 min postmortem; $L^*_{24\ h}$, muscle lightness value at 24 h postmortem; $a^*_{45\ min}$, muscle redness value at 45 min postmortem; $a^*_{24\ h}$, muscle redness value at 24 h postmortem; $b^*_{45\ min}$, muscle yellowness value at 45 min postmortem; $b^*_{24\ h}$, muscle yellowness value at 24 h postmortem. [1]Broilers were fed a basal diet supplemented with 0, 1, and 2 g/kg betaine. [2]Standard error of the mean.

Table 5. Effect of dietary betaine supplementation on the proximate composition (%, fresh basis) of breast and leg muscles of broiler chickens.

Treatment[1] Parameters	Muscle Type	Betaine levels (g/kg) 0	1	2	SEM[2]	P value Main	Linear	Quadratic
Dry Matter	Breast	24.02	23.82	23.88	0.055	0.309	0.291	0.264
	Leg	25.24	25.17	25.07	0.071	0.624	0.339	0.926
Crude Ash	Breast	1.15	1.10	1.13	0.030	0.809	0.805	0.551
	Leg	0.96	1.01	1.06	0.029	0.372	0.165	0.940
Crude Fat	Breast	0.80^{b}	1.44^{a}	1.11^{ab}	0.093	0.013	0.126	0.009
	Leg	1.00^{c}	2.43^{a}	1.70^{b}	0.146	<0.001	0.002	0.001
Crude Protein	Breast	22.96	22.61	22.99	0.170	0.188	0.946	0.071
	Leg	20.67	20.33	20.64	0.164	0.666	0.945	0.375

Means with different superscript letters within the same row are significantly different (P<0.05).
[1]Broilers were fed a basal diet supplemented with 0, 1, and 2 g/kg betaine.
[2]Standard error of the mean.

3.5. Fatty Acid Composition

Dietary betaine inclusion significantly altered several individual fatty acids (P<0.05), showing both linear and quadratic response patterns depending on the specific fatty acid (Table 6). Among the SFA in breast muscles, the level of myristic acid (C14:0) decreased in birds treated with 2 g/kg betaine compared with that in other groups (P<0.05). The palmitic acid (C16:0) content was lower in the 1 g/kg betaine group than in the control and 2 g/kg betaine groups (P<0.05). Stearic acid (C18:0) showed a similar pattern, with a reduced content in the 1 g/kg group relative to the control and 2 g/kg betaine groups (P<0.05). The behenic acid content (C22:0) also decreased with betaine inclusion (P<0.05), showing linear and quadratic responses. The total SFA was significantly reduced by betaine supplementation (40.84 vs. 37.59 and 41.18% for control vs. betaine groups; P<0.05), with this reduction showing a quadratic pattern (P_{quad} < 0.05). For MUFA in breast muscle, the levels of palmitoleic acid (C16:1) and vaccenic acid (C18:1n-7) increased in a dose-dependent manner, with the highest values observed in the 1 g/kg betaine group (P<0.05). However, the oleic acid (C18:1n-9) content was slightly reduced in the 2 g/kg group compared to the control and 1 g/kg group (P<0.05). The gadoleic acid (C20:1) content increased with dietary betaine supplementation (P<0.05). Dietary inclusion of 1 g/kg betaine significantly increased total MUFA levels (57.06%) compared with the control (53.98%) and 2 g/kg betaine groups (53.85%) (P<0.05). Regarding PUFA in breast muscles, linolenic acid (C18:3n-3) content significantly increased in the 2 g/kg group compared with the other treatments (P<0.05). Betaine supplementation significantly increased total PUFA (4.02 *vs.* 4.85% for control *vs.* 2 g/kg betaine group; P<0.05). The highest n-3 fatty acid content was observed in the 2 g/kg betaine-supplemented group (P<0.05). Moreover, the n-6/n-3 ratio was markedly improved by betaine supplementation, particularly in the 2 g/kg group (2.91), compared to the control (5.00) and 1 g/kg group (5.10) (P<0.05).

Table 6. Effect of dietary betaine supplementation on breast muscle fatty acid composition (g/100 g fatty acids) in broiler chickens.

Treatment[1]	Betaine levels (g/kg)				P value		
Paramaters	0	1	2	SEM[2]	Main	Linear	Quadratic
C14:0 Myristic acid	0.83^{a}	0.79^{a}	0.34^{b}	0.076	0.005	0.003	0.111
C15:0 Pentadecanoic acid	0.16	0.10	0.12	0.012	0.147	0.196	0.131
C16:0 Palmitic acid	26.99^{ab}	26.14^{b}	27.94^{a}	0.253	0.006	0.061	0.005
C16:1 Palmitoleic acid	5.18^{c}	6.72^{a}	6.00^{b}	0.194	0.001	0.019	0.001
C17:0 Heptadecanoic acid	0.27	0.22	0.21	0.021	0.493	0.269	0.691
C18:0 Stearic acid	12.24^{a}	10.14^{b}	12.34^{a}	0.280	<0.001	0.785	<0.001
C18:1n-7 Vaccenic acid	0.10^{b}	0.43^{a}	0.36^{a}	0.047	0.003	0.007	0.012
C18:1n-9 Oleic acid	48.45^{a}	49.05^{a}	46.54^{b}	0.315	<0.001	0.001	0.002
C18:2n-6 Linoleic acid	0.01	0.02	0.02	0.005	0.252	0.110	0.930
C18:3n-3 Linolenic acid	0.67^{b}	0.75^{b}	1.24^{a}	0.086	0.005	0.002	0.151
C20:0 Arachidic acid	0.25	0.18	0.22	0.015	0.175	0.411	0.094
C20:1 Gadoleic acid	0.25^{b}	0.86^{a}	0.95^{a}	0.089	<0.001	<0.001	0.028
C20:2n-6 Eicosadienoic acid	0.78	1.04	1.11	0.076	0.177	0.081	0.543
C20:3n-6 Dihomo-γ-linolenic acid (DGLA)	0.81	0.78	0.95	0.068	0.584	0.428	0.512
C20:4n-6 Arachidonic acid	1.75	1.98	1.52	0.109	0.238	0.388	0.144
C22:0 Behenic acid	0.10^{a}	0.02^{b}	0.01^{b}	0.011	<0.001	<0.001	0.005
Σ SFA	40.84^{a}	37.59^{b}	41.18^{a}	0.454	<0.001	0.577	<0.001
Σ MUFA	53.98^{b}	57.06^{a}	53.85^{b}	0.391	<0.001	0.747	<0.001
Σ PUFA	4.02^{b}	4.57^{ab}	4.85^{a}	0.134	0.024	0.008	0.570
Σ PUFA / Σ SFA	0.10	0.12	0.12	0.010	0.639	0.418	0.638
Σ n-3	0.67^{b}	0.75^{b}	1.24^{a}	0.086	0.005	0.002	0.151
Σ n-6	3.35	3.82	3.61	0.116	0.268	0.364	0.178
Σ n-6 / Σ n-3	5.00^{a}	5.10^{a}	2.91^{b}	0.270	0.001	0.003	0.010

Means with different superscript letters within the same row are significantly different (P<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6; n-3, omega-3 fatty acids. [1]Broilers were fed a basal diet supplemented with 0, 1, and 2 g/kg betaine. [2]Standard error of the mean.

The fatty acid profile of the leg muscles of broilers showed significant modifications in response to dietary betaine supplementation (Table 7). Among the SFA in the leg muscles, the pentadecanoic acid (C15:0) content increased linearly with increasing dietary betaine levels (P<0.05). Palmitic acid (C16:0) levels were reduced in birds fed 1 g/kg and 2 g/kg betaine, with the lowest value observed in the 1 g/kg group (26.18%) compared with the control (28.57%; P<0.05). Similarly, the levels of heptadecanoic acid (C17:0) and stearic acid (C18:0) were lower in the betaine-supplemented groups than in the control group (P<0.05). The total SFA content also decreased in the 1 g/kg betaine group (38.57%) compared with that in the control group (42.93%; P<0.05). For MUFA in leg muscles, the highest level of palmitoleic acid (C16:1) was observed in the control group compared with that in the betaine-supplemented groups (P<0.05). The oleic acid (C18:1n-9) content was increased by 1 g/kg betaine inclusion (48.55%) relative to that of the control (43.05%) and 2 g/kg betaine groups (43.37%) (P<0.05). The gadoleic acid (C20:1) content was significantly lower in the 2 g/kg group (0.25%) than in the control (0.90%) and 1 g/kg betaine groups (0.77%) (P<0.05). This trend was reflected in the total MUFA content, which was highest in the 1 g/kg group (57.10%) (P<0.05). In contrast, total PUFA, n-3, and n-6 fatty acid levels remained unaffected by the dietary treatment (P>0.05). However, the n-6/n-3 ratio was significantly higher in the 1 g/kg group (6.17) than in the 2 g/kg group (5.49) and the control (5.74) (P<0.05).

Table 7. Effect of dietary betaine supplementation on leg muscle fatty acid composition (g/100 g fatty acids) in broiler chickens

Treatment[1]	Betaine levels (g/kg)				P value		
Paramaters	0	1	2	SEM[2]	Main	Linear	Quadratic
C14:0 Myristic acid	1.14	0.91	1.10	0.074	0.428	0.830	0.206
C15:0 Pentadecanoic acid	0.02^{c}	0.08^{b}	0.14^{a}	0.015	<0.001	<0.001	0.930
C16:0 Palmitic acid	28.57^{a}	26.18^{c}	26.93^{b}	0.195	<0.001	<0.001	<0.001
C16:1 Palmitoleic acid	9.23^{a}	7.70^{b}	7.74^{b}	0.216	0.013	0.010	0.095
C17:0 Heptadecanoic acid	0.28^{a}	0.15^{b}	0.15^{b}	0.023	0.017	0.012	0.128
C18:0 Stearic acid	12.64^{a}	10.88^{c}	11.37^{b}	0.136	0.001	0.015	0.002
C18:1n-7 Vaccenic acid	0.17^{a}	0.08^{b}	0.16^{a}	0.016	0.036	0.809	0.012
C18:1n-9 Oleic acid	43.05^{b}	48.55^{a}	43.37^{b}	0.686	<0.001	0.699	<0.001
C18:2n-6 Linoleic acid	0.66	0.64	0.39	0.084	0.405	0.227	0.532
C18:3n-3 Linolenic acid	0.66	0.63	0.67	0.093	0.987	0.995	0.872
C20:0 Arachidic acid	0.19	0.18	0.17	0.013	0.837	0.557	0.996
C20:1 Gadoleic acid	0.90^{a}	0.77^{a}	0.25^{b}	0.083	<0.001	<0.001	0.094
C20:2n-6 Eicosadienoic acid	0.95	0.62	0.59	0.079	0.109	0.059	0.340
C20:3n-6 Dihomo-γ-linolenic acid (DGLA)	0.96	1.14	1.26	0.082	0.349	0.156	0.868
C20:4n-6 Arachidonic acid	1.21	1.50	1.37	0.071	0.248	0.356	0.163
C22:0 Behenic acid	0.14	0.19	0.22	0.017	0.145	0.055	0.769
Σ SFA	42.93^{a}	38.57^{b}	40.08^{b}	0.535	<0.001	0.002	0.001
Σ MUFA	53.35^{b}	57.10^{a}	52.52^{b}	0.551	<0.001	0.251	<0.001
Σ PUFA	4.45	4.53	4.22	0.117	0.275	0.246	0.261
Σ PUFA / Σ SFA	0.10	0.13	0.11	0.013	0.654	0.764	0.391
Σ n-3	0.66	0.63	0.67	0.053	0.660	0.387	0.808
Σ n-6	3.79	3.89	3.68	0.072	0.528	0.545	0.345
Σ n-6 / Σ n-3	5.74^{b}	6.17^{a}	5.49^{b}	0.098	0.008	0.193	0.004

Means with different superscript letters within the same row are significantly different (P<0.05). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6; n-3, omega-3 fatty acids. [1]Broilers were fed a basal diet supplemented with 0, 1, and 2 g/kg betaine. [2]Standard error of the mean.

4. Discussion

The inclusion of betaine in broiler diets significantly enhanced growth performance, with consistent increases in BW and ADG and improved FCR throughout all phases of the study. Birds supplemented with 1 and 2 g/kg betaine exhibited approximately 5% improvement in final BW and ADG compared to the control group. Furthermore, betaine supplementation increased ADFI by 2–3% and improved the overall FCR by approximately 3%, demonstrating enhanced nutrient utilization efficiency. These results are consistent with those reported by Chen et al. (2018), who observed significant improvements in BW and ADG at comparable inclusion levels following betaine supplementation. Similarly, Chen et al. (2022) demonstrated enhanced growth performance, notably final BW and FCR, with both anhydrous betaine and betaine hydrochloride. The beneficial effects of supplementary betaine on broiler growth performance have also been widely documented (Shakeri et al., 2019; Nutautaitė et al., 2020; Song et al., 2021; Eldamrawy et al., 2023), confirming the consistent positive impact of betaine on poultry performance under various production conditions. Suliman et al. (2023) demonstrated that betaine supplementation improved growth performance under heat stress conditions, further supporting its efficacy in diverse rearing environments. In line with our results, Yang et al. (2022) reported that dietary betaine supplementation specifically improved the FCR of slow-growing broiler chickens. Ratriyanto et al. (2009) reported that betaine enhances feed intake, FCR, and overall growth performance by regulating cellular osmoregulation and protein metabolism. These results are also consistent with those of Shakeri et al. (2020), who demonstrated that betaine reduces the negative effects on performance by maintaining cellular osmotic balance and supporting protein synthesis pathways. Furthermore, the fundamental role of betaine as an osmolyte and methyl donor may help explain the observed improvement in BW gain, specifically by upregulating the expression of myogenic regulatory factors (MRFs) and promoting protein turnover (Lever & Slow, 2010; Chen et al., 2019). Therefore, the combined metabolic and cellular mechanisms of betaine, which ultimately optimize nutrient utilization efficiency and enhance growth performance, can likely be attributed to the observed improvements in BW and FCR in the present study.

Carcass characteristics and organ development are important indicators of production efficiency and overall health in broiler chickens (Özdoğan et al., 2023). Dietary betaine supplementation significantly improved carcass yield by approximately 2% compared with the control group. These results are consistent with those of Suliman et al. (2023), who reported that under heat stress conditions, betaine supplementation enhanced carcass yield in broiler chickens. Similarly, Arif et al. (2022) demonstrated that betaine supplementation improved quail carcass yield. However, the results of the current study do not support most previous research. Several previous studies have reported no significant effect of dietary betaine supplementation on carcass yield (Chen et al., 2019, 2022; Nutautaitė et al., 2020; Al-Sagan et al., 2021; Eldamrawy et al., 2023). Moreover, this study demonstrated that betaine supplementation significantly increased the leg meat yield by approximately 4–6% compared to that in the control group. However, Al-Sagan et al. (2021) and Chen et al. (2022) reported no significant changes in leg meat yield following dietary betaine supplementation. Conversely, Suliman et al. (2023) determined that betaine supplementation decreased leg meat yield in broiler chickens under heat stress. Furthermore, the current study found that the breast meat yield was unaffected by the dietary treatments. But several studies have shown that betaine supplementation enhances breast muscle yield. Al-Sagan et al. (2021) revealed that 0.15% betaine supplementation significantly improved breast yield. Similarly, Nutautaitė et al. (2020) demonstrated enhanced breast yield after the addition of betaine to the diet. Awad et al. (2014) observed a significant increase in breast muscle yield in betaine-supplemented ducks (0.5 and 1.0 g/kg) under summer conditions. These inconsistent findings may be attributed to variations in betaine inclusion levels, bird genotypes, environmental conditions, dietary compositions, or duration of betaine administration across studies. Interestingly, betaine supplementation resulted in a substantial increase in the relative small intestine weight compared with that in the control group. Arif et al. (2022) reported a similar increase in small intestine weight following dietary betaine supplementation at 1.5 and 2.25 g/kg in broiler quails. The fundamental role of betaine as an osmolyte in supporting intestinal development and function explains this result. As an osmolyte, betaine maintains cellular integrity and promotes cell proliferation in intestinal tissues, thereby enhancing intestinal growth and development. This finding is consistent with the improved nutrient absorption and feed efficiency observed in the growth performance results of the this study.

Meat quality characteristics are crucial factors influencing consumer acceptance and commercial value of broiler meat production. This study found that dietary betaine supplementation significantly influenced several meat quality parameters, which notably varied by muscle type and betaine inclusion level. Betaine supplementation significantly reduced squeezable water loss (SWL) in breast meat from 6.28% (control) to 5.51% at 1 g/kg and 5.81% at 2 g/kg betaine group, indicating improved water-holding capacity (WHC). These results are consistent with those of previous studies that reported similar effects of betaine on meat WHC (Yang et al., 2022; Chen et al., 2020). Conversely, the leg muscle showed increased squeezable water loss (from 2.22% in control to 2.63% at 2 g/kg), demonstrating tissue-specific responses to betaine supplementation. Shakeri et al. (2020) also reported muscle-specific responses, emphasizing the role of fiber type differences and redox balance in postmortem water-holding dynamics. Consistent with changes in water-holding characteristics, our results revealed a dose-dependent reduction in the ultimate pH of both breast and leg muscles with increased betaine supplementation. In the breast muscle, the pH decreased from 5.97 (control) to 5.70 at 2 g/kg betaine, whereas a similar trend was observed in the leg muscles. This observation contrasts with the results of Shakeri et al. (2020), who reported increased muscle pH with betaine supplementation under different conditions. These variations may stem from differences in the timing of pH measurements, muscle fiber type, or betaine interaction with stress responses. The lower ultimate pH in the current study likely contributed to observed increased drip and thawing losses, which are typical of enhanced postmortem glycolysis. Moreover, betaine supplementation resulted in higher thawing and drip losses in breast meat. Thawing loss increased from 1.80% in the control to 2.63% in both betaine groups, while drip loss showed a comparable pattern. However, these results contradict those of Chen et al. (2022) and Shakeri et al. (2019), who demonstrated reduced drip and thawing losses due to improved WHC with betaine. Such inconsistencies could be attributed to variations in environmental conditions, bird genotype, and thermoneutral or stress exposure among studies. Interestingly, cooking loss presented a quadratic response, significantly decreasing in both breast and leg muscles at 1 g/kg betaine. These results are consistent with those of Chen et al. (2022) and Yang et al. (2022), who noted that moderate betaine supplementation enhanced water retention and thermal stability. However, cooking loss values increased again at 2 g/kg, indicating a possible threshold effect, where higher betaine levels may alter the osmotic balance or protein structure integrity. Betaine significantly improved tenderness at 1 g/kg inclusion in both breast and leg muscles, reducing shear force from 15.31 N (breast) and 17.54 N (leg) in the control to 14.38 N and 15.64 N, respectively. These results are consistent with those of Yang et al. (2022) and Shakeri et al. (2019), who suggested that the role of betaine in enhancing myofibrillar fragmentation, reducing muscle fiber toughness, and mitigating oxidative damage could be attributed to improved tenderness. Shakeri et al. (2020) confirmed the robust effect of betaine supplementation on tenderness under various environmental conditions, indicating its broad applicability. The impact of betaine on meat color varied according to muscle type and postmortem time. At 45 min postmortem, breast meat color parameters (L*, a*, and b*) significantly decreased after 2 g/kg betaine supplementation, indicating a darker and less intense color. At 24 h postmortem, breast meat from birds that received 1 g/kg betaine displayed higher L*, a*, and b* values, indicating enhanced appearance and market acceptability. These results are in agreement with previous reports (Nutautaitė et al., 2020; Shakeri et al., 2019; Yang et al., 2022), which similarly linked enhanced meat color to increased antioxidant capacity. However, betaine supplementation did not affect leg muscle color parameters, consistent with previous findings suggesting more stable pigment dynamics in oxidative (red) muscles than in glycolytic (white) muscles (Nutautaitė et al., 2020; Chen et al., 2022). Overall, dietary betaine significantly enhanced meat quality parameters in broiler chickens through its multifaceted roles as an osmoprotectant, antioxidant, and methyl donor. Moderate betaine inclusion (1 g/kg) was optimal for improving tenderness, WHC, and color stability, whereas higher levels may disrupt osmotic balance or protein stability.

In the present study, dietary betaine supplementation significantly increased the crude fat content in both the breast and leg muscles, showing a clear quadratic response with the highest values observed at 1 g/kg, followed by a decline at 2 g/kg. The observed increase in crude fat may reflect enhanced intramuscular fat (IMF) deposition, as the proximate crude fat content generally correlates closely with IMF levels (Alirezaei et al., 2012). Ma et al. (2015) reported that betaine supplementation significantly improved IMF content in the breast and thigh muscles of broilers compared to that in the control group. This enhancement might be attributed to the role of betaine as a methyl donor involved in methionine metabolism, which potentially influences lipogenic gene expression and lipid storage nutrient partitioning (Eklund et al., 2005). However, contrasting results have also been reported by Leng et al. (2016) and Nutautaitė et al. (2020), who observed significant reductions in IMF due to increased fatty acid β-oxidation pathways activated by betaine supplementation. Additionally, Chen et al. (2022) and Alirezaei et al. (2012) reported no significant effects on IMF content. These inconsistencies may result from variations in genetic strains, dietary formulations, thermal environments, and lipid extraction methods, highlighting the complexity of the role of betaine in lipid metabolism.

Dietary betaine supplementation significantly influenced the fatty acid profiles of breast and leg muscles of broiler chickens. However, limited research exists on betaine effects on fatty acid composition in poultry muscle, restricting direct comparisons. In the present study, dietary betaine inclusion significantly altered the levels of individual fatty acids, displaying both linear and quadratic responses depending on the specific fatty acid and muscle type. Betaine supplementation reduced the total SFA content in the breast muscle, primarily due to decreases in palmitic (C16:0) and stearic (C18:0) acids, with the most pronounced effects observed at the 1 g/kg inclusion level. Similarly, betaine supplementation decreased the total SFA content in leg muscles, mainly due to reductions in palmitic and stearic acids. This reduction in SFA content could be beneficial, because a lower SFA content in poultry meat is desirable from a human health perspective. Moreover, betaine inclusion significantly increased the total MUFA content in both muscle types. This increase was driven primarily by increases in palmitoleic (C16:1) and vaccenic acids (C18:1n-7) in the breast muscle, whereas the enhancement was particularly notable for oleic acid (C18:1n-9) in the leg muscle. These results are consistent with those of Ma et al. (2015), who reported increased MUFA levels in the breast muscles of betaine-supplemented broilers compared with those of the controls. In addition, dietary betaine enhanced the total PUFA content in the breast muscle, particularly by elevating linolenic acid (C18:3n-3) concentrations at higher supplementation levels. Most importantly, betaine supplementation resulted in a marked improvement in the Σn-6/Σn-3 ratio in the breast muscle, with the 2 g/kg group showing the most favorable ratio (2.91) compared to the control (5.00). This result aligns with that of Du et al. (2018), who observed increased PUFA content in the muscle tissues of betaine-supplemented mice, and Yang et al. (2022), who reported similar improvements in fatty acid profiles in slow-growing broiler chickens. Conversely, no significant changes in PUFA content were observed in the leg muscles, indicating tissue-specific responses to betaine supplementation. These inconsistent results could be attributed to the inherent differences in lipid metabolism pathways between muscle tissues. The observed shifts toward increased MUFA and PUFA concentrations and decreased SFA levels may be explained by the potential role of betaine in enhancing fatty acid oxidation pathways, possibly via activation of peroxisome proliferator-activated receptor alpha (PPARα), as proposed by Du et al. (2018). Furthermore, the function of betaine as a methyl donor may influence lipid metabolism through methylation-dependent alterations in the expression of genes related to lipid biosynthesis and oxidation (Yang et al., 2022). These findings demonstrate that betaine supplementation can beneficially modify the fatty acid composition, particularly in the breast muscle, resulting in a more favorable fatty acid profile.

5.Conclusion

Dietary betaine, particularly at 1 g/kg, was identified as the optimal level, resulting in significant improvements in final body weight, average daily gain, feed conversion ratio, carcass yield, and key meat quality parameters, including water-holding capacity and tenderness. Moreover, dietary betaine supplementation beneficially modified muscle fatty acid composition by reducing saturated fatty acids and increasing monounsaturated fatty acids and polyunsaturated fatty acids, especially in the breast muscle, thus enhancing the nutritional quality of broiler meat. Consequently, betaine may be considered a functional feed additive to improve broiler performance and meat quality. However, given the limited number of existing studies on the effects of betaine on fatty acid metabolism in poultry, further research is required to clarify the precise mechanisms underlying these beneficial effects.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

The experimental protocols implemented in this study were fully compliant with the European directive on animal welfare and ethical use of animals in scientific research (Directive 2010/63/EU) and were approved by the Ethics Committee of Dicle University, Diyarbakır, Türkiye (approval date: March 26, 2025, protocol code: 2025/06).

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THE EFFECT OF DIFFERENT IRRIGATION LEVELS ON THE YIELD AND SOME QUALITY TRAITS OF MAIZE CULTIVARS

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Abstract: This study was conducted in 2023 under the conditions of Eskisehir to determine the effects of different irrigation levels on the yield and quality traits of maize cultivars. Three maize cultivars (P0937, P0900, DKC5812) were subjected to five different irrigation levels (171, 342, 513, 684, and 855 mm). The experiment was designed as a split-plot arrangement with maize varieties as the main plots and irrigation levels as the subplots, replicated three times. According to the results obtained from the experiment, statistically significant differences were found among the cultivars in terms of plant height, first ear height, ear length, thousand-kernel weight, test weight, protein content, and starch content. Additionally, statistically significant differences were observed among the irrigation levels in terms of plant height, first ear height, ear length, ear diameter, thousand-kernel weight, test weight, fat content, protein content, and starch content. Concerning irrigation levels, plant height ranged from 206.0 cm (171 mm) to 282.3 cm (855 mm), first ear height ranged from 97.4 (171 mm) to 128.3 cm (513 mm), ear diameter ranged from 44.8 (171 mm) to 50.6 mm (855 mm), grain yield ranged from 800.7 (171 mm) to 1606.0 kg da^{-1} (855 mm), thousand-kernel weight varied from 232.2 (171 mm) to 316.3 g (855 mm), test weight ranged from 76.0 (171 mm) to 78.3 kg (684 mm), ash content varied between 1.30 (855 mm) and 1.35% (171 and 342 mm), fat content ranged from 3.54 (855 mm) to 3.80% (171 mm), protein content varied from 8.03 (855 mm) to 9.72% (171 mm), and starch content ranged from 73.42 (171 mm) to 74.61% (855 mm). Increasing irrigation levels generally had positive effects on yield components such as plant growth, ear development, and kernel yield. However, negative effects of irrigation were also observed in quality components such as protein and fat content. This highlights the necessity of considering not only yield improvement but also product quality when determining irrigation strategies. Therefore, when formulating irrigation strategies, a balance between yield and quality must be achieved, and the water response of each maize cultivar should be considered.

Keywords: Corn, Grain, Yield, Quality, Irrigation, Variety

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1. Introduction

Maize (*Zea mays* L.) is an important crop used for food, animal feed, and as a raw material in various industrial sectors. It is the leading cereal crop worldwide in terms of both total and per-unit-area yield. With a cultivation area of 205.8 million hectares, maize ranks second globally in terms of area planted, yet it holds first place in production volume with 1.2 billion tons (FAO, 2022). In Türkiye, maize ranks third among cereals after wheat and barley in terms of cultivated area. It is utilized in a wide range of applications, including food, feed, industrial products, and bioenergy production. Additionally, it plays a significant role in decorative uses, paper manufacturing, matting, nut and fat industries, sweetener production, and bioenergy generation (Ozturk et al., 2019).

To achieve high grain yields in maize, proper management of irrigation, fertilization, pest and disease control, and harvesting is crucial. While potential yield is determined by the genetic makeup of the cultivar, actual yield is shaped by the interaction of agronomic practices and environmental conditions (Fischer et al., 2014). The effects of irrigation and water stress during different developmental stages on maize growth and grain yield have been extensively evaluated in various studies (Cakır, 2004). Short-term water deficits during the vegetative stages can lead to reductions of 28–32% in final biomass. However, water shortages during critical growth stages such as tasseling and grain filling may result in yield losses of up to 40% (NeSmith and Ritchie, 1992; Cakır, 2004). Water availability, whether through irrigation or rainfall, significantly affects maize yield and profitability.

Irrigation is one of the most effective methods for increasing crop yields and improving quality in agricultural production. In water-sensitive crops such as maize, determining optimal irrigation strategies not only boosts yield but also enhances quality parameters. However, both insufficient and excessive irrigation can adversely affect plant performance, resulting in losses in yield and quality. Therefore, considering the scarcity and cost of water resources, it is essential to use water efficiently (Fereres and Soriano, 2007). In situations where water resources are limited, applying deficit irrigation strategies instead of fully meeting crop water requirements during the growing season can help save water without significantly compromising yield. Although a slight reduction in yield per unit area may occur under such conditions, it becomes possible to irrigate larger areas with the available water, leading to an overall increase in total production.

Alongside other agronomic practices, achieving high yield and quality in maize production requires the efficient implementation of irrigation, including the use of drip irrigation systems and accurate scheduling of irrigation timing and amounts. Farmers generally base their irrigation practices on phenological observations rather than technical criteria, which often leads to over-irrigation due to the absence of water requirement-based management (Ucan, 2000). Uncontrolled irrigation practices may cause soil salinization and result in portions of land becoming unproductive each year due to excessive water use. Therefore, efficient use of soil and water resources and the evaluation of irrigation system performance are of great importance (Cakmak, 2002).

This study was conducted to determine the effects of different irrigation levels on the yield and quality characteristics of maize cultivars under the ecological conditions of Eskisehir.

2. Materials and Methods

This research was conducted in 2023 in the Alpu district of Eskişehir. In the study, hybrid maize cultivars P0937 (FAO 550) and P0900 (FAO 550) from the PIONEER company, and DKC5812 (FAO 550) from the DEKALB company were used. The experiment was established in a split-plot design with three replications. Cultivars were assigned to the main plots and irrigation levels to the sub-plots. Plots, each 6 meters in length, were sown with six rows at 70 × 16 cm inter-row and intra-row spacing. In the experiment, irrigation was applied ten times at 7-day intervals with five different irrigation durations: I6 – 6 hours (171 mm), I12 – 12 hours (342 mm), I18 – 18 hours (513 mm), I24 – 24 hours (684 mm), and I30 – 30 hours (855 mm). At sowing, 25 kg da^{-1} of di-ammonium phosphate (DAP) was applied as base fertilizer. For top-dressing, a total of 30 kg da^{-1} of urea fertilizer was divided into three parts: the first application was made at the 4–6 leaf stage, the second at the 8–10 leaf stage, and the final one at the beginning of flowering. After sowing, emergence irrigation was applied to all plots using a sprinkler irrigation method. Following plant emergence and the first hoeing, a drip irrigation system was installed, and irrigation was carried out every 7 days according to the treatments. Weed control was conducted through pre-emergence herbicide application and hoeing. During harvest, to eliminate border effects, the first rows and 50 cm sections from the beginning and end of the plots were excluded. Harvest was done manually, and the ears were shelled.

Measurements in the study were made on 15 randomly selected plants and ears from each plot. Grain yield, measurement, and analyses were performed based on a grain moisture content of 12%. Subsequently, physical and chemical analyses were conducted. Seeds were ground using a grinder with a 0.5 mm sieve and stored at +4 °C until analysis. In the study, 11 different traits were determined: plant height, first ear height, ear length, ear diameter, grain yield, thousand-kernel weight, hectoliter weight, ash content, fat content, protein content, and starch content. Morphological characteristics were evaluated according to the technical guidelines for maize value determination trials (Anonymous, 2018). Among the chemical properties, ash content was determined by AACC 08-01.01, hectoliter weight by AACC 55-10.01, protein content by AACC 46-11.02, starch content by AACC 76-13.01, and fat content by AOAC 920.39 (AOAC, 2012; AACC, 2020).

The obtained data were analyzed using the MSTAT-C statistical package program based on the split-plot experimental design, and the differences between the means were determined by Duncan's multiple comparison test.

2.1. Climatic and Soil Characteristics of the Experimental Site

During the maize growing season in Alpu district, where the study was conducted, the total precipitation, average temperature, and relative humidity were measured as 323.7 mm, 6.8 ºC, and 66.1%, respectively (Table 1).

Table 1. Climatic data of the experimental site for the year 2023

Months	Rainfall (mm)	Temperature (^{0}C)	Relative humidity (%)
March	90.2	7.1	78.3
April	58.8	10.1	71.1
May	68.1	14.4	74.5
June	77.2	19.3	69.8
July	21.1	22.6	57.0
August	0.0	25.2	53.4
September	8.3	19.3	58.9
Total	323.7	-	-
Average	-	16.8	66.1

According to the soil analysis results of the experimental field, the texture ratio of the soil is 86.9%, indicating that the soil is classified as "clayey." The lime ($CaCO_3$) content is 7.5%, which places it at a moderate lime level. The total salt concentration is measured at 0.0061 mS/cm, indicating no salinity issue in the soil. The soil pH value is 7.93, showing a slightly alkaline reaction. The phosphorus (P) content is very low at 1.71 ppm. The potassium (K) content is 266.4 ppm, which is considered adequate. The organic matter content is 1.87%, indicating a low level of organic matter in the soil.

3. Results

In this study, conducted in the 2023 growing season under Eskisehir conditions, the effects of different irrigation levels on the yield and quality of certain maize varieties were determined. The data for the examined traits are presented in Tables 2 and 3. According to the results obtained from the experiment, significant statistical differences were found between the varieties for plant height, first ear height, ear length, thousand kernel weight, hectoliter weight, protein content, and starch content. Moreover, significant statistical differences were observed between the irrigation levels for plant height, first ear height, ear length, ear diameter, thousand kernel weight, hectoliter weight, fat content, protein content, and starch content. Additionally, the interaction between cultivar and irrigation level (C×I) had a statistically significant effect on plant height, first ear height, ear length, thousand kernel weight, ash content, fat content, protein content, and starch content (Tables 2 and 3).

Table 2. Mean values and statistical significance groups of the examined traits for cultivars and irrigation levels

	PH	FEH	EL	ED	GY	TKW	HW	AC	FC	PC	SC
Cultivar (C)	*	**	**	ns	ns	**	*	ns	ns	**	**
DKC5812	268.1a	127.1A	17.7B	48.4	1285.0	290.6A	76.5B	1.36	3.70	9.21A	72.73B
P0937	263.0b	118.1B	19.1A	48.4	1277.5	279.9B	76.9B	1.32	3.67	8.69B	74.38A
P0900	262.3b	117.2B	18.9A	47.3	1272.4	266.5C	78.2A	1.31	3.73	8.60B	74.54A
Irrigation (I)	**	**	**	**	**	**	**	ns	*	**	**
I_6	206.0C	97.4B	16.9E	44.8B	800.7D	232.2C	76.0B	1.35	3.80A	9.72A	73.42C
I_{12}	272.3B	125.3B	17.6D	45.6B	977.4C	242.0C	76.6AB	1.35	3.67AB	9.39B	73.57C
I_{18}	281.0A	126.1B	18.4C	49.7A	1464.5B	299.8B	78.1A	1.31	3.75AB	8.30D	73.70C
I_{24}	280.7A	126.8B	19.0B	49.6A	1542.9AB	304.9AB	78.3A	1.33	3.70AB	8.70C	74.11B
I_{30}	282.3A	128.3A	20.9A	50.6A	1606.0A	316.3A	77.1AB	1.30	3.54B	8.03D	74.61A

*: significant at the P<0.05 probability level, **: significant at the P<0.01 probability level, ns: non-significant, PH= plant height (cm), FEH= first ear height (cm), EL= ear length (cm), ED= ear diameter (mm), GY= grain yield (kg da^{-1}), TKW= thousand-kernel weight (g), HW= hectoliter weight (kg), AC= ash content (%), FC= fat content (%), PC= protein content (%), SC= starch content (%).

Table 3. Mean values and significance groups of the examined traits for the cultivar × irrigation level interactions

Cultivar	Irrigation	PH	FEH	EL	ED	GY	TKW	HW	AC	FC	PC	SC
		**	**	**	ns	**	**	ns	**	*	**	**
DKC5812	I_6	218.3d	111.7e	15.6j	45.0cd	885.7de	260.2de	75.9	1.37ab	3.94a	10.06a	71.72c
	I_{12}	272.7bc	128.7a-d	16.4ij	46.1cd	1001.7cd	263.2d	76.3	1.43a	3.65ab	10.12a	71.91c
	I_{18}	282.7a	130.0abc	18.4efg	49.5ab	1423.2b	307.5abc	77.4	1.34ab	3.77ab	8.63c-g	72.03c
	I_{24}	284.0a	130.7ab	18.1fg	50.7a	1566.0ab	307.1abc	77.5	1.33ab	3.90a	9.10cde	73.24b
	I_{30}	283.0a	134.7a	19.8bc	50.8a	1548.5ab	315.4ab	75.7	1.32ab	3.28b	8.14fgh	74.76a
P0937	I_6	203.7e	92.3f	18.2efg	45.7cd	819.0ef	218.2f	75.0	1.33ab	3.73ab	9.29bc	74.01ab
	I_{12}	271.7c	125.2bcd	17.7gh	45.7cd	868.0def	230.3f	75.2	1.32ab	3.66ab	9.26bcd	74.25a
	I_{18}	279.0abc	126.3bcd	19.2c-f	49.9ab	1484.4b	307.6abc	78.4	1.35ab	3.76ab	8.39fgh	74.54a
	I_{24}	279.0abc	123.7cd	19.3cde	50.4ab	1510.4b	314.3ab	78.8	1.36ab	3.66ab	8.60d-g	74.54a
	I_{30}	281.7ab	123.0d	20.9ab	50.5a	1706.1a	329.3a	77.4	1.20b	3.58ab	7.89h	74.55a
P0900	I_6	196.0e	88.3f	16.9hi	43.9d	697.5f	218.2f	77.2	1.34ab	3.90a	9.81ab	74.53a
	I_{12}	272.7bc	122.3d	18.6d-g	45.1cd	1062.7c	232.5ef	78.5	1.29ab	3.72ab	8.79c-f	74.54a
	I_{18}	281.3abc	122.0d	17.7gh	49.6ab	1486.0b	284.2cd	78.6	1.25b	3.74ab	7.87h	74.54a
	I_{24}	279.0abc	126.0bcd	19.7cd	47.6bc	1552.3ab	293.2bc	78.8	1.31ab	3.53ab	8.47e-h	74.53a
	I_{30}	282.3ab	127.3bcd	21.9a	50.4ab	1563.3ab	304.3abc	78.2	1.36ab	3.78ab	8.07gh	74.53a

*: significant at the P<0.05 probability level, **: significant at the P<0.01 probability level, ns: non-significant, PH= plant height (cm), FEH= first ear height (cm), EL= ear length (cm), ED= ear diameter (mm), GY= grain yield (kg da^{-1}), TKW= thousand-kernel weight (g), HW= hectoliter weight (kg), AC= ash content (%), FC= fat content (%), PC= protein content (%), SC= starch content (%).

3.1. Plant Height (cm)

The average plant height for the varieties was measured as 262.3 cm for P0900, 263.0 cm for P0937, and 268.1 cm for DKC5812. The highest plant height was observed at the S30 irrigation level (282.3 cm), while the lowest was observed at the S6 irrigation level (206.0 cm) (Table 2). According to the C×I interaction, the lowest plant height (196.0 cm) was observed in the P0900 cultivar with the S6 application, while the highest plant height (284.0 cm) was observed in the DKC5812 cultivar with the S24 application (Table 3). Plant height in maize is an important morphological characteristic that directly affects agricultural performance, such as yield, photosynthesis capacity, resistance to lodging, and ease of harvest. According to Vartanlı and Emeklier (2007), plant height is influenced by both genetic and environmental factors, ranging between 196.0 cm and 284.0 cm. In our study, statistical differences were found between the varieties (Table 2). In a study conducted under the ecological conditions of Konya, where the yield potential of 14 hybrid maize varieties was measured, the plant height ranged from 162.1 cm to 214.9 cm (Ayrancı and Sade, 2004). In our study, it was determined that as the irrigation water increased, plant height also increased (Table 2). In a study by Kuscu (2010) examining the effects of different irrigation levels on maize plant height, it was observed that the highest plant heights were obtained with full irrigation, and plant height decreased as the irrigation amount was reduced. Yang et al. (2024) found that the highest plant height was achieved at the full irrigation level in their study examining the effects of different irrigation and nitrogen applications on maize.

3.2. First Ear Height (cm)

The average first ear height for the varieties was measured as 117.2 cm for P0900, 118.1 cm for P0937, and 268.1 cm for DKC5812. The highest first ear height was observed at the S30 irrigation level (126.1 cm), while the lowest was observed at the S6 irrigation level (97.4 cm) (Table 2). According to the C×I interaction, the lowest first ear height (88.3 cm) was observed in the P0900 cultivar with the S6 irrigation application, while the highest first ear height (134.7 cm) was observed in the DKC5812 cultivar with the S24 application (Table 3). First ear height is one of the factors that determine yield and quality in maize. It plays a critical role in the development of the maize plant and is strongly related to yield. First ear height is influenced by the plant's genetic characteristics, climate conditions, as well as agricultural practices such as irrigation regimes and fertilization (Han et al., 2016). The height of the first ear is an important breeding criterion for harvest suitability and resistance to lodging. If the first ear is positioned higher, it allows the plants to utilize nutrients more efficiently, while a lower first ear height can result in yield loss (Song et al., 2019). In particular, the relationship between ear height and the plant's consumption of water and nutrients indicates that irrigation and fertilization practices should be applied in the most optimal manner. Yang et al. (2024) reported that as irrigation level increases, first ear height also increases (120-150 cm under full irrigation), whereas at lower irrigation levels, ears develop at lower positions (restricted irrigation 90-120 cm).

3.3. Ear Length (cm)

The average ear length of the varieties was measured as 17.7 cm for DKC5812, 18.9 cm for P0900, and 19.1 cm for P0937. The highest average ear length was recorded at the S30 irrigation level (20.9 cm), while the lowest was observed at the S6 irrigation level (16.9 cm) (Table 2). According to the cultivar × irrigation level interaction, the smallest ear length (15.6 cm) was found in the DKC5812 cultivar under the S6 irrigation treatment, while the largest ear length (21.9 cm) was recorded in the P0900 cultivar under the S30 irrigation treatment (Table 3). Ear length is an important morphological characteristic that directly affects the total yield of maize. The size of the ear varies depending on the plant’s photosynthetic capacity, agricultural practices, climatic factors, and genetic traits (Marković et al., 2017). Ear length is considered an indicator of maize's yield potential, and larger ears typically contain more kernels, thereby increasing yield. In our study, it was observed that as the irrigation duration increased, ear length also increased (Table 2). Karasu et al. (2015) reported that increasing irrigation amounts resulted in larger ear lengths.

3.4. Ear Diameter (mm)

The average ear diameter of the varieties was measured as 47.3 mm for P0900, 48.42 mm for DKC5812, and 48.47 mm for P0937. The ear diameter varied between 44.8 mm (S6) and 50.6 mm (S30) according to the irrigation levels. In maize, ear diameter is an important characteristic in terms of yield and quality. This trait is influenced by factors such as the plant’s genetic makeup, environmental conditions, and agricultural practices. Ear diameter is generally directly related to the total grain yield of maize. Arıoglu and Erekul (2022) reported that restricted irrigation practices narrowed ear diameter, leading to significant yield losses, and emphasized that ear diameter, a critical trait for yield, is sensitive to agricultural practices like water management. In our study, the applications with lower water amounts resulted in smaller ear diameters (Table 2). Karasahin and Sade (2011) also reported that different irrigation methods affected ear diameter.

3.5. Grain Yield (kg da^{-1})

The average grain yield of the varieties was determined as 1272.4 kg da^{-1} for P0900, 1277.5 kg da^{-1} for P0937, and 1285.0 kg da^{-1} for DKC5812. The highest grain yield was recorded at the S30 irrigation level (1606.0 kg da^{-1}), while the lowest was observed at the S6 irrigation level (800.7 kg da^{-1}) (Table 2). According to the cultivar × irrigation level interaction, the lowest grain yield (697.5 kg da^{-1}) was obtained in the P0900 cultivar under the S6 irrigation treatment, while the highest yield (1706.1 kg da^{-1}) was recorded in the P0937 cultivar under the S30 irrigation treatment (Table 3). Grain yield is determined by the interaction of various factors such as genetic traits, environmental conditions, and agricultural techniques. In particular, cultural practices such as irrigation, fertilization, planting density, and soil cultivation are critical factors that directly affect maize grain yield (Wanjura et al., 2003). In our study, the highest grain yield was achieved with a 30-hour water application (Table 2). Pinnamaneni et al. (2023) reported that full irrigation conditions increased yield by 15.3% compared to non-irrigated conditions. Ashine et al. (2024) found that as the irrigation level increased, the yield also increased in their study on maize. Previous studies have shown that irrigation practices have significant effects on maize grain yield, with yields ranging between 6.5 and 12.8 t ha^{-1} (Demir et al., 2021; Pinnamaneni et al., 2023; Simić et al., 2023; Ashine et al., 2024).

3.6. Thousand-Kernel Weight (g)

The average thousand-kernel weight was measured as 266.5 g for P0900, 279.95 g for P0937, and 290.6 g for DKC5812. The highest thousand-kernel weight (316.3 g) was obtained with the S30 treatment, and both the S30 and S24 irrigation treatments were grouped in the same statistical category for grain yield (Table 2). The lowest thousand-kernel weight was recorded at the S6 (232.2 g) and S12 (242.0 g) irrigation levels. According to the cultivar × irrigation level interaction, the lowest thousand-kernel weight (218.2 g) was observed in the P0900 and P0937 varieties under the S6 irrigation treatment, while the highest weight (329.3 g) was recorded in the P0937 cultivar under the S30 irrigation treatment (Table 3). Wang et al. (2017) reported that thousand-kernel weight, ear number, and kernel number per ear are important factors determining maize grain yield. Thousand-kernel weight is a trait that reflects the genetic potential of the maize plant and the effects of environmental conditions on plant development. This trait plays a critical role in understanding the effects of irrigation, fertilization, and climatic conditions on kernel development. Thousand-kernel weight has been shown to depend on genotype, environmental factors, and agricultural practices such as planting time and irrigation (Idikut et al., 2020). Previous studies have indicated that irrigation practices increase thousand-kernel weight and subsequently enhance yield in maize (Demir et al., 2021; Gonulal et al., 2021; Ashine et al., 2024).

3.7. Hectoliter Weight (kg)

The average hectoliter weight was measured as 76.5 kg for DKC5812, 76.9 kg for P0937, and 78.2 kg for P0900. The highest hectoliter weight (78.3 kg) was recorded at the S24 irrigation level, while the lowest hectoliter weight (76.04 kg) was observed at the S6 irrigation level (Table 2). Hectoliter weight, one of the quality parameters of cereals such as maize, is of great importance, especially for the food industry, animal feed industry, and biotechnological applications. Hectoliter weight is a key factor affecting the density, kernel quality, processability, transportability, and storability of maize. This trait, directly related to the kernel size and shape, indicates larger and more robust kernels when higher. Hectoliter weight is a factor that enhances the durability and ease of transport of maize. Gonulal et al. (2021) reported that the lowest hectoliter weight was obtained with the least irrigation treatment. In our study, it was observed that as the irrigation level decreased, hectoliter weight also decreased. Additionally, it was determined that hectoliter weight varied across the different varieties (Table 2).

3.8. Ash Content (%)

Although no statistically significant differences were found between varieties and irrigation levels in terms of ash content, the ash content ranged from 1.31% to 1.36% among the varieties, and from 1.31% to 1.35% among the irrigation treatments (Table 2). According to the cultivar × irrigation level interaction, the lowest ash content (1.20%) was found in the P0937 cultivar under the S30 irrigation treatment, while the highest ash content (1.43%) was recorded in the DKC5812 cultivar under the S24 irrigation treatment (Table 3). The ash content of maize is influenced by factors such as cultivation conditions, soil properties, and applied fertilization strategies. Additionally, ash content is an important indicator in quality control during maize processing and is especially used in evaluating product purity and mineral content in the animal feed and food industries. It is reported that the ash content of maize generally ranges from 1.0% to 1.16%, and this value can vary depending on the soil and environmental conditions where the maize is grown (Ali et al., 2010). Irrigation can directly affect the plant’s water and nutrient uptake, influencing mineral accumulation and, therefore, ash content. Water restriction may limit the plant's mineral uptake, reducing ash content. Kale et al. (2018) reported that ash content increased with higher irrigation levels in maize.

3.9. Fat Content (%)

The average fat content was measured as 3.67% for P0937, 3.70% for DKC5812, and 3.73% for P0900. According to the irrigation treatments, fat content ranged from 3.54% (S30) to 3.80% (S6). All irrigation levels, except for S30, were grouped in the same statistical category regarding this trait (Table 2). According to the cultivar × irrigation level interaction, the lowest fat content (3.28%) was found in the DKC5812 cultivar under the S30 irrigation treatment, while the highest fat content (3.94%) was recorded in the DKC5812 cultivar under the S6 irrigation treatment. In terms of fat content, all varieties, except for DKC5812 under the S30 treatment, were statistically in the same group (Table 3). It is known that maize has a relatively high fat content among cereals, second only to oats. Ullah et al. (2010) reported that the fat content in maize varies widely, ranging from 3.21% to 7.71%. The fat content in maize can vary due to several factors, including genotype (Cetin and Soylu, 2021), environmental conditions (Mut et al., 2022), and agricultural practices (Dag et al., 2024). Adequate irrigation can enhance the plant’s ability to absorb nutrients more efficiently, which is thought to increase fat content. However, water stress may inhibit fat synthesis and reduce fat content. Arıoglu and Erekul (2022) reported that irrigation practices have significant effects on fat content. In our study, it was determined that as the irrigation level decreased, fat content increased (Table 2). Ulus and Koca (2023) reported that fat content in maize ranged from 2.82% to 3.59%.

3.10. Protein Content (%)

The average protein content was determined to be 8.6% for P0900, 8.69% for P0937, and 9.21% for DKC5812. The highest protein content (9.72%) was found at the S6 irrigation level, while the lowest (8.03%) was observed at the S30 irrigation level (Table 2). According to the cultivar × irrigation level interaction, the lowest protein content (7.89%) was found in the P0937 cultivar under the S30 irrigation treatment, while the highest (10.12%) was found in the DKC5812 cultivar under the S12 irrigation treatment (Table 3). Protein content is an important quality trait in maize. In a study by Mut et al. (2022), it was reported that the protein content in maize is influenced by both genetic and environmental factors. Arıoglu and Erekul (2022) found that irrigation treatments have significant effects on protein content, with protein levels being higher under limited irrigation compared to full irrigation. On the other hand, Kresović et al. (2018) reported that increased irrigation levels reduced the protein content in maize kernels.

3.11. Starch Content (%)

The average starch content was determined to be 72.73% for DKC5812, 74.38% for P0937, and 74.54% for P0900. The highest starch content (74.61%) was found at the S30 irrigation level, while the lowest (73.42%) was observed at the S6 irrigation level (Table 2). According to the cultivar × irrigation level interaction, the lowest starch content (71.72%) was found in the DKC5812 cultivar under the S6 irrigation treatment, while the highest (74.76%) was found in the DKC5812 cultivar under the S30 irrigation treatment (Table 3). Starch, a primary digestible carbohydrate in plants, is an important energy source in human and animal nutrition. Beckles and Thitisaksakul (2014) reported that factors such as cultivar, rainfall, temperature, soil type, and growth conditions may have a greater effect on starch content in grains than genetic factors. Arıoglu and Erekul (2022) found that starch content in maize under 100% irrigation was higher compared to 60% irrigation. Kaplan et al. (2019) reported that as the amount of water applied increased, the starch content in maize kernels also increased. In our study, it was observed that as the irrigation time increased, the starch content in the kernels also increased (Table 2).

4. Conclusion

This study was conducted in the 2023 growing season under the conditions of Eskisehir to determine the effects of different water levels on the agricultural traits and quality parameters of various maize cultivars. The findings revealed that the applied irrigation levels had significant effects on all examined traits, except for ash content. Increasing irrigation levels generally had positive effects on yield components such as plant growth, ear development, and kernel yield. However, negative effects of irrigation were also observed in quality components such as protein and fat content. This highlights the necessity of considering not only yield improvement but also product quality when determining irrigation strategies. Therefore, when formulating irrigation strategies, a balance between yield and quality must be achieved, and the water response of each maize cultivar should be taken into account. Since different maize varieties respond differently to irrigation, it is recommended to develop irrigation management strategies tailored to each cultivar. Such studies will serve as an important guide to enhance the effectiveness of irrigation management in maize farming and contribute to sustainable agricultural practices.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	Z.M	N.D.	O.D.E.K.
C	40	60	
D	50	50	
S	100		
DCP		60	30
DAI	60	20	20
L	20	80	
W	30	40	30
CR	40	20	40
SR	30	40	30
PM	40	60	
FA	40	60	

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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UNVEILING THE GENETIC WEALTH: CHARACTERIZATION OF WILD HAZELNUT (*Corylus avellana* L.) POPULATIONS IN DÜZCE PROVINCE FROM TÜRKIYE

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Abstract: This study was carried out in Yığılca, Gümüşova, Cumayeri and Akçakoca districts of Düzce province in 2023. In the study, pomological and morphological characteristics of 129 genotypes in natural hazelnut populations and orchards were determined. Genotypes were distributed in the altitude range of 25-760m, 90.5% were detected in orchards and 9.5% in areas such as roadsides, stream beds and mountain slopes. When the genotypes were grouped in terms of plant height, it was determined that 75.39% were taller than 4 m, 22.24% were between 3-4 m, 1.58% were between 2-3 m and 0.79% were between 1-2 m. In the examined genotypes, nut length was measured between 15.0-25.4 mm, nut width was measured between 14.5-28.1 mm, nut size was measured between 14.19-20.61 mm and nut shape index was measured between 0.75-1.84. Leaf length varied between 8.92-15.36 mm, leaf width between 6.85-12.89 mm, and petiole length varied between 0.5-2.68 mm. While 79.13% of the genotypes have a husk longer than the nut, 66.09% have a husk slit. The number of hazelnut husk, one of the basic elements of yield, was determined to be 3 and above in 50% of the genotypes. In the study, it was evaluated that the hazelnut genetic resources in Düzce have a wide variation and that it is important to protect and use them in breeding studies. It was emphasized that especially genotypes with a nut size of 20 mm and above, round-shaped and number of nuts in cluster is over four in new cultivar development.

Keywords: *Corylus avellana*, Biodiversity, Climate change, Wild hazelnut

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1. Introduction

The Corylus genus of the Betulaceae family is in the form of hazelnut bushes or trees, and is among the hard-shelled fruits with the highest economic value. Species in tree form are highly valued as timber. Molnar (2011) reported that there are 10 polymorphic species in the Corylus genus as a result of morphological and molecular studies. The most studied and best known of these species is *Corylus avellana* L. Hazelnut is an endemic plant in the northern hemisphere and is widespread in countries such as Türkiye, Italy, Spain and the USA (Özçağıran et al., 2014). Commercial production in Türkiye is largely concentrated in the Black Sea Region, and important cultivars such as ‘Tombul’ and ‘Palaz’ have been developed as a result of local selection (Balık and Arif, 2023). Türkiye has an important place in terms of plant biodiversity, being located at the intersection of the Mediterranean and Near East Gene Centers (Vavilov, 1994). Anatolia is the origin of hazelnuts (*C. avellana* L. and *C. colurna* L.), as well as many other fruit species (Molnar, 2011). Significant variations are observed among hazelnut cultivars in orchards and natural populations in the Black Sea Region (Balık et al., 2014). Some researchers note that in addition to the variation among Turkish hazelnut cultivars, there is also a rich variation within the cultivar. They even classify Turkish hazelnut cultivars as a collection of clones (İslam, 2008; Balık et al., 2018; Bostan and Karakaya 2024). In order to take advantage of this clonal variation in Türkiye, many clonal selection have been carried out in different cultivars and locations (Demir, 1997; İslam and Özgüven 2001; Turan, 2007; Çayan, 2019; Karakaya, 2021; Uzun, 2021; Bilgen, 2023). The main characteristics taken into consideration in hazelnut breeding are nut and yield characteristics, morphological and phenological characteristics, resistance, and rootstock (İslam, 2019).

Climate change has caused yield and quality problems in hazelnut production, as in other agricultural products, in recent years. Especially the risk of late spring frost, pollination and fertilization problems, and drought negatively affect yield (Beyhan and Odabaş, 1996; Ustaoğlu, 2009; Balık et al., 2021). Determination of genotypes tolerant to biotic and abiotic stresses is of great importance in hazelnut production. Population growth, urbanization and climate change threaten hazelnut genetic resources, making new cultivar development studies necessary (Erat and Balık, 2022). Türkiye, the world's number one hazelnut producer, has 70% of the world's hazelnut production area and 80% of its trade, but lags behind countries such as the USA and China in terms of yield. In Türkiye, hazelnut cultivation is carried out as licensed production in 16 provinces and 123 districts on an area of 760.000 hectares. While Ordu, Samsun and Giresun are at the top in terms of production area and quantity, the highest yield is in Sakarya. Düzce produces approximately 83 thousand tones of hazelnuts, accounting for 11% of the country's hazelnut production; most of the production takes place in the Akçakoca, Merkez and Yığılca districts (Ünver, 2024).

Genes possessed by genetic resources are valuable in the process of increasing yield in agriculture and developing cultivars that are resistant to diseases and pests and have high adaptability. Therefore, genetic resources offer significant advantages, especially against climate change. In order to benefit from the advantages of plant biodiversity against the negativities caused by climate change, strategies for the survey, collection, characterization and evaluation of genetic resources are needed. Genetic resources research in Anatolia, a place where three important gene centers intersect, has been carried out since the 1930s. Research on hazelnut genetic resources was initiated by the Hazelnut Research Institute in 1969. 460 genotypes are preserved in the field gene bank (Anonymous, 2024a). However, no studies on hazelnut genetic resources conducted in Düzce province, which is an important hazelnut production region, have been found in the literature. Biodiversity studies will not only protect valuable hazelnut genetic resources but will also enable the development of new cultivars through the use of genotypes with different characteristics in selection and hybridization.

The aim of this research was to determine and characterize wild hazelnut types found in natural populations and orchards in Yığılca, Gümüşova, Cumayeri and Akçakoca districts of Düzce province.

2. Materials and Methods

The material of the research consists of 129 hazelnut genotypes determined as a result of surveys conducted in the districts of Düzce province in 2023 (Figure 1). 33 of the genotypes were identified in Akçakoca, 35 in Cumayeri, 32 in Gümüşova and 29 in Yığılca district. Genotypes are distributed in the altitude range of 25-760m. 90.5% were detected in orchards and 9.5% in areas such as roadsides, stream beds and mountain slopes.

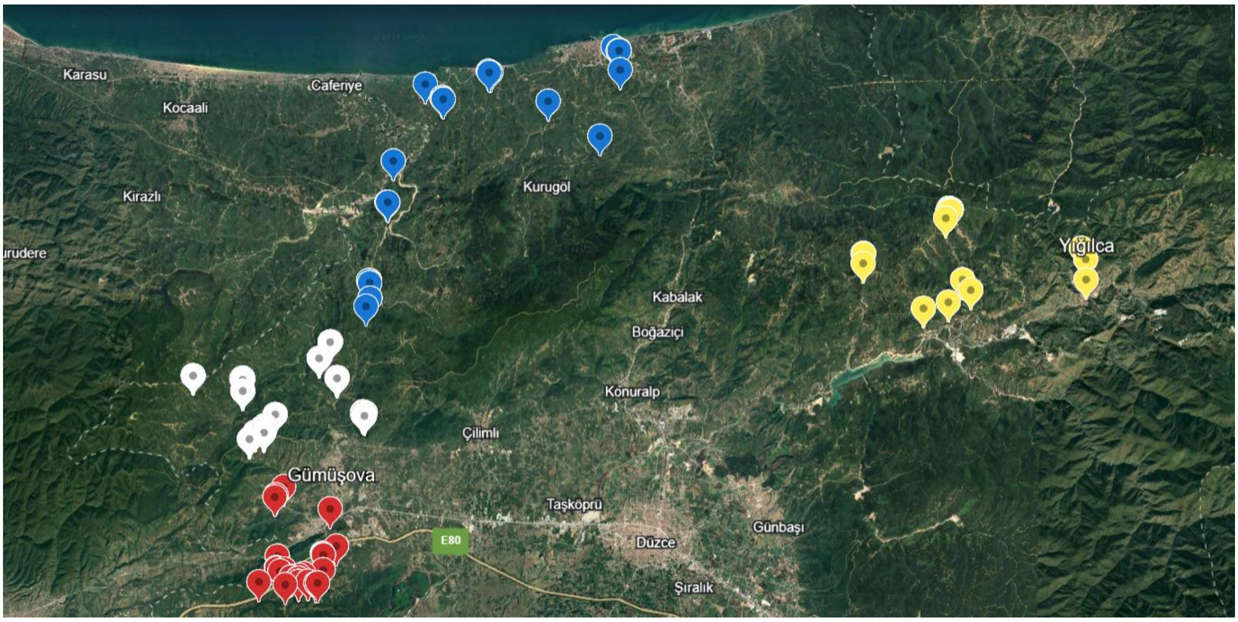


Figure 1. Locations of genotypes (blue: Akçakoca, white: Cumayeri, red: Gümüşova, yellow: Yığılca)

2.1. Surveys

Before the survey, contact was made with Düzce province Akçakoca, Cumayeri, Gümüşova and Yığılca Agriculture and Forestry District Directorates, Chambers of Agriculture, leading farmers and producers, and potential villages where wild hazelnut types could be found were determined and determinations were made by sampling method in areas with different altitudes, directions and locations. The location and altitude of the points where samples were taken were determined and recorded with the GPS location application. Tree, trunk, husk and nut photos were taken to be used in the identification of hazelnut types. Additionally, leaf, nut and shoot samples of the genotypes were taken and characterized (UPOV, 2025).

2.2. Nut characteristics

Nut sizes were determined on 30 randomly selected nuts using a digital caliper sensitive to 0.01 mm (Figure 2). Nut shape is classified from 1 to 6 (Figure 3). The number of nuts in cluster was calculated by counting the fruits in each of 10 randomly selected clusters and the average was taken. Curvature of basal scar is divided into 3 classes as concave, flat and convex according to the criteria specified (Figure 4). Shape of apex was according to the criteria specified, it is divided into 4 classes: narrow acute, broad acute, obtuse, truncate (Figure 5).

2.3. Morphological characters

Plant height is divided into four classes as 1-2m, 2-3m, 3-4m and longer than 4m, measured from the soil level to the tip of the top shoot. The suckers were counted and divided into four classes: 1-3, 3-5, 5-7 and more than 7. Leaf dimensions were measured as in Figure 6. Leaf color is divided into four classes yellow, light green, green and dark green. Leaf shape was divided into four classes (Figure 7).

The size of the involucre is divided into three classes: shorter, equal and longer (Figure 8). Density of hairs are divided into three classes: sparse, medium, dense. Additionally, depth of indentation and serration of indentation of the involucre were evaluated based on observation.

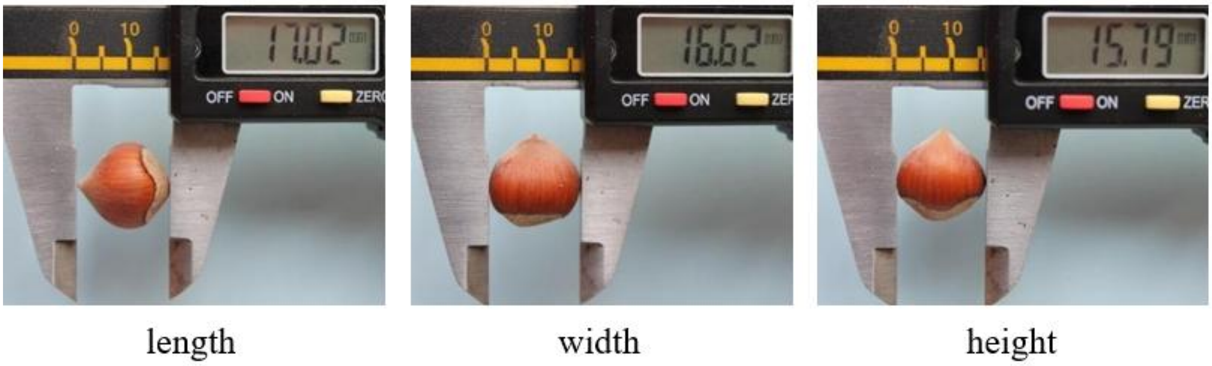


Figure 2. Measuring nut dimensions.

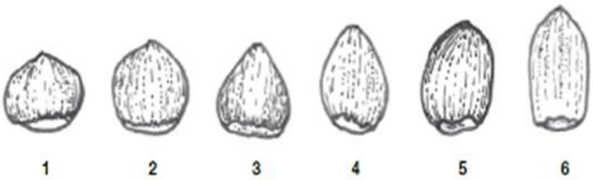


Figure 3. Classification of genotypes according to nut shape (1: globose 2: conical, 3: ovoid, 4: obloid, 5: Short-sub cylindrical, 6: Long-sub cylindrical) (UPOV, 2025).

Figure 4. Curvature of basal scar; concave (1), flat (2) and convex (3) (UPOV, 2025).

Figure 5. Classification of genotypes according to shape of apex, narrow acute (1), broad acute (2), obtuse (3), truncate (4) (UPOV, 2025).

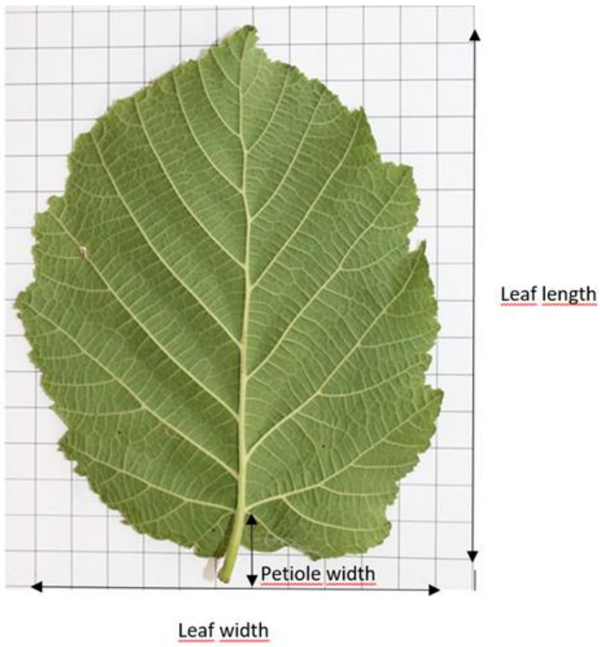


Figure 6. Leaf dimensions (leaf length, leaf width, petiole width).

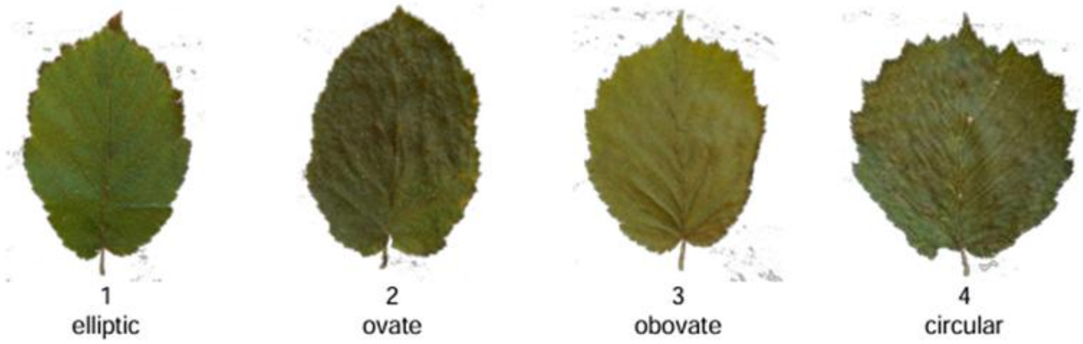


Figure 7. Leaf shape (elliptical, ovate/obovate and circular) (UPOV, 2025).

Figure 8. Involucre categories: shorter, equal and longer.

3. Results

In the research, nut length, nut width, nut height, nut size, nut shape index, nut oblateness index, nut shape, curvature of basal scar, shape of apex and number of nuts in cluster of the genotypes were determined (Table 1).

Table 1. Nut characteristics of genotypes

Characteristics	Max	Min	Average
Nut length (mm)	25.4	15	19.02
Nut width (mm)	28.1	14.5	18.07
Nut height (mm)	27.6	11.8	15.57
Nut size (mm)	20.61	14.19	17.44
Nut shape index	1.84	0.75	1.14
Nut oblateness index	1.39	1.04	1.17

Nut length of the genotypes was determined between 15.0-25.4 mm. While Köksal (2002) determined the nut length as 19.6 mm in Turkish hazelnut cultivars, Ünver (2024) determined the nut length as 13.5-15.94 mm in the 'Mincane' grown in the Akçakoca region. In the same region, nut length of 'Yomra' (Sny. Yomrali) was determined to be between 16-75-20.43 mm by Akça and Söylemezoğlu (2024). Nut width of the examined genotypes was determined between 14.5-28.1 mm. While Köksal (2002) determined the nut width as 17.6 mm in Turkish hazelnut cultivars, Ünver (2024) determined the nut width as 15.26-17.90 mm in the 'Mincane' grown in the Akçakoca region. Nut width in 'Yomra' (Sny. Yomrali) was determined to be between 16.89-20.61 mm by Akça and Söylemezoğlu (2024). The nut heigth of the genotypes was determined to be between 11.8-27.6 mm. While Köksal (2002) determined the nut heigth as 15.5 mm in Turkish hazelnut cultivars, Ünver (2024) determined the nut heigth as 16.34-18.67 mm in the 'Mincane' grown in the Akçakoca region.

Nut size is an important quality parameter in terms of marketability, and genetic and environmental factors play a role in the formation of nut size. Nut size was determined between 14.19-20.61 mm. Nut size was determined as 16.43-17.90 mm in 'Palaz' by Balık and Beyhan (2007); 15.38-18.91 mm in 'Tombul' and 'Palaz' by İşbakan and Bostan (2020); 16.54-21.81 mm in standard hazelnut cultivars by Balık et al. (2016); and 16.34-18.67 mm in 'Mincane' by Ünver (2024).

The nut shape index was determined between 0.75-1.84. While Köksal (2002) determined the nut shape index between 0.8-1.7 in Turkish hazelnut cultivars, Ünver (2024) determined the nut shape index between 0.97-1.16 in the 'Mincane' grown in the Akçakoca region. Nut shape index is especially important for export. It is an important factor affecting the marketing, processing, quality control and storage processes of hazelnuts. By choosing symmetrical and regular shaped hazelnuts, companies can improve product quality and thus achieve a higher market value. It is also extremely important in terms of addressing consumer preferences and ensuring product segmentation (Anonymous, 2024b).

Nut shape was classified according to UPOV; 73% were globose, 16% short-sub cylindrical, 7% long-sub cylindrical, 2% ovoid and 2% conical. Curvature of basal scare was evaluated under 3 classes and 53% was determined as concave, 33% as flat and 14% as convex.

Shape of apex was evaluated under 4 classes and 47% was determined as narrow acute, 34% as broad acute, 14% as obtuse and 5% as truncate. When genotypes were grouped according to the number of nuts in cluster, genotypes with one nut in the cluster were determined as 13%, genotypes with two nuts as 17%, genotypes with three nuts as 42%, genotypes with four nuts as 17%, and genotypes with more than four nuts as 11% (Figure 9).
Çalışkan (1992) determined the number of nuts in cluster of genotypes preserved in the Hazelnut Research Institute field gene bank between 1.42-6.40. While Beyhan and Demir (1998) determined the number of nuts in cluster in the 'Palaz' to be between 1.82-2.15, İslam and Özgüven (2001) determined it to be between 3.25-4.67. Although a higher number of nuts in cluster generally means higher yield, this is an indication of a balance between yield and quality. Balık (2018) noted that the number of nuts in cluster may change due to the effect of pollen, and if there are too many, the shape of the nut may deteriorate. Bak et al. (2024) reported that nut weight and nut length decreased when the number of nuts in cluster was more than four in 'Foşa'.
When the genotypes were grouped in terms of plant height, it was determined that 75.39% were taller than 4m, 22.24% were between 3-4m, 1.58% were 2-3m and 0.79% were between 1-2m (Figure 10). Çalışkan (1992) grouped 676 genotypes preserved in the field gene bank of the Hazelnut Research Institute into 8 classes ranging from very short (< 150cm) to very tall (601cm <) and 4 of the genotypes were categorized as very short (< 150cm), 108 as very short-short (151-250cm), 202 as short (251-300cm), 234 as short-medium (301-350cm), 57 as medium-long (401-450cm), 26 as long (451-500cm), 28 as long-very tall (501-600cm) and 17 as very tall (601cm <). In the same study, the habitus of the genotypes was also evaluated and 60.5% were classified as spreading, 28.7% as semi-erect, 10.5% as upright, 0.24% as drooping and 0.12% as very upright. In recent years, the development of cultivars suitable for mechanization has become more important in developing cultivars with strong growth and upright growing habitus (Anonymous, 2024a).

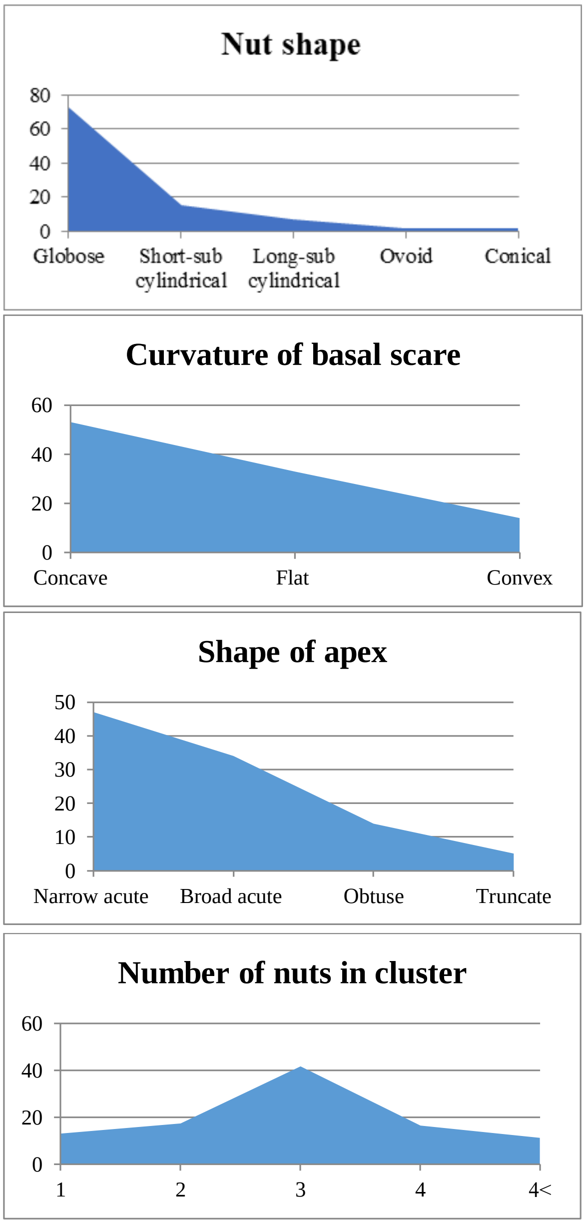


Figure 9. Classification of genotypes according to nut characteristics (%).

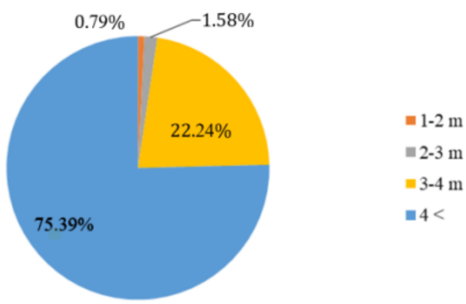


Figure 10. Classification of genotypes according to plant height (%).

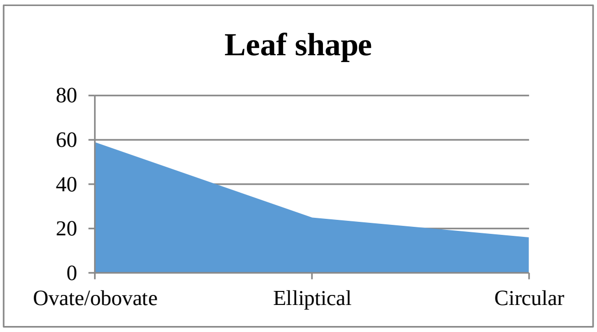
Leaf length, leaf width, petiole length and leaf color of the genotypes were determined (Table 2). Leaf length varied between 8.92-15.36 cm. Çalışkan (1995) determined the leaf size (width+length/2) in Turkish hazelnut cultivars as between 7.9-11.8cm. Balık and Beyhan (2007) determined the leaf length in the 'Palaz' as 10.23-13.21mm. Bostan and Karakaya (2024) determined the leaf length as 11.6cm in the hazelnut types they examined in Gürgentepe district of Ordu province. Leaf width of the genotypes was determined between 6.85-12.89cm. Balık and Beyhan (2007) found the leaf width to be between 9.09-12.19 cm in 'Palaz', while Bostan and Karakaya (2024) found the leaf width to be 9.9 cm on average in the hazelnut types they examined in the Gürgentepe district of Ordu province. The leaf petiole length of the genotypes was determined between 0.5-2.68 cm. Balık and Beyhan (2007) determined the leaf petiole length in 'Palaz' to be between 1.18-2.72 cm.

Table 2. Leaf characteristics of genotypes

Characteristics	Max	Min	Average
Leaf length (cm)	15.36	8.92	11.09
Leaf width (cm)	12.89	6.85	8.86
Petiole length (cm)	2.68	0.5	1.37

In the study, the leaf shape of the genotypes was evaluated under 3 classes according to UPOV and it was determined that 59% of them had ovate/obovate, 25% elliptical and 16% circular leaf shape. It was determined that 54% of the genotypes had dark green, 37% green and 9% light green leaf color. Yellow leaf color was not recorded (Figure 11).

The size of the involucre is divided into three classes: shorter, equal and longer. It was found that 79% of the time it was longer than the nut, 18% of the time it was equal to the nut and 3% of the time it was shorter. In Turkish hazelnut cultivars, the involucre is longer and tightly wraps the nut. This feature of the cultivars makes mechanical harvesting difficult. Therefore, there is a need to develop new cultivars suitable for mechanical harvesting, which are separated from the involucre spontaneously when ripe and where the husk is shorter than the nut and wraps loosely (Anonymous, 2018) (Figure 12). Balık and Beyhan (2019) stated that involucre characteristics may differ depending on the pollinators.



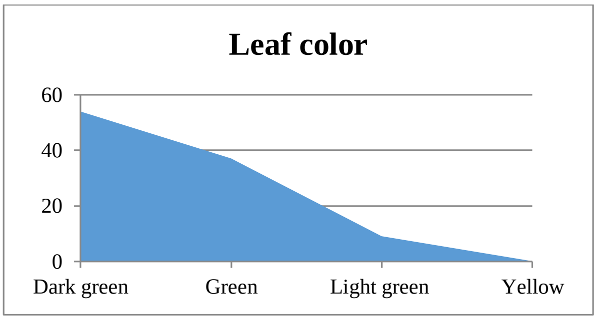


Figure 11. Classification of genotypes according to leaf characteristics (%).

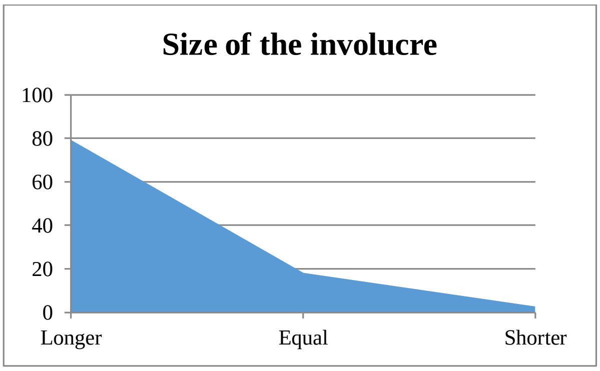


Figure 12. Classification of genotypes according to involucre characteristics (%).

4. Conclusion

In this study conducted to determine the hazelnut diversity in Düzce province, it was determined that the hazelnut genotypes in Akçakoca, Cumayeri, Gümüşova and Yığılca districts have a wide diversity in terms of the examined traits. Considerable variations were detected among genotypes in terms of nut size, nut shape, leaf characteristics and involucre structure. It has been determined that genotypes with globose nut shape and high number of nuts in cluster can be evaluated commercially. Research on the protection of hazelnut diversity in the region should be expanded, genotypes should be preserved in gene banks, characterised and evaluated in breeding programmes.

Author Contributions

The percentages of the authors' contributions are presented below. All author reviewed and approved the final version of the manuscript.

	E.D	H.İ.B
C		100
D		100
S		100
DCP	100	
DAI	40	60
L	80	20
W	20	80
CR		100
SR		100
PM		100
FA	60	40

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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PHYTOPLANKTON COMPOSITION OF BULANCAK STREAM, INTERPRETATION OF WATER QUALITY BASED ON INDICES AND FUNCTIONAL GROUPS

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Abstract: This study aims to analyze the phytoplankton community of Bulancak Stream, determine the functional groups of dominant algae and reveal the ecological status of the stream with the help of indicator species. Phytoplankton samples were collected monthly from three stations between February 2018-January 2019 and environmental parameters were measured. Bacillariophyta is the dominant division, constituting 72% of the total abundance comprising 37 taxa. *Cocconeis pediculus*, *Cymbella affinis* and *Ulnaria ulna* species dominate at all stations. In the study, changes in the phytoplankton composition of Bulancak Stream were also examined with the most common diversity indices, Shannon Index, Simpson Index and Margalef Index and it was concluded that the species were equally distributed. The study identifies 15 functional phytoplankton groups that are commonly found in shallow and organically rich streams. To assess the water quality of Bulancak Stream, the Q(r) index developed for rivers was used in addition to the calculated WQI and the results were found to be consistent. According to both indices, the water quality at Station 1, which is close to the city center and empties into the sea was classified as moderate while the upstream stations were assessed as having good water quality.

Keywords: WQI, Phytoplankton, Q(r) index, Functional group, Diversity, Bulancak

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1. Introduction

Rivers are basic resources with various uses in agriculture, irrigation, animal husbandry, transportation, recreation and industry. Recently, the need for water has increased with industrialization and population growth. The amount of usable water is also restricted due to pollution from various reasons of water resources. Water pollution is the changes that limit or completely prevent water use and disrupt the ecological balance (Sürücü and Demir, 2024). In preventing water pollution, knowing the water quality variables, the sum of a water body's physical, chemical and biological properties is essential. The parameters that make up the physicochemical structure of waters vary depending on the anthropogenic activities affecting the source and the geological structure of the waterbed. For this reason, every freshwater source requires effective monitoring (Mdee et al., 2024). Regulations such as the Water Pollution Control Regulation, the Urban Wastewater Treatment Regulation and the Basin Protection Action Plans set water quality standards for monitoring water pollution in Türkiye (WPCR-TESR, 2021). Türkiye has made significant progress in recent years in aligning its water policies with integrated river basin management principles, particularly through efforts to align with the EU Water Framework Directive. While some occasional challenges have been encountered in practice, significant strides have been made in the EU harmonization process.

The use of bioindicators in determining water quality has become quite widespread recently. The potential of phytoplankton as bioindicators is often overlooked, but they are essential for monitoring water quality in an aquatic ecosystem (Kumar et al., 2024). Phytoplankton are organisms that act as primary producers in aquatic ecosystems and form the basis of the food web (D'Costa and Naik, 2019). Phytoplankton abundance in waters allows information about environmental conditions to be obtained (Chandel et al., 2024; Suganti et al., 2024). Studies focused on phytoplankton in determining water quality in river ecosystems are less common than lakes (Gao et al., 2024) but there are also indices developed to measure algal conditions over time. Phytoplankton biomass is important in interpreting the indicator of water quality and pollution conditions. Streams are extremely sensitive to human pressures such as urbanization, agriculture and deterioration of habitat. Because of this problem, biologically oriented indices are becoming more utilized to assess rivers water quality. The Q(r) index, which a phytoplankton based river assessment tool is a modification of the original Q index applied to rivers. The relationships between organisms and their environment and their general distributions have been frequently studied in recent years. In addition to similar studies around the world, numerous studies are being carried out on the phytoplankton flora of Turkish freshwaters and their scope is gradually expanding over time (Burdis et al., 2025; Çelekli et al., 2025; Gao et al., 2024; İpek, 2022; Memiş, 2019; Öğrü et al., 2025; Tohumcu, 2020; Temizel, 2022; Uysal, 2019; Ye et al., 2024; Yilmaz et al., 2025). The functions of phytoplankton species in the ecosystem, their structural adaptations and the factors affecting changes in the food chain are being made more understandable by grouping species according to their function (Hochfeld and Hinners, 2024). Phytoplankton species are commonly classified by their functions to make generalizations about their role in ecosystems, role in the environment and position in the food web. One of the more prominent is the functional group system developed by Reynolds and Padisák. This system uses ecological strategies shared by the phytoplankton species to classify these groups that use letters as an abbreviation such as C, J, P, S1 and Lo. These groups are defined by different terms including the species growth strategies, which include fast or slow growth (but persistent), light needs, nutrient needs, tolerances to environmental conditions (e.g. flow, temperature, pH) ability to stay in suspension and traits such as toxic or competitive advantages (Akkoyunlu and Akiner, 2012; Borics et al., 2014; Padisák et al., 2009; Reynolds et al., 2002).

In line with these objectives, besides determining the physical and chemical factors affecting the enumeration and species distribution of the phytoplankton biomass of Bulancak Stream, changes in the phytoplankton association structure were examined by calculating Shannon, Simpson's and Margelef Indexes and an attempt was made to summarize the community structure. Q(r) index, which has recently started to be used in Turkish river systems is accepted as a strong indicator for determining the pollution in different parts of the stream. Based on this, the validity of the Q(r) index to assess the water quality of Bulancak Stream and the relationship between nutrient richness and pollution using Functional Groups (FGs), which facilitate the interpretation of water quality by grouping phytoplankton species with similar ecological characteristics were investigated. Phytoplankton functional groups of Bulancak Stream were determined, and the water quality status of the stream was assessed with the Q(r) index developed for rivers. WQI was also calculated for water quality and the results were observed to support each other (Borics et al., 2007).

This study aims to analyze the phytoplankton community structure of Bulancak Stream, a stream whose algal flora has not previously been identified, to identify potential dominant taxon functional groups and to assess the ecological status of Bulancak Stream using characteristic indicator taxa and biotic abundance indices. This was achieved by combining a planktonic based assessment with environmental parameters and using both the Q(r) index and water quality indices to better understand water quality dynamics. Also, by determining the phytoplankton biodiversity of Bulancak Stream, it will contribute to Türkiye's algal flora. Furthermore, by determining some of the water's physicochemical properties, it will be possible to comment on its pollution and potential for active agricultural use.

2. Materials and Methods

2.1 Study Area

Phytoplankton collections and water quality measurements were carried out simultaneously at three sampling stations on the Bulancak Stream between February 2018 and January 2019. Sampling stations were selected to represent the upstream, middle and downstream areas of the stream. The sampling stations selected for the study were determined to represent the ecological status of the stream. Station 1 (40°56'23"N and 38°14'11"E) is where physical, chemical and biological interactions occur between the river and seawater, where it flows into the Black Sea. Therefore, it is a mixed area where freshwater and saltwater organisms live together. The sampling area, which has a muddy bottom structure, includes a location heavily affected by domestic waste and waste from small scale agricultural activities. Station 2 (40°56'03 'N and 38°14'13 'E) covers the transition zone close to the district center, and Station 3 (40°55'43 'N and 38°14'18 'E) covers the upper basin, a rural, forested area under minimum anthropogenic impact. At each station, water samples were collected for analysis of physicochemical parameters and phytoplankton samples were collected and stored using standard techniques. The sampling design allowed direct comparison of biological communities with environmental conditions. Surface water samples (0-20 cm) were taken from the designated stations to determine phytoplankton density in the Bulancak Stream and to examine seasonal changes (Figure 1). On the sampling date, temperature, dissolved oxygen content, electrical conductivity and pH of the stream water were measured using a YSI 556 MPS multiprobe.

Figure 1. The locations of the sampling stations in the research area.

2.2 Collection and Analysis of Phytoplankton Samples

Water samples brought to the laboratory were shaken and poured into 100 mL measuring cylinders for each station. For the organisms in the water to settle to the bottom and be identified by staining, 10 mL of Lugol was added to each measuring cylinder and left for 24 hours. Then, the upper clear part was siphoned with a thin U-shaped glass tube without shaking the measuring cylinders and the remaining part was taken into counting tubes (Utermöhl, 1958). After waiting (4-6 hours) for the organisms to settle, identification and counting were made with a Leica inverted microscope at x40 and x100 magnifications. The results were calculated according to the method of Lund (Lund et al., 1958). The average of three counting replicates was used to determine the final abundance of each species. Algal biovolume was calculated using formulas for geometric shapes (Hillebrand et al., 1999). Standard diagnostic books were used to identify algal species (Cox, 1996; Krammer and Lange Bertalot, 1986, 1991a,b, 1999a,b). The establishment of systematic groups and the validity of the synonymous status of the identified species were updated in accordance with the AlgaeBase database (Guiry and Guiry, 2024).

2.3. Determination of Functional Groups and Evaluation of Water Quality (WQI), Q(r) and Biodiversity Indices

To assess the water quality of Bulancak Stream, the WQI value was calculated, along with the Q(r) index developed for rivers. Phytoplankton community diversity was calculated using the Shannon-Wiener Diversity Index (H'), Simpson Diversity Index (D) and Margalef Diversity Indices using the PAST program, a comprehensive statistical analysis tool for ecological and paleoecological data (Hill, 1973; James and Adejare, 2010; Krebs, 1998; Kwak and Peterson, 2007; Shannon, 1948). Total phytoplankton species in Bulancak Stream were classified into functional groups according to established methods (Borics et al., 2007; Padisák et al., 2009; Reynolds et al., 2002). The Minimum Water Quality Index (WQI_{min}) value was calculated using the Excel program to obtain the Water Quality Index (WQI), using the method developed by Akkoyunlu and Akiner (2012). In this context, the pH, temperature, dissolved oxygen and electrical conductivity parameters of Bulancak Stream were used to calculate the WQI_{min} value in our study.

The Q(r) index, used to assess the ecological quality of river systems based on phytoplankton communities, was calculated by combining the relative biomass of each functional group with its assigned ecological weight. For each sampling point, the biomass ratio (pi) of each group was multiplied by the sensitivity factor (Fi), as recommended by Borics et al. (2007). The resulting index ranges from 0 to 5, with higher values reflecting better water quality; it ranges from 'poor' (0-1) to 'excellent' (4-5). Reynolds et al. (2002), identified various species communities based on the functional associations of species represented in phytoplankton to define vegetation based on the structure of freshwater plankton. In the system later updated by Padisak et al. (2009) and used to define environmental conditions, phytoplankton species were divided into 33 groups, designated by alphanumeric codes, based on their survival strategies, tolerances and sensitivities. Functional groups symbolized by alphanumeric symbols represent different water types and different phytoplankton genera. Total phytoplankton species in Bulancak Stream were divided into FGs according to established methods (Borics et al., 2007; Padisák et al., 2009; Reynolds et al., 2002).

2.4. Statistical Analysis

To evaluate the relationships between environmental parameters and phytoplankton abundance across seasons, a series of multivariate analyses, including Pearson correlation, Principal Component Analysis (PCA) and Canonical Correlation Analysis (CCA) were conducted with SPSS 20.0 program.

3. Results

3.1. Physical and Chemical Properties of Bulancak Stream

In Bulancak Stream, between February 2018 and January 2019, water temperatures ranged from 6.13°C to 19.4°C, dissolved oxygen (DO) ranged from 8.1 to 9.2 mg/L, pH ranged from 6.95 to 7.84 and electrical conductivity (EC) ranged from 342.28 to 496 µS/cm. These physicochemical parameters had similar values for each sampling station. Seasonal variations revealed that water temperatures were highest in summer and lowest in winter. However, dissolved oxygen concentrations remained relatively constant throughout the year, with a slight increase in winter and autumn compared to other months. Furthermore, pH values varied little between seasons. The seasonal overlap of temperature and biological activity, as well as the solubility of oxygen, conforms to the characteristics of temperate freshwater systems. In general, the largest seasonal variation was seen in electrical conductivity, with the highest (~500 µS/cm) and lowest values observed in summer and winter respectively. While general water parameters such as pH and dissolved oxygen are similar across stations, the slightly higher conductivity at S1 likely indicates local nutrient input from upstream or nearby human activities. These factors combined likely explain why Station 1 exhibits lower ecological quality than the others. The seasonal distribution of the average values of some physicochemical measurements measured in Bulancak Stream between February 2018- January 2019 is given in Figure 2.

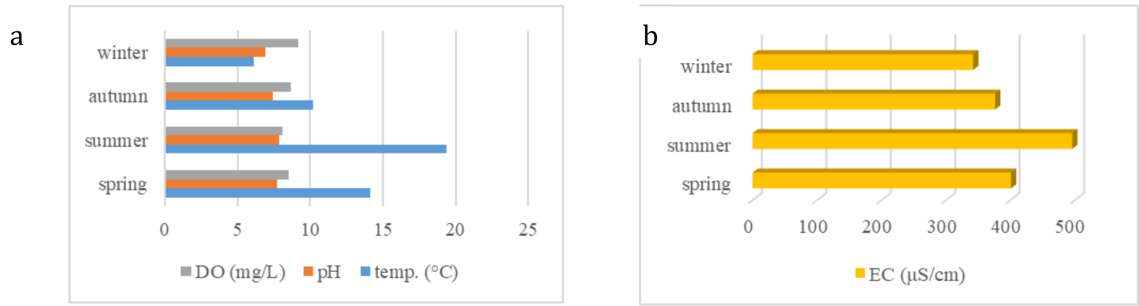

Figure 2. Seasonal average values of physicochemical measurements in Bulancak Stream: **a)** Dissolved Oxygen (DO, mg/L), pH, temperature (°C), **b)** Electrical Conductivity (EC, µS/cm).

3.2. Composition of Phytoplankton

A total of 53 phytoplankton taxa, representing five major taxonomic groups, were identified in the Bulancak Stream. The percentage distribution of species according to divisions is as follows: Bacillariophyta (72%), Chlorophyta (20%), Euglenozoa (5%), Cryptophyta (2%) and Cyanobacteria (1%). The list of available species is given in the Table 1 according to divisions. The phytoplankton composition of Bulancak Stream is given in Figure 3. The lowest number of organisms was at station 1 in March and April 2018, the highest amount of organisms was at station 3 in July and August 2018. Seasonally, the highest number of individuals occurred in summer (37%), followed by autumn (27%), winter (21%) and spring (15%). Seasonal changes and percentage distributions of total organism abundances at the three selected stations are shown in Figure 5B.

Table 1. List of available species according to divisions

Bacillariophyta		
Asterionella formosa Hassall	*Encyonema silesiacum* (Bleisch) D.G.Mann	*Navicula cincta* Pantocsek
Cocconeis pediculus Ehrenberg	*Epithemia adnata* (Kützing) Brébisson	*Navicula radiosa* Kützing
Cocconeis placentula Ehrenberg	*Epithemia sorex* Kützing	*Navicula rhynchocephala* Kützing
Cymbella affinis Kützing	*Epithemia turgida* (Ehrenberg) Kützing	*Navicula salinarum* Grunow
Cymbella aspera (Ehrenberg) Cleve	*Fragilaria capucina* Desmazières	*Navicula veneta* Kützing
Cymbella cistula (Ehrenberg) O.Kirchner	*Fragilaria crotonensis* Kitton	*Nitzschia amphicephala* Grunow
Cymbella helvetica Kützing	*Fragilaria tenera* (W.Smith) Lange-Bertalot	*Nitzschia gracilis* Hantzsch
Cymbella hungarica Grunow Pantocsek	*Gomphonema gracile* Ehrenberg	*Nitzschia linearis* W.Smith
Cymbella lanceolata C.Agardh	*Gomphonema minutum* (C.Agardh) C.Agardh	*Nitzschia palea* (Kützing) W.Smith
Diatoma moniliformis (Kützing) D.M.Williams	*Gomphonema parvulum* (Kützing) Kützing	*Pantocsekiella ocellata* (Pantocsek) K.T.Kiss and Ács
Diatoma tenuis C.Agardh	*Mallomonas caudata* Iwanoff [Ivanov] 1899	*Ulnaria ulna* (Nitzsch) Compère
Diatoma vulgaris Bory	*Navicula angusta* Grunow	
Dinobryon divergens O.E.Imhof	*Navicula cari* Ehrenberg	
Chlorophyta		
Ankistrodesmus bernardii Komárek	*Desmodesmus abundans* (Kirchner) E.H.Hegewald 2000	*Tetradesmus dimorphus* (Turpin) M.J.Wynne
Botryococcus braunii Kützing	*Desmodesmus communis* (E.Hegewald) E.Hegewald	*Tetradesmus lagerheimii* M.J.Wynne and Guiry
Chlamydomonas globosa J.W.Snow	*Scenedesmus aciculatus* P.González	*Tetradesmus obliquus* (Turpin) M.J.Wynne
Euglenozoa		
Euglena gracilis G.A.Klebs	*Euglena* sp.	*Trachelomonas armata* (Ehrenberg) F.Stein
Euglena viridis (O.F.Müller) Ehrenberg 1830	*Trachelomonas similis* A.Stokes 1890	
Cyanobacteria		
Oscillatoria limosa C.Agardh ex Gomont		
Cryptophyta		
Cryptomonas ovata Ehrenberg		

The significant dominance of Bacillariophyta may indicate that environmental conditions in the river were suitable for diatoms, which are generally associated with well oxygenated and moderately nutrient rich conditions. Bacillariophyta, the dominant division in our study area, was mostly represented by moderately pollution tolerant taxa such as *Ulnaria ulna, Cymbella affinis* and *Cocconeis pediculus*. In many studies conducted on streams in our country, Bacillariophyta was the dominant division and these species were frequently encountered (Çelekli et al., 2023; Çelik, 2022; Sevindik et al., 2021). *U. ulna*, which was seen in large numbers at all stations, is a species common in waters shifting from mesotrophic to eutrophic and is both sensitive and tolerant to pollution in water (Cox, 1996). It has been reported that it is widespread in fresh and salt waters, both in plankton and benthos around the world (Williams, 2025). *C. affinis* is a species common in all seasons, especially in silica waters. *C. pediculus* is also a medium quality water class organism and has been reported to be found in clean and moderately polluted streams (Çelekli et al., 2021). Species such as *Ulnaria ulna, Cymbella affinis, Navicula rhynchocephala*, *Gomphonema parvulum*, which are common in our study area, have a cosmopolitan distribution and are reported to be indicators of nutrient rich waters (Bhatt et al., 2025). The movement of phytoplankton from the Bulancak Stream to the Black Sea, particularly during the rainy season, can have significant consequences on coastal ecosystems. In this study was observed a decrease in biomass for all groups in the Bulancak Stream during the rainy season (spring). Phytoplankton biovolume reached its highest level year round in summer. Biovolume generally tended to decrease in winter and especially in spring (Figure 4). It was observed that species diversity was higher in the 3rd station. In the measured pH values, it was determined that a small number of the species frequently seen in Bulancak Stream, which is slightly alkaline, are species that can live in polluted waters. Waters with slightly alkaline pH values (between 7.5 and 8.5) provide a favorable habitat for many freshwater microalgae. In recent years, industrial, agricultural, and population growth have increased salinity and alkalinity in aquatic environments. This fluctuation in waters affects the differences among species tolerant to salinity and alkalinity, leading to significant changes in the structure of algal communities. In light of this data, pollution in aquatic ecosystems is bound to negatively impact species that are particularly sensitive to biodiversity (Gasiūnaitė et al., 2005; Kirst, 1990; Sui et al., 2016).

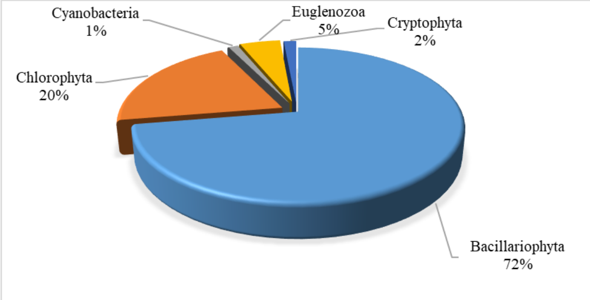

Figure 3. Phytoplankton composition of Bulancak Stream.

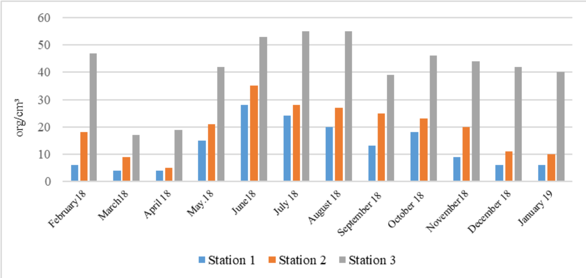

Figure 4. Monthly change in total organism abundance by stations.

Phytoplankton biomass showed clear seasonal and spatial differences among the three stations. Station 3 consistently had the highest biomass throughout the year, peaking at about 160 org/cm^3 in summer, probably because of warmer temperatures and more available nutrients. Biomass dropped during autumn and winter and was lowest in spring (Figure 5A). Stations 1 and 2 followed similar seasonal trends but always had lower biomass than Station 3. These results suggest that temperature, light and nutrients play a key role in phytoplankton growth, while local environmental factors likely explain the differences between stations.

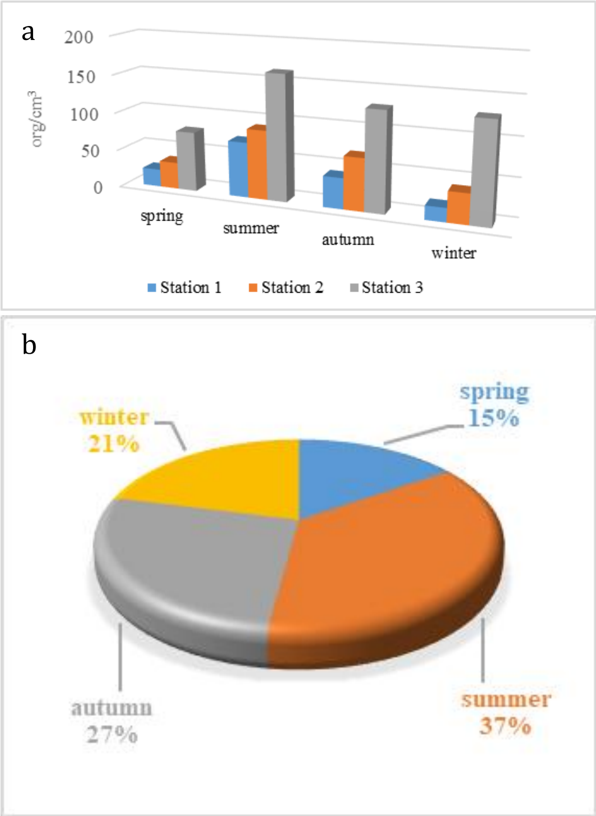

Figure 5. A. Seasonal variation of total organism abundance, B. Percentage distribution of total individuals in the study area according to seasons.

3.4. Q(r) Index Values and Functional Groups

Station 1 had a lower Q(r) score (2.93) compared to Stations 2 and 3, which were in the good category, indicating moderate ecological quality. This difference appears to be due to both biological structure and local environmental conditions. The identified taxa were categorized into River Functional Groups (RFGs) based on their ecological preferences and indicator values (Fi). The presence of high Fi values (4-5) in groups such as TB and MP indicate that the taxa are adapted to lotic, nutrient rich, or physically disturbed conditions and indicate recovery or moderate-high ecological integrity in some parts of the system. In contrast, groups W0 and W1, which have low Fi values (Fi = 1) and consist of species tolerant to organic pollution (*Euglena* sp., *Chlamydomonas globosa*), indicate local organic enrichment or degraded conditions. Also, it observed a higher proportion of these tolerant species, commonly found in waters with high organic content, in S1. Pollution sensitive diatom species were less common here, indicating some ecological stress, indicating the spatial heterogeneity of the ecological status of the study area. The species collected from three sampling stations in Bulancak Stream were divided into 15 functional groups: C, D, E, F, J, MP, P, TB, TC, TD, W0, W1, W2, X1 and Y (Table 2). Among these functional groups, species belonging to groups J, MP, and TB were the most common species. During the study period, functional groups MP (37%) and TB (22%) exceeded 10% of the total phytoplankton biomass. The most dominant functional group was MP, represented by species frequently encountered in the flora. The dominant functional groups (MP and TB) include species frequently encountered in streams, rivers and inorganically mixed and turbid waters. Group W1 was observed to increase with increasing temperature in summer after a significant decrease in spring. Members of this group, which were never seen in station 3, were more dominant in station 1, where water quality also decreased.

Adopting phytoplankton-based indices for streams is important step for assessing water quality in river ecosystems. These indices have been used in many studies worldwide for a long time and have been very useful for interpreting phytoplankton dynamics and water quality in streams (Borics et al., 2014; Li et al., 2022; Wu et al., 2012; Zhu et al., 2021). Although there are many studies on phytoplankton composition in Türkiye (Çelekli et al., 2023; Dalkıran et al., 2024; Fakıoğlu et al., 2023; Gönülol and Şahin, 2024; Maraşlıoğlu and Bektaş, 2022), there are almost no studies using phytoplankton communities as indicators of river water quality through indices (İpek and Maraşlıoğlu, 2024). Regardless of the season in which the sampling was carried out, the lowest Q(r) value was determined at station 1. The water quality at station 1, which is located in the city center and is poorer in terms of general species composition, was determined as 'medium' according to the Q(r) index, while the water quality at the other stations was determined as 'good'. The average Q(r) index of Bulancak Stream is 3.56, indicating 'good' water quality. The values and means of the Q(r) index determined by the stations are also given in Table 3. At each station in the Bulancak Stream, Q(r) index values are observed to be lower during the rainy season. Increased rainfall intensity alters the stream ecosystem and negatively impacts water quality. Streams in the Black Sea Region, where slopes are high, flow rapidly during the rainy season, resulting in increased turbidity. As a result, a decrease in photosynthetic pigment concentration is observed (Taş et al., 2021). In the Bulancak Stream, decreases in the number of taxa across the entire flora were observed during March and April, when rainfall increased (GPDEU, 2019).

Table 2. Reynolds Functional Groups, habitat features, taxa in Bulancak Stream and Fi scores based on (Gao et al., 2024)

RFG	Habitat features	Taxa in this study	F_i
C	Mixed eutrophic	*Asterionella formosa*	4
D	Shallow, nutrient-enriched, turbid	*Encyonema silesiacum, Pantocsekiella ocellata*	4
E	Small oligotrophic and nutrient-rich	*Dinobryon divergens, Mallomonas caudata*	3
F	Mixed, meso-eutrophic	*Botryococcus braunii*	3
J	Shallow, mixed, organic-enriched	*Desmodesmus abundans, Desmodesmus communis, Scenedesmus aciculatus, Tetradesmus dimorphus, Tetradesmus obliquus, Tetradesmus lagerheimii*	2
MP	Mixed and inorganically turbid	*Cocconeis pediculus, Cocconeis placentula, Cymbella affinis, Cymbella aspera, Cymbella cistula, Cymbella helvetica, Cymbella hungarica, Cymbella lanceolata, Epithemia adnata, Epithemia sorex, Epithemia turgida, Ulnaria ulna*	4
P	Mixed, eutrophic waters	*Fragilaria crotonensis*	3
T_B	Streams and rivulets (Highly lotic environments)	*Fragilaria capucina, Fragilaria tenera, Gomphonema gracile, Gomphonema minutum, Gomphonema parvulum, Navicula angusta, Navicula cari, Navicula cincta, Navicula radiosa, Navicula salinarum, Navicula rhynchocephala, Navicula veneta, Nitzschia amphicephala, Nitzschia gracilis, Nitzschia linearis, Nitzschia palea*	5
T_C	Eutrophic waters	*Oscillatoria limosa*	2
T_D	Slow flowing rivers, mesotrophic waters	*Diatoma moniliformis, Diatoma tenuis, Diatoma vulgaris*	3
W_0	High organic contents	*Chlamydomonas globosa*	1
W_1	High organic content	*Euglena gracilis, Euglena* sp., *Euglena viridis, Trachelomonas similis*	1
W_2	Mixed, meso-eutrophic	*Trachelomonas armata*	3
X_1	Shallow, nutrient-enriched	*Ankistrodesmus bernardii*	3
Y	Small-Nutrient-enriched	*Cryptomonas ovata*	3

Table 3. Station-based Q(r) scores of phytoplankton species

Station	St1	St2	St3	Mean
Q(r) scores	2.93	3.90	3.85	3.56
Ecological quality	medium	good	good	good

Since the average values of environmental parameters were in close ranges at all stations during our study, WQI values also took close values. According to the calculated WQI values, it was determined that Bulancak Stream was in the 'good' water category on average at all stations throughout the study (Figure 6). 'Moderate' water quality was rarely encountered at different time intervals, especially at station 1.

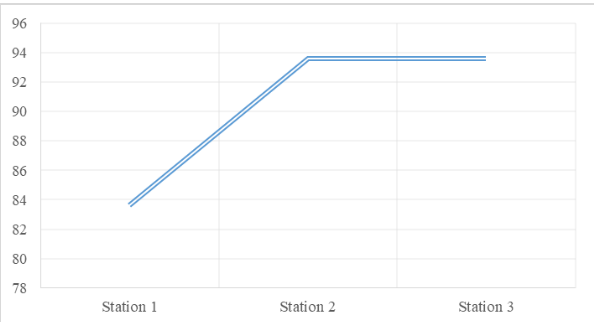

Figure 6. Change in water quality index according to stations.

3.5. Biodiversity Indices

Diversity indices varied slightly among stations. More specifically, the Shannon-Wiener and Simpson Indices were similar across all three stations, indicating approximately similar species diversity and stability. Shannon-Wiener and Simpson's Index, moderate phytoplankton biodiversity was observed at all stations. Margalef's index determined slightly higher values than the others. The Margalef Index at Stations 2 and 3 was elatively higher than at Station 1, indicating better species richness at these stations (Figure 7). This suggests that species diversity was relatively stable, but species richness was highest in the least impacted areas. A high diversity index indicates balanced communities (Shantala et al., 2009). Species have similar distributions and close values within the stations. Therefore, the calculated diversity indexes show that species are distributed equally and the probability of any species dominating the community is low.

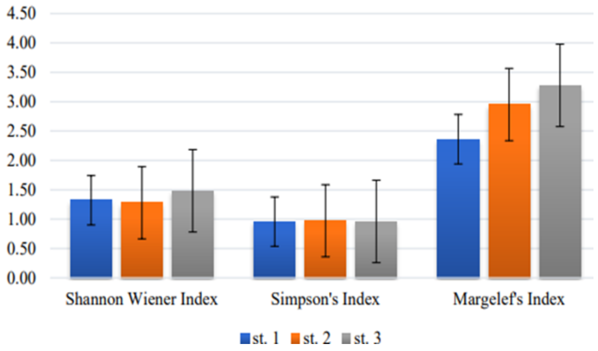

Figure 7. Different Diversity indices (Shannon, Simpson, and Margalef Indices) of the Bulancak Stream phytoplankton community.

3.6. Statistical Analysis

The Pearson correlation matrix revealed several significant relationships among the variables (Figure 8). Temperature showed a strong negative correlation with dissolved oxygen (DO) (r = -0.99), indicating that oxygen levels decrease with increasing temperature, a phenomenon frequently observed in aquatic systems due to decreased gas solubility at higher temperatures (Zhou et al., 2025). Electrical conductivity EC showed strong positive correlations with all three phytoplankton stations, especially Station 1 (r = 0.90), indicating that increased ion concentration could support or overlap with higher phytoplankton densities. Furthermore, Q(r) values were positively correlated with phytoplankton biomass, especially Station 3 (r = 0.78), highlighting its potential ecological sensitivity.

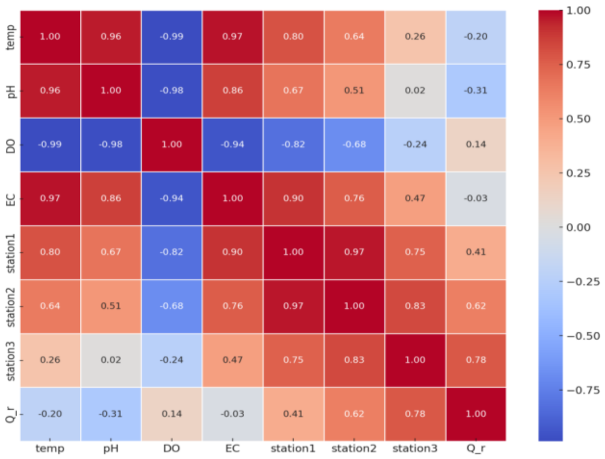

Figure 8. Pearson correlation analysis heat map of the seasonal relationship between the averages of physicochemical measurements of Bulancak Stream water samples and phytoplankton community parameters. (Station 1= Average number of phytoplankton species at Station 1, Station 2= Average number of phytoplankton species at Station 2, Station 3= Average number of phytoplankton species at Station 3).

PCA analysis was performed to reveal data patterns (Figure 9). The first two principal components explained most of the variance among the samples. PC1 sharply demarcated summer from winter, demonstrating seasonal contrasts driven predominantly by temperature, EC and biological productivity. PC2 helped distinguish spring from autumn. Variables such as temperature, EC and phytoplankton densities loaded positively on PC1, while DO loaded negatively, reinforcing the inverse relationship between temperature and oxygen availability. CCA was used to investigate the interdependence between environmental variables (temperature, pH, DO, EC) and biological parameters (phytoplankton abundance at three stations). The first canonical dimension (CCA1) captured the underlying common variance between the two datasets (Figure 10).

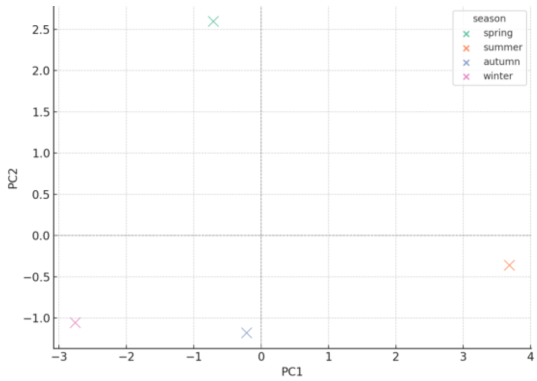

Figure 9. PCA plot showing seasonal clustering based on environmental and phytoplankton variables.

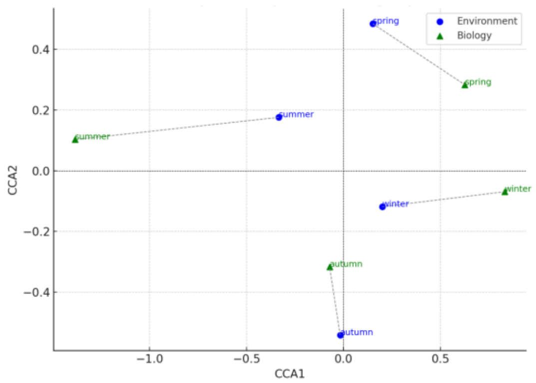

Figure.10. CCA plot showing seasonal relationships between environment and phytoplankton.

The multivariate analysis demonstrates that seasonal variation strongly influences both environmental dynamics and biological structure in the studied aquatic ecosystem. The close associations between EC and phytoplankton suggest that conductivity may serve as a proxy for nutrient availability or eutrophication status. These findings underscore the value of combining correlation-based and ordination techniques for a holistic understanding of aquatic ecosystem functioning.
The multivariate analyses revealed clear relationships between environmental factors and phytoplankton abundance across seasons. The correlation heatmap showed a strong negative relationship between

temperature and dissolved oxygen (r = -0.99), which aligns with established knowledge that oxygen solubility decreases in warmer water (Wetzel, 2001). Additionally, electrical conductivity EC was positively correlated with phytoplankton densities, especially at Station 1, suggesting that higher ion concentrations might support algal growth (Wang et al., 2017). Principal Component Analysis (PCA) grouped seasonal data effectively. Summer was clearly separated along the first principal component, driven by high temperature, EC and phytoplankton abundance. This supports the idea that phytoplankton communities respond sensitively to seasonal environmental changes (Padisak et al., 2006). The Canonical Correlation Analysis (CCA) further clarified the relationship between environmental variables and phytoplankton abundance. Temperature and EC emerged as key drivers, particularly influencing phytoplankton at Stations 1 and 2. This indicates a coupled effect of thermal and ionic conditions on biological productivity (Ter Braak and Prentice, 1988).

4. Conclusion

As a result, differences in species diversity were observed depending on the location of the stations in Bulancak Stream. The relationship between the Q(r) index and FGs indicated that the area where Bulancak Stream flows into the sea is most likely to be affected by both eutrophication and organic matter pollution. The lower WQI values at the same station support this finding. The continuity of long term monitoring programs across different river basins is essential for the sustainable management and protection of freshwater resources in Türkiye. The findings of this study contribute valuable information to the freshwater algal flora of Türkiye. Several taxa identified across different seasons and habitats highlight the ecological and floristic richness of the region. Some species, particularly those associated with specific environmental conditions, may represent new or rarely reported records for local aquatic systems. Moreover, the use of functional groups provides an ecological perspective that complements traditional taxonomic approaches and supports their potential as bioindicators. Overall, the study enhances the current understanding of algal diversity in Turkish inland waters and serves as a useful reference for future floristic and ecological research.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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ARTIFICIAL INTELLIGENCE MODELS FOR PREDICTING ROOT TRAITS OF CHOKEBERRY UNDER SALT STRESS

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Abstract: Chokeberry (*Aronia melanocarpa*) is a recently introduced functional berry in Türkiye. It has a high health-promoting potential and growing commercial value. However, limited information is available regarding its physiological responses to abiotic stresses such as salinity. This study aimed to investigate the effects of salt stress on the root architecture of chokeberry plants grown in different growing media (soil and peat) and irrigated with five different salinity levels (0.65-10 dS m^{-1}). Root traits including fresh and dry weight, total root length, surface area, volume, average diameter, number of tips, forks, and crossings were measured using WinRhizo software. Additionally, the study employed machine learning algorithms XGBoost, Multilayer Perceptron (MLP), and Gaussian Process Regression (GPR) to predict root traits based on salinity levels and identify the most accurate predictive model. The results showed that increasing salinity significantly reduced all root growth parameters. Among the tested models, XGBoost achieved the highest predictive performance ($R^2 > 0.9$), followed by MLP and GPR. Fresh and dry root weights were predicted with 98% and 97-98% accuracy, respectively, while MLP was most effective in estimating surface area and root tips. However, predictions for average diameter, root volume, and root crossings showed lower accuracy (MAPE > 10%). The findings indicate that artificial intelligence-based models can successfully estimate chokeberry root responses to salt stress and offer a powerful tool for sustainable cultivation.

Keywords: Root architecture, Machine learning, XGBoost, Multilayer perceptron

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1. Introduction

Chokeberry (*Aronia melanocarpa*) is a berry fruit that naturally grows in North America (Poyraz Engin and Boz, 2023). It was introduced to Russia and various parts of Europe from North America in the early 20th century (Chrubasik et al., 2010). It has been cultivated commercially in countries such as Poland, the Czech Republic, and Germany since the 1950s (Šnebergrová et al., 2014).

Morphologically, *A. melanocarpa* (Michx.) Elliot is a deciduous shrub characterized by a dense, suckering habit, typically reaching a height of 1.2 to 2 meters. Its fruits form mid-July and reach maturity in August-September (Brand, 2010). Thanks to its rich bioactive compounds and antioxidant capacity, it can help to treat metabolic diseases (Jeppsson and Johansson, 2000). It has been shown to positively affect diabetes, cardiovascular diseases, urinary tract inflammation, eye inflammation, and various viral diseases. It also helps prevent some cancer cell proliferation (Poyraz Engin et al., 2018).

Chokeberry cultivation is still relatively new in Türkiye, despite its many health benefits and a growing global market. As more people are interested in cultivating this plant, knowing what it needs to thrive well in diverse climates and soils is becoming more important. Sustainable water management is one of the most significant problems in modern farming, especially in places like Türkiye where water scarcity increases. The General Directorate of State Hydraulic Works (DSİ, 2025) states that, according to the 2021 data, Türkiye uses about 58.41 billion cubic meters of water each year, and 77% of that (45.05 billion m^3) is used for irrigation in agriculture. This situation highlights the urgent need to optimize irrigation practices and improve plant resilience to abiotic stresses such as drought and salinity.

Salinity stress is a major abiotic factor limiting plant growth and productivity. It mainly affects the plants in arid and semi-arid regions where soil salinization is progressively increasing due to improper irrigation practices and climate change. Root systems are crucial to a plant's ability to absorb water and nutrients from the soil. It also plays a key role in determining a plant's tolerance to saline conditions. The architecture of the root system, such as total root length, root surface area, diameter, volume, and the number of tips and forks, is a crucial indicator of how plants respond to such stress factors.

In recent years, machine learning (ML) techniques have emerged as powerful tools for modeling complex biological systems. Traditional statistical methods often fall short when dealing with highly non-linear, multidimensional datasets typical of plant-environment interactions (Camacho et al., 2018). In contrast, machine learning algorithms such as Random Forest, Support Vector Machines, Gradient Boosting, and Artificial Neural Networks can detect subtle patterns and interactions within large datasets without requiring prior assumptions (Ma et al., 2014; Mahood et al., 2020; Soltis et al., 2020). These models have been successfully used to predict crop yield, classify stress responses, and optimize irrigation and fertilization regimes based on various physiological and morphological parameters (Jeong et al., 2016; Singh, 2016; Van Dijk et al., 2021; Tütüncü, 2024).

Applying machine learning to root architecture analysis under salinity stress offers several advantages. It allows for the accurate prediction of plant responses based on measurable traits. There is a significant lack of comprehensive literature studies that evaluate the root architecture of *A. melanocarpa*.

This study aimed to investigate the effects of salt stress on the root architecture of chokeberry and to model the plant's morphological responses using artificial intelligence machine learning techniques.

2. Materials and Methods

The research was conducted in the greenhouse of Samsun Ondokuz Mayıs University, Faculty of Agriculture, between 2021-2022. In the study, 1-year-old plants of 'Viking' chokeberry cultivar were used. The plants were transferred into 15 litter pots and placed on a set 10 cm above the soil level (Figure 1).

Figure 1. Experimental setup of *A. melanocarpa* plants grown under controlled greenhouse conditions.

The study was conducted to evaluate the effects of irrigation water quality on plant root development. A control and four different levels of saline irrigation water were applied (0.65 dS m^{-1}(control), 2 dS m^{-1}, 4 dS m^{-1}, 8 dS m^{-1}, 10 dS m^{-1}). Peat and soil were used as growing mediums for all treatments. The saline solutions were prepared using three different salts. $MgSO_4$ with 99% purity, $CaCl_2$ with 99% purity and NaCl with 99.5% purity with high water solubility were used. The required electrical conductivity (EC) value for each treatment subject was determined in QBASIC computer program so that Sodium Adsorption Ratio (SAR) <5 and Ca/Mg ratio 1/1. The amounts of salt used per 1L of water according to the application subjects are stated in Table 1.

Table 1. Amounts of salt used in salt stress (g L^{-1})

Treatments	NaCl	$MgSO_4$	$CaCl_2$
2 dS m^{-1}	0.56	0.45	0.31
4 dS m^{-1}	0.87	1.75	0.75
8 dS m^{-1}	1.50	5.00	1.75
10 dS m^{-1}	2.00	6.12	2.5

2.1. Assessment of Root Architecture

At the end of the experimental period, the plants were carefully uprooted and removed from the pots in October. The roots were carefully washed and dried with paper towels. They were weighed on a precision balance to determine fresh root weight (g). The roots were then placed on A3 size acetate, and the roots were carefully separated to be detected in detail by the device. The roots, which were prepared for scanning, were placed on the scanner (Epson Expression 10000XL, Epson America Inc., Long Beach, CA, USA) of the device and transferred to the computer environment in three dimensions at 400 dpi resolution. WinRhizo root analysis program (ver. 2013, Regent Instruments, QC, Canada) (Sarıbaş et al. 2019) was used to examine the root architecture. Total root length (cm), root surface area (cm^2), root volume (cm^3), average root diameter (mm), number of root tips, forks, and crossings parameters of chokeberry plant were examined by WinRhizo root analysis program. After the analysis roots were placed in an oven at 80 ^{0}C for 72 hours. When they reached a constant weight, they were weighed to determine the root dry weight (g).

2.2. Modeling Procedure

The root architecture of chokeberry plants was predicted using three machine learning (ML) models: a multilayer perceptron (MLP) representing artificial neural networks (ANN), extreme gradient boosting (XGBoost), and Gaussian process regression (GPR). The dataset was randomly split into training (70%) and testing (30%) subsets to evaluate the performance of the machine learning models.

Fresh root weight, dry root weight, total root length, surface area, average diameter, root volume, number of tips, forks, and crossings were each treated as target (dependent) variables in separate models. For each trait, machine learning algorithms including Extreme Gradient Boosting (XGBoost), Multilayer Perceptron (MLP), and Gaussian Process Regression (GPR) were applied using the remaining eight traits as input features. For the MLP model, a feed-forward neural network was implemented with two hidden layers consisting of 16 and 8 neurons, respectively. The ReLU (Rectified Linear Unit) activation function was used in the hidden layers, while a linear activation function was applied in the output layer. To avoid overfitting, early stopping was applied during the training process. For the XGBoost model, the main hyperparameters were as follows: maximum tree depth (max_depth = 6), learning rate (eta = 0.1), and number of boosting rounds (nrounds = 100). Hyperparameter tuning was carried out using a grid search strategy combined with 5-fold cross-validation, and the best-performing configuration was selected for model evaluation. For the GPR model, the Radial Basis Function (RBF) kernel was employed as the covariance function. Kernel parameters were optimized through cross-validation starting from default values. Although a polynomial kernel was also tested, the RBF kernel provided higher predictive accuracy and was therefore selected as the final kernel function. All machine learning models were implemented using the R programming (version 4.3.3) with the assistance of the "caret", "xgboost", "neuralnet", and "kernlab" packages. For model training and evaluation, each root trait was treated as a separate dependent variable, and the remaining traits were used as predictors. The performance of each model was evaluated using commonly accepted regression metrics. These included the Mean Absolute Error (MAE), the Root Mean Square Error (RMSE), the Coefficient of Determination (R^2), and the Mean Absolute Percentage Error (MAPE) (equations 1, 2, 3 and 4).

$$\text{MAE}=\frac{1}{n}\sum_{i=1}^{n}|yi-\hat{y}i| \tag{1}$$

$$\text{RMSE}=\sqrt{\frac{1}{n}\sum_{i=1}^{n}(yi-\hat{y}i)2} \tag{2}$$

$$R^2=1\frac{\sum_{i=1}^{n}(yi-\hat{y}i)2}{\sum_{i=1}^{n}(yi-\bar{y}i)2} \tag{3}$$

$$\text{MAPE}=\frac{100}{n}\sum_{i=1}^{n}|\frac{yi-\hat{y}i}{yi}| \tag{4}$$

The experiment was conducted using a randomized plot design with three replications, each consisting of three plants. The experiment was repeated for two years with different plants. For Machine Learning the data of two years were combined and analyzed together. Descriptive analyses were done by using Microsoft Office 2015 Excel package program.

3. Results

3.1. Evaluation of the Effects of Treatments on Root Development

Descriptive statistics of root fresh weight, root dry weight, root length, root surface area, root volume, average root diameter, root tips, forks and crossings training values are given in Table 2. Root fresh weight (g) was varied between 25.6 g (10 dS m^{-1}) and 138.7 g (control) in training data. The highest average root dry weight (40.1 g) was obtained in the control treatment. Root length was varied between 490.2 cm (10 dS m^{-1}) and 1942.2 cm (control). Also, surface area was varied between 636.3 cm^2 (10 dS m^{-1}) and 2439 cm^2 (control). The highest average diameter (5.4 mm) and the highest root volume (247.4 cm^3) was obtained in the control application. Besides, the highest root tips (5453), forks (25970) and crossing (7135) were obtained from the control application (Table 2).

Table 2. Descriptive statistics of training dataset

	Root Fresh Weight (g)			Root Dry Weight (g)			Root Length (cm)		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	66.7	138.7	103.2±30	28.2	54.7	40.1±10	1056.5	1942.2	1520±357
2 dS m^{-1}	57.6	127.6	88.4±29	21.1	46.8	32.7±10	903.9	1788.9	1336±388
4 dS m^{-1}	41.2	91.9	67.3±19	17.1	34.2	25.3±6.6	724.3	1462.1	1081±322
8 dS m^{-1}	33.6	65.4	45.9±11	10.9	23.9	16.4±4.2	666.6	1181.3	895±194
10 dS m^{-1}	25.6	43.4	34.5±6	10.4	17.1	12.7±1.9	490.2	1023.3	727±202
	Surf Area (cm^2)			Avgdiam (mm)			Root Volume (cm^3)		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	1404.2	2439.0	1934.8±418	3.2	6.9	5.4±1.2	219.4	268.1	247.4±12
2 dS m^{-1}	1102.1	2083.5	1497.1±348	3.0	6.7	4.6±1.4	134.7	227.1	189.6±35
4 dS m^{-1}	901.9	1785.4	1275.7±315	2.7	6.6	4.9±1.4	107.7	224.7	165.1±40
8 dS m^{-1}	753.6	1465.3	1076.8±278	2.6	6.4	4.8±1.2	85.9	177.6	137.1±38
10 dS m^{-1}	636.3	1081.7	878.1±132	2.8	6.9	4.6±1.3	63.4	155.5	111.8±37
	Root Tips			Root Forks			Root Crossing		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	4060	6623	5453±877	14923	39468	25970±8412	5699	8889	7135±881
2 dS m^{-1}	2348	6482	4389±932	12174	27155	19033±4329	2223	5671	4361±1038
4 dS m^{-1}	2422	5047	3859±912	12786	22384	17440±3346	1784	4891	3282±1252
8 dS m^{-1}	2263	4531	3237±802	10434	20315	15120±3856	1173	3546	2264±773
10 dS m^{-1}	2255	3802	2824±480	10267	17796	12083±2087	1195	3115	2095±693

In the test dataset (Table 3), root fresh weight ranged from 40.3 g (10 dS m^{-1}) to 134.6 g (control), while root dry weight ranged from 10.4 g (10 dS m^{-1}) to 50.6 g (control). The lowest root length (533.1 cm) was recorded at 10 dS m^{-1}, whereas the highest (1809 cm) was recorded in the control treatment. Root surface area values varied between 921.4 cm^2 (10 dS m^{-1}) and 2139 cm^2 (2 dS m^{-1}). Average diameter ranged from 2.2 mm (4 dS m^{-1}) to 6.9 mm (8 dS m^{-1}), while root volume ranged from 65.5 cm^3 (10 dS m^{-1}) to 231.6 cm^3 (control). The highest values for root tips (5827), forks (31,101), and crossings (8150) were also obtained from the control treatment (Table 3).

The descriptive statistic was done according to the years. For this purpose, root fresh weight, root dry weight, root length, root surface area, root volume, average root diameter, root tips, forks and crossings data were determined. In the experiment soil and peat mediums were used. Table 4 presents the results of the descriptive statistical analysis for chokeberry plants grown in soil in 2021.

In 2021 in the soil medium the root fresh weight values were ranged from 30.1 g (10 dS m^{-1}) to 81.3 g (control) (Table 4). Dry root weight values ranged from 13.2 g (10 dS m^{-1}) to 31.4 g (control), with the highest average value obtained in the control (30.9 g) application. The root length value was highest in the control application (1274.7 cm) and the lowest in the 10 dS m^{-1} irrigation water application (490.2 cm). Like the fresh root weight, dry root weight, and root length values, the root surface area was found to be highest in the control treatment (1623.9 cm^2) and lowest in the 10 dS m^{-1} irrigation water treatment (636.3 cm^2). The average root diameter value was ranged from 5.00 mm (2 and 10 dS m^{-1}) to 6.60 mm (4 dS m^{-1}), while the root volume was ranged from 150.1 cm^3 (10 dS m^{-1}) to 244 cm^3 (control). The highest value of the root tips parameter was obtained in the control application (5860), while the lowest was obtained in the 10 dS m^{-1} irrigation water application (2255). The root forks was ranged from 10267 (10 dS m^{-1}) to 24111 (control), while the root crossing value ranged from 1173 (8 dS m^{-1}) to 7399 (control). The root architecture results of the plants grown in peat medium in 2021 are presented in Table 5.

Table 3. Descriptive statistics of testing dataset

	Root Fresh Weight (g)			Root Dry Weight (g)			Root Length (cm)		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	70.0	134.6	102.3±45	31.1	50.6	40.8±13	1217.0	1809.0	1513±419
2 dS m^{-1}	58.1	119.5	88.7±43	23.6	44.8	34.2±15	876.6	1756.2	1316±621
4 dS m^{-1}	42.4	91.3	62.5±23	18.3	32.4	24.2±7	792.3	1482.1	1117±352
8 dS m^{-1}	50.5	58.1	53.3±40	16.1	25.6	20.1±5	658.8	1031.9	796±205
10 dS m^{-1}	40.3	42.8	41.5±10	10.4	17.5	13.9±5	533.1	829.6	681±209
	Surf Area (cm^2)			Avgdiam (mm)			Root Volume (cm^3)		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	1595.1	2088.0	1841±348	3.6	5.4	4.5±1.2	223.4	231.6	227±5
2 dS m^{-1}	1247.3	2139.0	1693±630	4.5	5.8	5.2±0.9	182.9	204.8	193±15
4 dS m^{-1}	970.4	1895.2	1325±422	2.2	5.8	4.3±1.7	108.6	192.1	162±38.9
8 dS m^{-1}	983.4	1406.7	1136±235	3.2	6.9	5.1±1.8	147.7	177.4	167±16
10 dS m^{-1}	921.4	944.5	933±16	3.6	4.6	4.1±0.7	65.5	146.7	106±57
	Root Tips			Root Forks			Root Crossing		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	4649	5827	5238±832	18368	31101	24734±9003	5229	8150	6689±2065
2 dS m^{-1}	2563	5421	3992±202	13733	22581	18157±6256	3546	4701	4123±816
4 dS m^{-1}	2842	5077	3643±1055	13252	20293	18425±3151	1780	4822	3072±1525
8 dS m^{-1}	3230	5541	4148±1226	15793	21474	17849±3148	2526	2990	2740±234
10 dS m^{-1}	2916	3466	3191±388	12348	13075	12711±514	1800	2851	2325±743

Table 4. Descriptive statistical values of chokeberry plants grown in soil medium in 2021

	Root Fresh Weight (g)			Root Dry Weight (g)			Root Length (cm)		
Soil/2021	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	74.5	81.3	78.9±3.8	30.4	31.4	30.9±0.5	1236.3	1274.7	1255±19.1
2 dS m^{-1}	57.6	60.7	59.5±1.6	21.1	21.6	21.3±0.2	945.9	1096.7	1012.4±76
4 dS m^{-1}	41.2	43.1	42.2±0.9	17.1	18.3	17.9±0.7	792.3	862.6	831.9±35
8 dS m^{-1}	33.6	36.2	34.9±1.3	14.7	16.0	15.2±0.7	666.6	726.5	694.5±30
10 dS m^{-1}	30.1	30.7	30.3±0.3	13.2	13.7	13.4±0.3	490.2	527.8	504.5±20
	Surf Area (cm^2)			Avgdiam (mm)			Root Volume (cm^3)		
Soil/2021	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	1404.2	1623.9	1527.7±112.3	5.5	6.3	5.9±0.3	242.4	244.0	243±0.9
2 dS m^{-1}	1102.1	1260.4	1189.9±80.5	5.0	6.0	5.7±0.5	203.4	227.1	218±12.9
4 dS m^{-1}	901.9	1041.0	971.1±69	5.7	6.6	6.1±0.4	190.3	197.6	193±3.7
8 dS m^{-1}	753.6	788.1	768.9±17.6	5.6	6.4	6.1±0.4	171.8	177.6	174±2.8
10 dS m^{-1}	636.3	745.3	701.2±57.3	5.0	6.4	5.6±0.7	150.1	155.5	152±2.8
	Root Tips			Root Forks			Root Crossing		
Soil/2021	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	5451	5860	5639±206	20059	24111	21472±2287	6845	7399	7162±285
2 dS m^{-1}	2348	3828	3139±745	12174	16001	14706±2193	2223	4693	3645±1276
4 dS m^{-1}	2422	2856	2706±246	12786	14572	13536±926	1780	1807	1790±14.5
8 dS m^{-1}	2263	2509	2426±141	10434	10909	10610±260	1173	1555	1400±201
10 dS m^{-1}	2255	2636	2449±190	10267	11397	10715±599	1195	1544	1341±181

Table 5. Descriptive statistical values of chokeberry plants grown in peat medium in 2021

	Root Fresh Weight (g)			Root Dry Weight (g)			Root Length (cm)		
Peat/2021	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	132.0	138.7	134±3.6	48.7	49.1	48.9±0.1	1838.5	1942.2	1883±53
2 dS m^{-1}	119.5	127.6	122±4.2	44.5	46.8	45.4±1.2	1724.8	1788.0	1756±31
4 dS m^{-1}	91.3	91.9	91.7±0.3	32.4	34.2	33.1±0.9	1438.8	1482.0	1461±21
8 dS m^{-1}	58.1	65.4	61.4±3.6	22.5	25.6	24±1.5	1007.3	1031.9	1021±12
10 dS m^{-1}	25.6	30.7	28.9±2.8	10.5	11.7	11.2±0.6	800.4	886.0	831±47
	Surf Area (cm^2)			Avgdiam (mm)			Root Volume (cm^3)		
Peat/2021	Min	Max	Mean±SD	Min	Max	Mean+SD	Min	Max	Mean±SD
Control	2318.3	2439.0	2390.6±63	3.2	5.8	4.6±1.2	252.4	254.2	253±1.0
2 dS m^{-1}	2051.0	2139.0	2091.2±44.4	3.0	4.5	3.7±0.7	202.7	207.6	205±2.4
4 dS m^{-1}	1682.9	1895.2	1787.8±106	3.0	4.9	3.8±1.0	160.4	172.7	167±6.2
8 dS m^{-1}	1406.7	1465.3	1439.9±30	3.2	4.5	3.9±0.6	141.8	147.7	145.2±3
10 dS m^{-1}	963.0	1081.7	1016.3±60	3.5	4.6	4.1±0.5	90.1	102.5	96.1±6.2
	Root Tips			Root Forks			Root Crossing		
Peat/2021	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	5837	6288	6035±238	28016	39468	35551±6527	7412	8889	7936±826
2 dS m^{-1}	5421	6482	5921.6±533	22388	27155	24041±2698	4701	5671	5265±504
4 dS m^{-1}	5018	5077	5047.3±29.5	20293	22384	21290±1048	4757	4891	4823±67
8 dS m^{-1}	4167	5541	4746±711	19182	21474	20323±146	2705	3546	3112±421
10 dS m^{-1}	2430	3246	2733±446	10627	13150	11640±1332	2016	2916	2409±460

The root fresh weight was ranged from 25.6 g (10 dS m^{-1}) to 138.7 g (control), while root dry weight was ranged from 10.5 g (10 dS m^{-1}) to 49.1 g (control). In both fresh and dry root weight parameters, increasing salinity levels led to a noticeable reduction in root weight. Increasing salinity levels resulted in a progressive decline in root length. In the control application, root length was measured as 1942.2 cm, while in the 10 dS m^{-1} salt application, it was measured as 800.4 cm. On the other hand, root area ranged from 963 cm^2 (10 dS m^{-1}) to 2439 cm^2 (control), while root volume ranged from 90.1 cm^3 (10 dS m^{-1}) to 254.2 cm^3 (control). Both parameters exhibited a declining trend as salt concentration increased. The average root diameter was lowest at 2 and 4 dS m^{-1} salinity levels (3.0 mm), while the highest value was observed in the control treatment (5.8 mm). Root tips, forks, and crossing values showed a decreasing trend with increasing irrigation water salinity. The highest values for all three parameters were obtained in the control application, while the lowest values were obtained in the 10 dS m^{-1} irrigation water application (Table 5).

The descriptive statistical values of the analysis results from the second year of the experiment are presented in soil medium (Table 6) and peat medium (Table 7).

In 2022, in the soil medium, the root fresh weight was ranged from 42.8 g (10 dS m^{-1}) to 70 g (control) (Table 6), while in the peat medium it was ranged from 36.8 g (10 dS m^{-1}) to 134.6 g (control) (Table 7). Similar to the observations in 2021, root dry weight decreased with increasing salt concentrations in both peat and soil growing media, with the lowest value recorded at 10 dS m^{-1}. The average dry root weight was determined to be 29.6 g in the soil medium and 51.5 g in the peat medium. Root length values were ranged from 533.1 cm (10 dS m^{-1}) to 1217.2 cm (control) in the soil environment and from 829.6 cm (10 dS m^{-1}) to 1882 cm (control) in the peat environment. Root surface area and root volume values decreased with increasing salt concentration in both peat and soil mediums. The average root surface area was recorded as 1590.8 cm^2 in the soil and 2336.4 cm^2 in the peat medium. The average root volume was measured as 249.1 cm^3 in the soil and 231.2 cm^3 in the peat medium. The average root diameter value ranged from 4.60 to 6.90 mm in the soil and from 2.20 to 4.80 mm in the peat medium. Root tips, forks, and crossing values decreased with increasing salt levels in both peat and soil mediums. Root tips were determined as a maximum of 4649 (control) in the soil and 6623 (control) in the peat mediums. The average maximum values of the root forks were obtained from the control application in both growing mediums. Root crossing values ranged from 1523 (10 dS m^{-1}) to 6011 (control) in soil and from 2150 (8 dS m^{-1}) to 8150 (control) in peat.

Table 6. Descriptive statistical values of chokeberry plants grown in soil medium in 2022

	Root Fresh Weight (g)			Root Dry Weight (g)			Root Length (cm)		
Soil/2022	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	66.7	70.0	68.6±1.6	28.2	31.1	29.6±1.4	1056.5	1217.2	1134.6±80
2 dS m^{-1}	58.0	65.4	61.1±3.8	22.4	26.8	24.2±2.2	876.6	939.9	906.8±31
4 dS m^{-1}	52.7	53.3	53±0.3	18.5	21.9	20.4±1.7	724.3	785.4	761.2±32
8 dS m^{-1}	48.4	51.3	50.1±1.4	16.2	18.5	17.3±1.1	658.8	710.5	688.9±26
10 dS m^{-1}	42.8	43.3	43.1±0.3	14.7	17.5	16.4±1.5	533.1	586.8	557±27
	Surf Area (cm^2)			Avgdiam (mm)			Root Volume (cm^3)		
Soil/2022	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	1545.4	1632.1	1590.8±43	5.4	6.9	6.4±0.8	223.4	268.1	249.1±23
2 dS m^{-1}	1247.3	1277.9	1260.3±15	5.8	6.7	6.1±0.4	182.9	215.1	189±16
4 dS m^{-1}	1020.1	1146.4	1082.6±63	5.0	6.5	5.8±0.7	165.4	224.7	189.1±31
8 dS m^{-1}	953.3	1018.1	984.9±32	5.3	6.9	5.9±0.8	154.7	177.4	169.4±12
10 dS m^{-1}	856.8	921.4	879.5±36	4.6	6.9	5.4±1.2	142.5	146.7	143.9±2.3
	Root Tips			Root Forks			Root Crossing		
Soil/2022	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	4060	4649	4281±320	14923	18661	17317±2078	5229	6011	5646±393
2 dS m^{-1}	2563	3835	3328±674	13733	17794	15871±2039	3546	4499	3958±489
4 dS m^{-1}	2954	3704	3422±408	15103	15362	15222±130	1875	2878	2397±502
8 dS m^{-1}	3006	3674	3303±339	13972	16281	15348±1216	2455	2990	2657±290
10 dS m^{-1}	2708	2916	2783±115	11464	13075	12180±820	1523	1800	1682±143

The findings of this study show that increasing salinity levels significantly negatively affect root morphology parameters in *A. melanocarpa*. All root growth indicators, such as total root length, surface area, root volume, average root diameter, and root tip number, decreased significantly with increasing salt concentration. Similarly, the literature widely reports that salt stress inhibits plant root development. For example, a study on the chokeberry indicated that increasing salt concentration progressively reduced plant height, fresh/dry weight, and root length (Mahmood and Pırlak, 2025). Similarly, it is stated that salinity negatively affects both the above-ground and root system growth of apple rootstock seedlings, causing a significant decrease in root length and biomass (Li et al., 2024). The general scientific consensus is that plants under salt stress show significant declines in morphological indicators. In particular, salt damages the roots results in a reduction in root length and mass (Li et al., 2024). Studies conducted on the model plants *Arabidopsis thaliana*, rice, and soybeans have shown that both main root length and lateral root formation are significantly inhibited in the presence of salt (Liu et al., 2025). Additionally, high salt concentrations have been reported to seriously affect root architecture in species such as corn, wheat, and apple (Rahnama et al., 2011; Li et al., 2021; Li et al., 2024). Indeed, salt stress in wheat seedlings significantly reduced total root length and root surface area, while also decreasing root biomass and weakening overall root development. In a study where 0, 50, 100, and 150 mM NaCl was applied to 'Viking' chokebery plants in MS and semi-MS media, it was determined that root length, root age, and dry weight decreased with increasing salt concentrations (Nas et al., 2025). Bryla and Scagel (2014) stated that the root weight of the 'Honeoye' strawberry variety that is treated with $CaCl_2$ decreased by 19-48% due to salt stress. Also, in another study conducted with strawberries, 35 mM NaCl salt dose reduced root volume by 37% (Aras and Eşitgen, 2018). This study also shows that the chokeberry root system is extremely sensitive to salt, and that salinity causes a decline in all root architecture parameters, which is consistent with the general findings in the literature.

3.2. Analysis of ML Models

The data obtained from the root architecture analysis of chokeberry plants grown under different irrigation qualities were analyzed using artificial neural network-based multilayer perceptron (MLP), XGBoost, and GPR machine learning algorithms. The results were validated and estimated using performance metrics such as root mean square error (RMSE), the coefficient of determination (R^2), mean absolute error (MAE) and mean absolute percentage error (MAPE) The changes in the modeled algorithms according to irrigation applications are presented in Table 8.

Table 7. Descriptive statistical values of chokeberry plants grown in peat medium in 2022

	Root Fresh Weight (g)			Root Dry Weight (g)			Root Length (cm)		
Peat/2022	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	124.9	134.6	130.3±4.9	49.4	54.7	51.5±2.7	1715	1882	1802±84
2 dS m^{-1}	108.3	112.1	110.4±1.9	38.8	42.8	40.9±2.0	1570	1788	1655±116
4 dS m^{-1}	73.1	78.5	75.7±2.7	27.0	30.2	28.3±1.6	1298	1356	1321±31
8 dS m^{-1}	41.3	47.7	44.5±3.2	10.9	14.2	12.9±1.8	996	1181	1075±95
10 dS m^{-1}	36.8	40.3	38.4±1.7	10.4	12.2	11.3±0.9	829.6	1023.3	950±105
	Surf Area (cm^2)			Avgdiam (mm)			Root Volume (cm^3)		
Peat/2022	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	2079.5	2336.4	2167±145	3.6	4.7	4.1±0.5	219.4	242.5	231.2±11
2 dS m^{-1}	1486.8	1692.1	1577±104	3.2	3.6	3.4±0.1	134.7	147.3	139.9±6.5
4 dS m^{-1}	1227	1395	1327±88	2.2	4.8	3.2±1.3	107.7	108.6	108.1±0.4
8 dS m^{-1}	1118	1210	1172±48	2.6	4.2	3.5±0.7	85.9	90.6	88.9±2.6
10 dS m^{-1}	903.1	944.5	929±23	2.8	3.6	3.1±0.4	63.4	70.6	66.5±3.7
	Root Tips			Root Forks			Root Crossing		
Peat/2022	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
Control	4694	6623	5714±969	24001	31101	28718±4085	6850	8150	7499±650
2 dS m^{-1}	4346	5972	4905±924	19601	22599	20930±1527	3521	5385	4418±933
4 dS m^{-1}	3800	4178	3873±190	17583	18837	18357±677	3298	4337	3838±520
8 dS m^{-1}	3028	3949	3385±493	15805	18293	16929±1261	2150	2543	2362±198
10 dS m^{-1}	2820	3802	3382±499	12241	17796	14128±3176	2586	3115	2850±264

Table 8. Performance metrics of MLP, XGBoost, and GPR Models for root trait prediction

Parameters	MLP and ML Models	R^2	RMSE	MAE	MAPE (%)
	XGBoost	0.98	4.83	3.76	7.04
Fresh Weight (g)	MLP	0.98	4.98	4.16	8.57
	GPR	0.97	6.38	5.57	10.96
	XGBoost	0.98	1.64	1.31	5.52
Dry Weight (g)	MLP	0.97	2.17	1.83	7.52
	GPR	0.97	2.26	2.05	9.01
	XGBoost	0.95	97.97	67.13	9.24
Length (cm)	MLP	0.96	85.40	79.10	8.91
	GPR	0.96	86.48	76.39	9.25
	XGBoost	0.92	135.54	90.73	7.01
Surface Area (cm^2)	MLP	0.95	106.62	84.13	6.33
	GPR	0.91	171.10	131.56	9.61
	XGBoost	0.76	0.63	0.56	13.23
Average Diameter (mm)	MLP	0.69	0.74	0.62	13.26
	GPR	0.70	0.80	0.69	15.86
	XGBoost	0.83	25.20	18.53	12.72
Root Volume (cm^3)	MLP	0.77	28.14	18.08	11.23
	GPR	0.79	28.75	22.09	14.75
	XGBoost	0.90	448.23	383.41	9.60
Root Tips	MLP	0.92	353.86	274.05	7.01
	GPR	0.87	531.00	413.06	9.84
	XGBoost	0.93	2910.56	1690.23	7.09
Root Forks	MLP	0.92	2888.59	1720.21	7.21
	GPR	0.88	3901.71	2113.70	8.20
	XGBoost	0.86	768.15	638.36	19.50
Root Crossing	MLP	0.83	874.05	689.89	19.98
	GPR	0.78	983.29	831.60	22.90

Based on the findings, it was observed that the models generally demonstrated high prediction accuracy (R^2 > 0.9). Among the models, XGBoost outperformed both MLP and GPR in many parameters. Although the MLP and GPR models exhibited similar R^2 values. However, the MLP model provided better results due to its lower error rates compared to GPR. When the machine learning models were evaluated based on individual parameters, differences were observed among them (Table 8). The fresh root weight parameter was predicted with 98% accuracy by both XGBoost and MLP models. With the lowest error (RMSE: 4.83), XGBoost can be considered the most successful model for predicting fresh root weight. For the dry root weight parameter, the MLP and GPR models achieved 97% accuracy, while the XGBoost model reached 98%, again showing the best performance with lower error values.

Both the MLP and GPR models predicted the root length parameter with high performance, achieving 96% accuracy. The root surface area parameter was predicted by the MLP model with 95% accuracy. The root surface area predicted by the MLP model had an RMSE value of 106.62, while the XGBoost and GPR models achieved R^2 values of 0.92 and 0.91, respectively. Regarding the prediction of average diameter and root volume, the XGBoost model showed the highest performance with R^2 values of 0.76 and 0.83, respectively. For the root tips parameter, the MLP model performed best with an R^2 value of 0.92.

In the prediction of root forks, R^2 values ranged between 0.88 and 0.93, with the highest performance obtained from the XGBoost model (0.93). Similarly, in the prediction of the root crossing parameter, the XGBoost model again showed the best performance with 86% accuracy. However, due to MAPE values exceeding 10% for the average diameter, root volume, and root crossing parameters, the prediction accuracy for these traits is considered low. Therefore, the models may not be suitable for accurately predicting these specific parameters.

The literature emphasizes that XGBoost performs particularly well with tabular data and is considered the “default powerful model” for many problems (Gill et al., 2022). Similarly, Duarte et al. (2022) reported that the SVM model performed best in predicting root architecture in 100 different soybean genotypes under two different water conditions, and that the MLP, RF, and XGBoost models also provided more successful results compared to linear models.

Since the data size in our study was relatively limited, MLP was used with a moderately complex architecture and was successful in certain parameters. Indeed, in the study by Aasim et al. (2023), which modeled the response of chickpea seeds to salt stress, it was noted that the MLP model could predict all germination and growth indices with high accuracy, thereby achieving a level of prediction success that could not be achieved with classical statistical approaches. In a study conducted by Tütüncü (2024), the root architecture of Primula plants was analyzed, and it was reported that the MLP model performed better than other models. While the study clearly showed the order MLP > GP > RF > XGBoost, this study emphasized the superiority of the XGBoost model. This difference may be due to various factors such as plant type, the level of optimization of model parameters, and the content of input variables.

In our study, the underperformance of GPR may have been due to the limited size of the dataset and the difficulty of modeling the relationship between root parameters and salt stress with a single global Gaussian kernel. Similarly, in Dong et al. (2023) sunflower root modeling study, GPR lagged RF and XGBoost; the researchers noted that RF and XGBoost made RLD (root length density) predictions with lower error than GPR.

Figures 2, 3, and 4 illustrate the comparative distributions of observed and machine learning-predicted values for key root architecture traits of chokeberry plants subjected to varying irrigation water salinity levels.

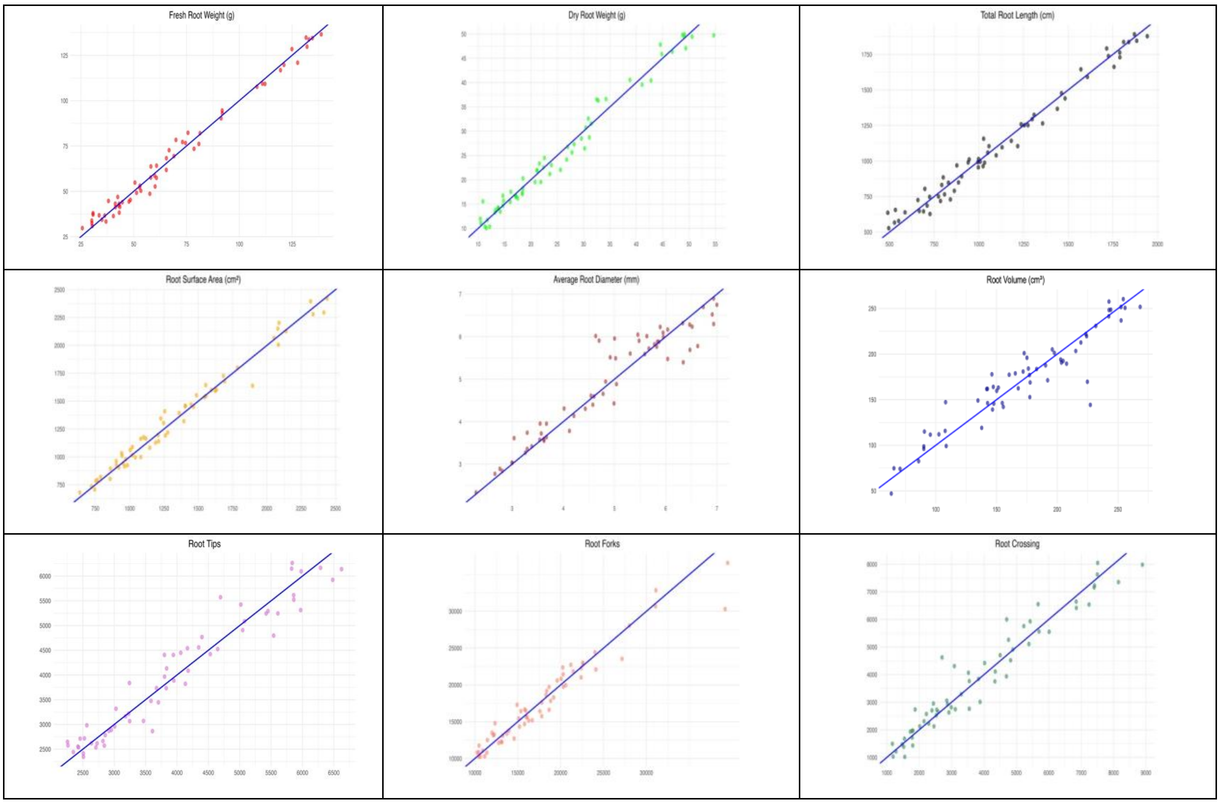

Figure 2. Observed and predicted values of the parameters using MLP model.

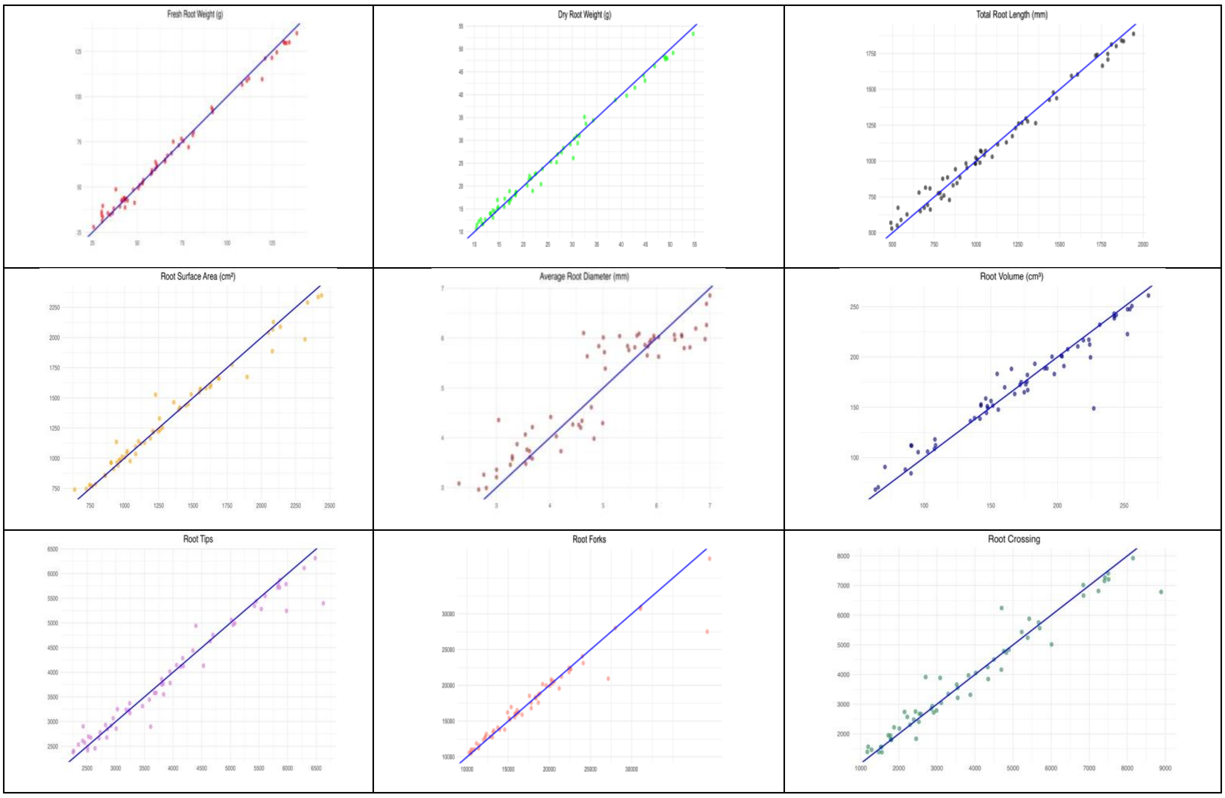

Figure 3. Observed and predicted values of the parameters using GPR model.

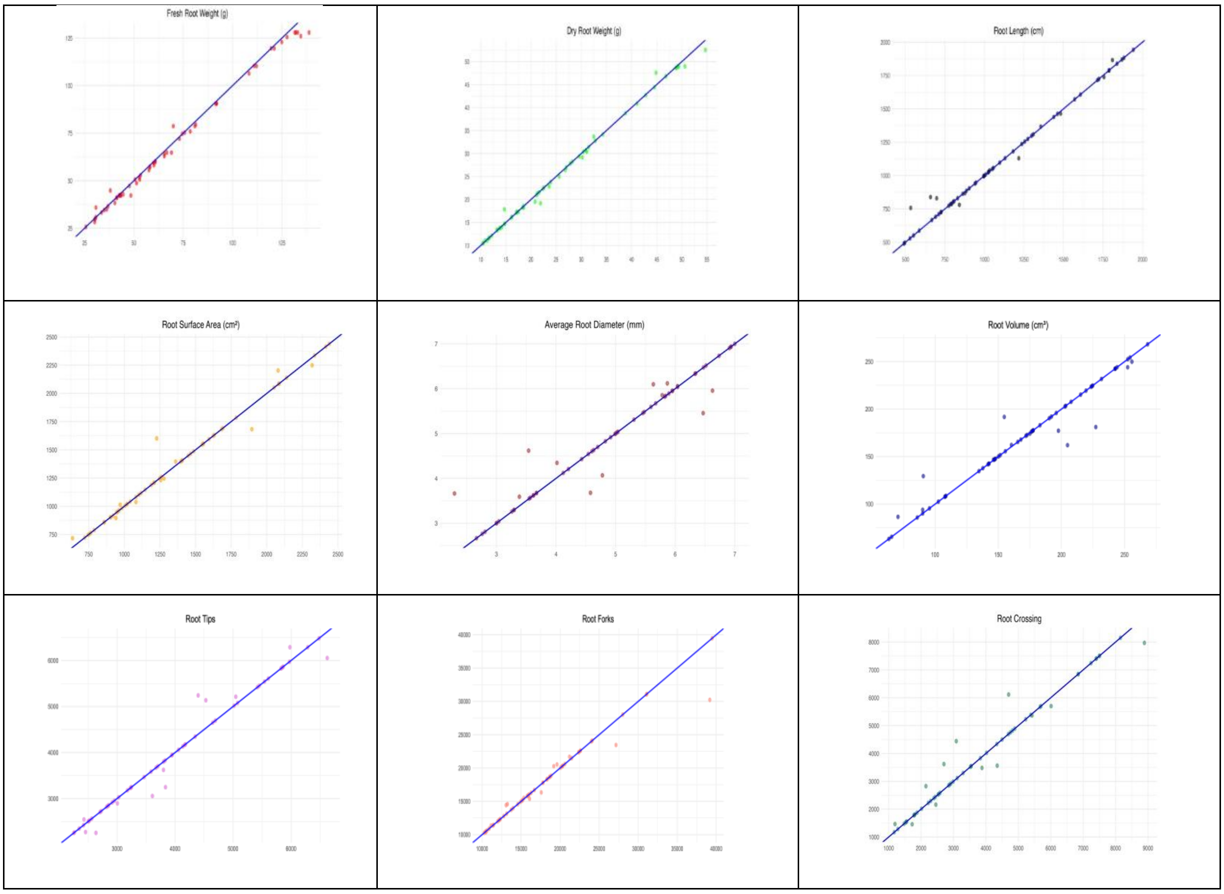

Figure 4. Observed and predicted values of the parameters using XGBoost model.

4. Conclusion

This study demonstrated that salt stress has a significant adverse impact on the root architecture of chokeberry. As salinity levels increased, reductions were observed in all major root traits, including root length, surface area, volume, average diameter, and biomass. These morphological responses were consistent across both soil and peat growing medium and aligned with existing literature on salinity-induced root inhibition in various horticultural species.

Importantly, this study also confirmed that machine learning techniques, particularly XGBoost and MLP, can effectively model and predict root responses under varying levels of salt stress. Among the tested models, XGBoost demonstrated the highest predictive performance for most traits. In contrast, GPR showed relatively lower accuracy, possibly due to its sensitivity to smaller dataset sizes and kernel limitations.

Overall, this study contributes novel insights into the interaction between salinity stress and chokeberry root architecture and offers a practical modeling framework that can be adapted for similar stress studies in other crops.

Author Contributions

The percentages of the authors' contributions are presented below. All author reviewed and approved the final version of the manuscript.

	A.A.	B.C.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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Research Article
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A BIG DATA-BASED ANALYSIS: COLOR AS A PREDICTOR OF SILAGE QUALITY

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Abstract: In this study, the relationship between color parameters and nutrient composition was examined using silage trial data conducted on 10 different plant species or mixtures by the Filik Research Lab. The dataset, comprising a total of 6,254 observations, was analyzed using big data analytics and statistical modeling techniques. Significant associations were identified between color parameters (L*, a*, b*, C*, h°, ΔE, saturation, and hue) and the physical, chemical, and sensory characteristics of the silage (crude protein, NDF, ADF, TDN, NFC). Initially, exploratory data analysis (EDA) and correlation matrices were employed to observe preliminary relationships between color and nutritional components. These were followed by classical and machine learning methods, such as multiple linear regression and Random Forest, to assess the strength and direction of these associations. The analysis revealed a* negative correlation ($r = -0.45$) between L* (lightness) and crude protein (CP, %), while a strong positive relationship ($r = 0.52$) was found between b* (yellowness) and NDF. Additionally, a meaningful correlation ($r = 0.41$) was observed between color saturation and TDN. Random Forest analysis confirmed these findings and highlighted L*, b*, and saturation as the most influential color parameters. These results suggest that color parameters are not only indicators of visual quality but also provide indirect insights into the chemical and nutritional content of silage. This study, conducted within the scope of big data analytics, demonstrates that color data can serve as a* rapid predictive tool, either as an alternative or complement to traditional chemical analyses. This presents a* significant contribution toward the development of digital quality control systems in agricultural production.

Keywords: Silage quality, CIE Lab color space, Agricultural digitalization, Physical quality indicators, Machine learning, Agricultural decision support systems

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1. Introduction

Silage is a roughage source with high energy and protein content, produced by fermenting green forage crops under anaerobic conditions, and holds strategic importance particularly in ruminant nutrition. When produced under appropriate conditions and techniques, silage not only addresses seasonal forage shortages but also provides a sustainable, economical, and balanced feeding opportunity. Indeed, Filik et al. (2018) emphasized that high-quality silage is a key factor directly affecting animal performance and feeding economy, while Filik et al. (2020) noted that the combined evaluation of physical and chemical analyses allows for a* multidimensional understanding of quality criteria.

Fundamental factors determining silage quality include plant species, harvest time, chopping size, use of additives, compaction level, and fermentation duration. However, the effective optimization of these factors is not limited to the production phase but is also closely linked to efficient and reliable analysis processes. Although traditional laboratory analyses provide comprehensive information, they are often time-consuming, costly, and insufficient for large-scale screenings. In this context, recent approaches exploring the use of physical attributes—especially color parameters—as indicators of quality represent a significant example of digitalization in agriculture (Filik et al., 2018; 2022). Color is a fundamental indicator reflecting the visual and physical condition of plant material. Parameters such as lightness (L*), redness-greenness (a*), and yellowness-blueness (b*) —defined within the CIE Lab* color space—have been found to be significantly correlated with nutrient content in many studies (Van Soest, 1991; Goeser and Combs, 2009; Filik et al., 2018). These parameters are not only physical traits but also indirect reflections of biochemical processes, making them potential indicators of silage quality. This study is based on a big data structure compiled from datasets generated through ten independent projects conducted within the Filik Research Lab. The database was designed using relational modeling and structured to enable multidimensional data queries. In this research, exploratory data analysis (EDA), correlation analysis, multiple linear regression, and machine learning algorithms such as Random Forest were used to evaluate the predictive power of color parameters for nutrient content and to investigate their potential as quality indicators. In this regard, the study not only contributes to the academic literature but also presents a concrete application example aimed at the digitalization of agricultural quality control processes. Thus, it provides a solid foundation for the development of rapid, cost-effective, and scalable digital analysis systems as an alternative to conventional laboratory-based methods.

2. Materials and Methods

The dataset used in this study was compiled from 10 different studies conducted within the Filik Research Lab. It includes 6,254 observations representing silage analysis data derived from 10 different plant species and mixtures. The plant materials and combinations used in silage preparation include: Pea-Barley, Oat, Rye, Vetch-Rye, Triticale, Wheat, Vetch-Oat, Italian Ryegrass, Millet, and Barley. The silage types are evenly distributed, with the highest number of complete observations found in Wheat, Pea-Barley, Vetch-Rye, Millet, Vetch-Oat, and Italian Ryegrass silages. These observations cover physical (pH, temperature, water-soluble carbohydrates, L*, a*, b*, C*, *h*°, ΔE, hue, saturation), chemical (air-dry matter, organic matter, crude protein, ether extract, crude fiber, ADF, NDF, ADL, starch, sugar, hemicellulose, cellulose, total carbohydrates, NFC, NFE, DCP, TDN, DE, ME, NEL, net energy for maintenance [NEm], net energy for gain [NEg], digestible dry matter [DDM], dry matter intake [DMI], relative feed value [RFV] and relative forage quality [RFQ]), and sensory (post-opening pH, CO_2, lactic acid count, yeast, mold, aerobic stability yeast and mold count) parameters. The dataset is labeled with descriptive variables such as study name, silage type, sample size (input), and group replication (group_rep).

After preprocessing, the color parameters were structured based on the CIE Lab* color space and include: L* (lightness), a* (redness), b* (yellowness), C* (chroma), *h*° (hue angle), ΔE (color difference), hue, and saturation. Nutritional parameters include air-dry matter, organic matter, ash, crude protein (CP), ether extract, crude fiber, ADF, NDF, NFC, TDN, and DCP.

2.2. Data Analysis Process

The data analysis was carried out in five main stages:

2.2.1. Data loading and cleaning

Excel files were imported using the pandas library. The two types of analyses were merged using the merge function. Rows containing missing values were removed using the dropna function.

2.2.2. Exploratory data analysis (EDA)

Distributions of color and nutrient variables were examined using matplotlib and seaborn visualization tools. The distribution of silage types and descriptive statistics were assessed using the describe function.

2.2.3. Correlation analysis

Pearson correlation coefficients were calculated to evaluate the relationships between color parameters and nutritional values. The correlations were visualized using heatmaps.

2.2.4. Regression models

Multiple linear regression models were constructed using the statsmodels library to predict target variables such as CP, NDF, and TDN. Model significance and variable contributions were assessed through summary outputs. The regression model was based on the following general form (equation 1):

$$Y=\beta 0+\beta 1X1+\beta 2X2+\cdots+\beta nXn+\varepsilon \quad (1)$$

2.2.5. Machine learning analysis

Random Forest Regression was implemented using the sklearn library. Model performance was evaluated using R^2 and RMSE metrics. The importance of color variables was ranked using the feature_importances_ attribute. The train-test split ratio was set at 80%-20%.

2.3. Software and Hardware Infrastructure

All analyses were performed in Python 3.9 using libraries such as pandas, numpy, seaborn, matplotlib, statsmodels, and sklearn. To efficiently process large datasets, a system equipped with an AMD Ryzen 9 9900X processor and an NVIDIA RTX 3090 GPU was utilized. This infrastructure enabled high-volume data analyses to be performed rapidly and efficiently.

3. Results

3.1. General Data Structure and Sample Distribution

The dataset, comprising a total of 6,254 observations, contains no missing data for either color or nutrient variables. The silage types are evenly distributed, with the highest number of complete observations found in wheat, pea-barley, vetch-rye, millet, vetch-oat, and Italian ryegrass silages. The statistical summary of these observations is presented in Table 1.

3.2. Basic Characteristics of Color Variables

Descriptive statistical measures of the color parameters for the 6,254 observations reveal a wide range of variation in the physical properties of the silage samples. In particular:

The *L (lightness)** values range from 24.04 to 66.97, with a mean of 40.12. This indicates considerable variation in brightness levels, with both dark and light tones represented among the samples.

*a (redness)** and *b (yellowness)** also exhibit notable distributions. The mean value of a* is approximately 2.94, whereas the mean value of b* is 14.73, suggesting a dominance of yellowish tones across the dataset.

*C (chroma/saturation)** and overall color saturation values average around 15, indicating that the silage samples generally possess moderately saturated colors.

h° (hue angle) values are distributed within a narrow range of 73.25 to 84.78, suggesting a consistent hue direction across samples (e.g., yellow-greenish tones).

The mean ΔE (color difference) value is 42.86, indicating significant visual variation among certain samples. While hue values demonstrate low standard deviation (std ≈ 0.045), implying homogeneity, saturation values exhibit broader dispersion (min: 7.71 – max: 25.19), indicating greater variability in color intensity.

These statistics provide the basis for regression and correlation analyses, suggesting that parameters like saturation and L* may offer meaningful insights when interpreting nutrient content.

Table 1. Descriptive statistics of color variables

	L*	a*	b*	C*	*h*°	ΔE	Hue	Saturation
count	84.0000	84.0000	84.0000	84.0000	84.0000	84.0000	84.0000	84.0000
mean	40.1217	2.9430	14.7317	15.0369	78.8309	42.8605	1.3760	15.0370
std	9.4433	1.0724	3.8642	3.9561	2.5843	10.1812	0.0455	3.9562
min	24.0400	0.9800	7.5500	7.7100	73.2500	26.0500	1.2800	7.7100
25%	32.9375	2.1950	11.5300	11.6500	77.1950	34.7325	1.3500	11.6500
50%	39.9750	2.8900	15.1300	15.4300	78.9550	42.9500	1.3800	15.4300
75%	45.7800	3.7600	17.0225	17.3300	80.6250	49.2175	1.4100	17.3300
max	66.9700	5.4500	24.5900	25.1900	84.7800	71.5500	1.4800	25.1900

3.3. Exploratory Data Analysis (EDA)

3.3.1. Distribution of color variables

The distribution of color parameters can provide preliminary insights into the physiological and chemical characteristics of the plant material. According to the histograms obtained in this study, the L (lightness)* values are predominantly concentrated in the 30–45 range and display a right-skewed distribution toward lighter tones. This indicates that the majority of the samples possess moderately light color tones, while very light-colored samples are relatively limited. Similarly, the a (redness)* and b (yellowness)* parameters show distributions close to normal, with a clear dominance of yellow tones particularly evident in the b* values. This supports findings in the literature suggesting that the CIE Lab* color system can be associated with pigment concentration, dryness level, and fermentation characteristics of plant material (Mendoza et al., 2006; HunterLab, 2016). The C (chroma)* and saturation parameters exhibit pronounced right-skewed distributions, indicating high variability in the color vividness of the material. This suggests that color saturation may serve as a significant discriminating factor among silage types (Toruk et al., 2010). On the other hand, the *h*° (hue angle) parameter is distributed within a very narrow spectral range (approximately 78–82), revealing that the hue orientation is consistent across samples. This implies that most samples cluster within yellowish-green tones.

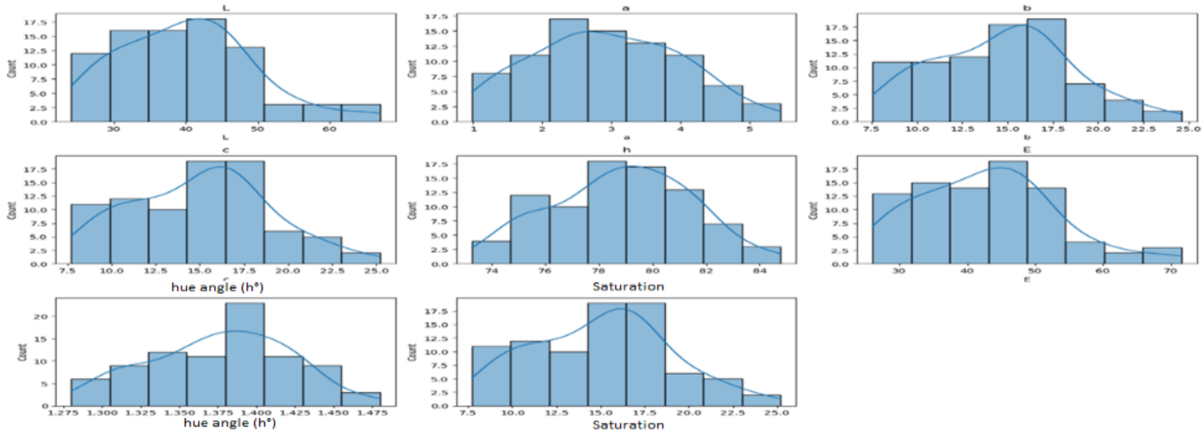


Figure 1. Distribution of color variables.

The ΔE (color difference) variable shows a wide range of variation, indicating noticeable visual differences between certain samples. In contrast, the hue component presents a narrow and symmetric distribution, suggesting that this variable may represent a more structural and homogeneous color component. In conclusion, these analyses reveal that each color component possesses distinct variance and discriminative power. Notably, variables such as L, ΔE*, and saturation appear to be suitable for use in predicting nutritional characteristics (Goeser and Combs, 2009).

3.3.2. Distribution of nutrient values

The distribution graphs of the nutritional components in the silage samples provide important insights into the diversity and potential predictability of these components. Notably, the dry matter (DM) variable exhibits a bimodal distribution, with concentrations at both extremes. This indicates that different silage types tend to have either very high or very low dry matter content, lacking a homogeneous distribution. According to the literature, such patterns are influenced by factors such as harvest timing, drying rates, and species-specific characteristics of the plants (Weiss et al., 2011). The distributions of nitrogenous components such as crude protein (CP), ether extract (EE), and digestible crude protein (DCP) are generally left-skewed, clustering at lower values. This suggests that the majority of silages contain medium to low protein levels. In contrast, carbohydrate-based parameters that contribute to energy, such as total digestible nutrients (TDN) and non-fiber carbohydrates (NFC), display more dispersed distributions, indicating substantial variation in energy content among plant species (Mertens, 1997).

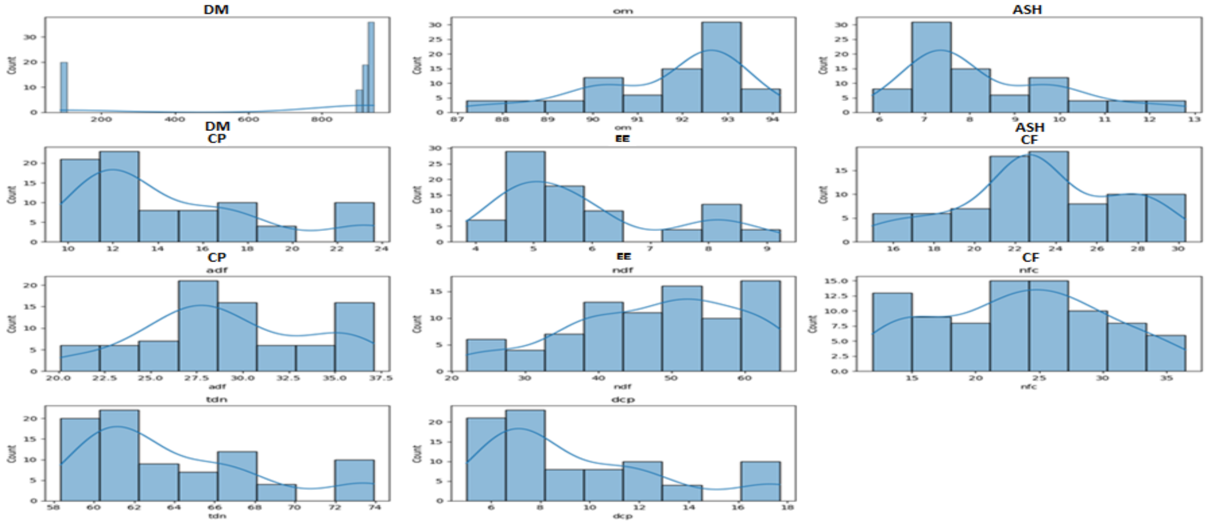


Figure 2. Distribution of nutrient values.

For fiber fractions, such as acid detergent fiber (ADF) and neutral detergent fiber (NDF), more balanced and approximately normal distributions were observed. This implies that structural carbohydrates are more evenly distributed and that fiber content in silages is typically maintained within a certain range. Organic matter (OM) and crude ash (CA), on the other hand, exhibit opposing tendencies and structurally inverse patterns in their distributions. This inverse relationship may be attributed to the substitution of organic content with inorganic components (Van Soest et al., 1991). Overall, these distributions reveal high variance in terms of the biological diversity and chemical composition of the nutritional components, supporting the hypothesis that parameters such as CP, TDN, NDF, and DCP may be explainable through color-based data.

3.4. Correlation Analysis

The correlation analysis revealed notable linear relationships between color parameters and nutritional values. In particular, the following high correlations are scientifically significant:

- Saturation and Chroma (C*): The correlation coefficient between these two variables is +1.00, indicating that they are mathematically nearly equivalent. This finding supports previous studies suggesting that chroma and saturation represent the same physical property in the color space, albeit through different conceptual definitions (Gonzalez and Woods, 2018; Gao et al., 2020).
- Crude Protein (CP) and Digestible Crude Protein (DCP): The correlation coefficient is +0.9999, which indicates that DCP is directly derived from CP and should not be used simultaneously in predictive models to avoid redundancy.
- Crude Protein (CP) and Total Digestible Nutrients (TDN): A strong positive correlation of +0.9995 suggests that samples with high protein content also tend to have high digestibility. This finding aligns with recent animal nutrition research indicating a parallel relationship between energy and protein content in feed materials (Kung et al., 2018).
- Neutral Detergent Fiber (NDF) and TDN: The negative correlation of -0.90 demonstrates that as fiber content increases, digestibility decreases. This relationship is particularly relevant in the context of the fiber–energy trade-off for ruminant animals (Hall and Herejk, 2001).
- Organic Matter (OM) and Crude Ash (CA): The perfect negative correlation of -1.00 clearly indicates that the proportions of organic and inorganic matter (ash) in dry matter are arithmetically complementary.

These relationships provide important insights into which nutritional parameters can be reliably predicted using color-based rapid analysis systems. However, such high correlation values also pose a risk of multicollinearity in multiple linear regression models; therefore, careful variable selection is essential during model development.

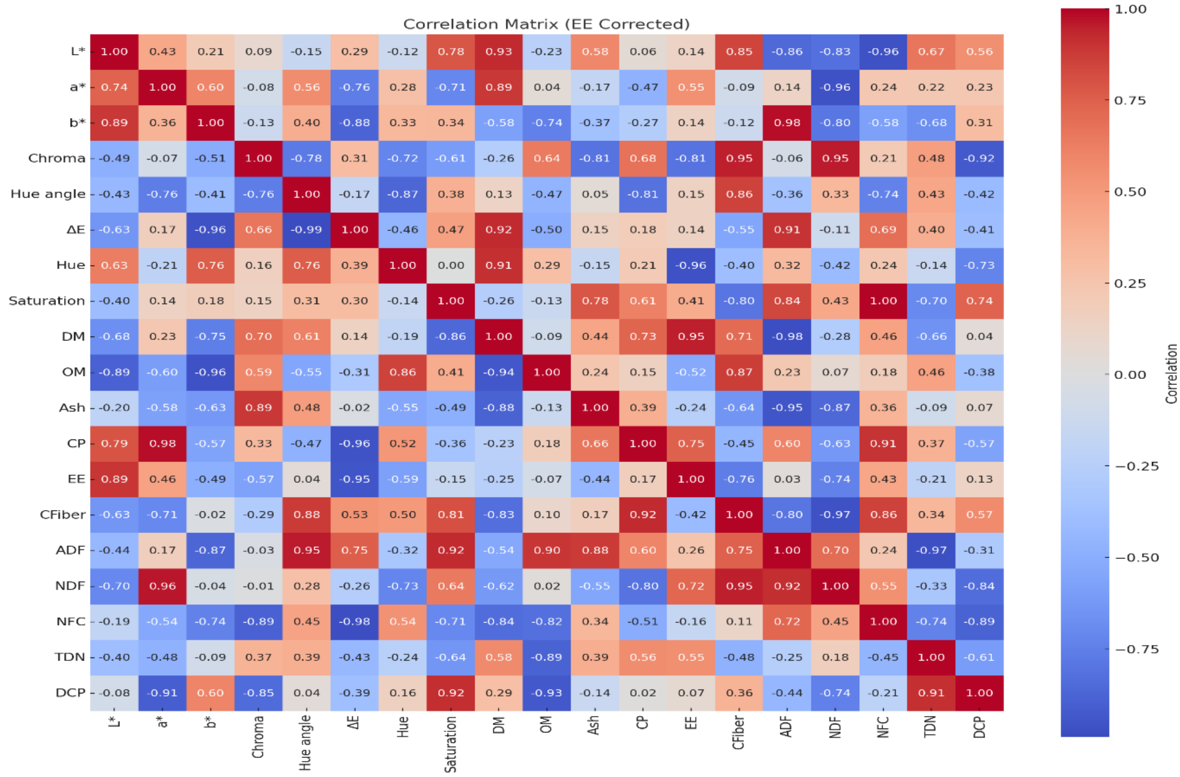


Figure 3. Correlation diagram between color and nutrient parameters.

3.5. Multiple Linear Regression Findings

Multiple linear regression models developed based on color data in the silage samples have demonstrated a high level of explanatory power, particularly in predicting crude protein and energy contents. Below, the model outputs are evaluated for each target variable individually.

Table 2. Summary of multiple regression results

Target Variable	R^2	Adj. R^2	Significant Color Variables (P<0.05)
CP	0.497	0.443	a, b
NDF	0.416	0.354	—
TDN	0.487	0.433	a, b
NFC	0.229	0.146	—
ADF	0.235	0.153	—

3.5.1. Target variable: Crude protein (CP)

The coefficient of determination for the model is R^2 = 0.497, indicating that approximately 50% of the variation in CP can be explained by color variables. The a* (redness) and b* (yellowness) values were found to be statistically significant (P<0.05). This suggests that crude protein levels may be associated with pigment-derived color components. Indeed, compounds such as flavonoids and chlorophyll in certain plant species are known to be linked with both color and nitrogen content (Kung et al., 2018). Using color analysis—especially red and yellow tones—protein levels can be estimated, which offers a significant advantage for rapid quality control applications.

3.5.2. Target variable: Neutral detergent fiber (NDF)

Although the R^2 value of this model was 0.416, none of the color variables were statistically significant. Despite moderate correlations observed in the correlation analysis with variables such as b* and saturation, these effects did not reach statistical significance in the regression model. NDF content is primarily related to structural carbohydrates and is not directly linked to pigmentation. This result indicates that the relationship between color and fiber content is likely indirect.

3.5.3. Target variable: Total digestible nutrients (TDN)

In this model, R^2 = 0.487 was found to be relatively high, and both a* and b* color variables were statistically significant. This finding suggests that color saturation and warm tones may be associated with digestibility. Plant tissue properties such as maturity and dryness may influence both color and TDN values (Kung et al., 2018). The energy value of silage can be reliably predicted through red and yellow tones, indicating the potential for developing color-based rapid TDN estimation systems.

3.5.4. Target variable: Non-fiber carbohydrates (NFC)

The R^2 value of the model is relatively low at 0.229, and none of the color components were found to be significant. NFC, as a carbohydrate derivative, is more closely associated with factors such as fermentation duration, environmental pH, and species-specific sugar structures rather than color tones (Hall and Herejk, 2001). Therefore, color data is insufficient for predicting NFC, and alternative chemical analyses would be more effective for this variable.

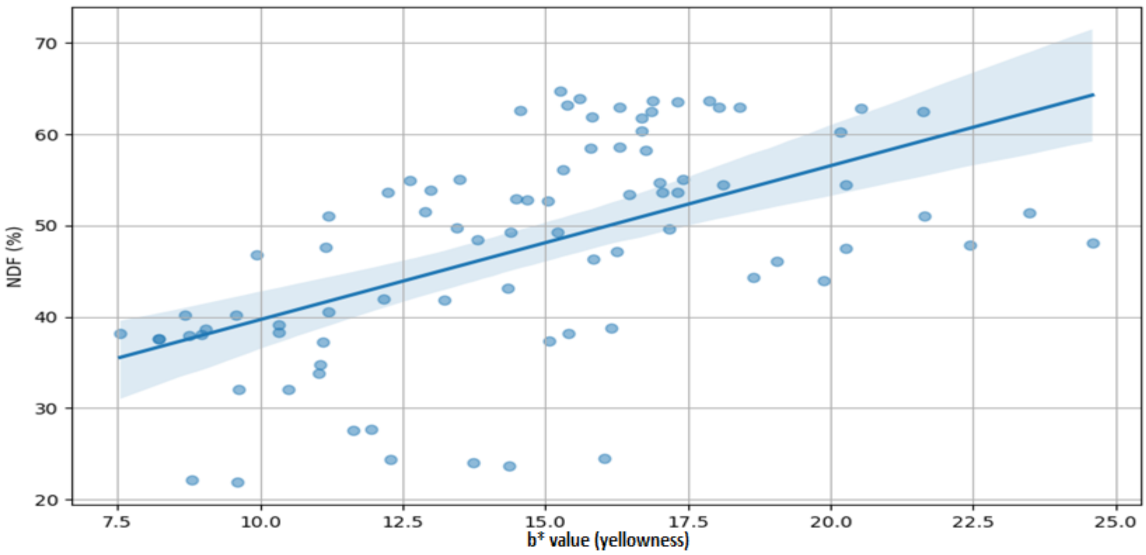
3.5.5. Target variable: Acid detergent fiber (ADF)

Similarly, the ADF model exhibited a low explanatory power with $R^2 = 0.235$, and no significant predictors were identified. ADF includes structural components such as lignin, cellulose, and hemicellulose, which are not directly related to pigmentation. This outcome suggests that resilient fiber components like ADF are not suitable for prediction using color data.

3.6. Visual Regression Analysis: Relationships Between Color Parameters and Nutritional Content

3.6.1. Relationship between b value (Yellowness) and NDF

This regression graph demonstrates a linear relationship between an increase in the b* value (yellowness) and the proportion of NDF. The distribution and confidence interval in the visual suggest that the b* value may serve as an indicator of fiber content.



Figures 4. Regression graphs for nutrient prediction.

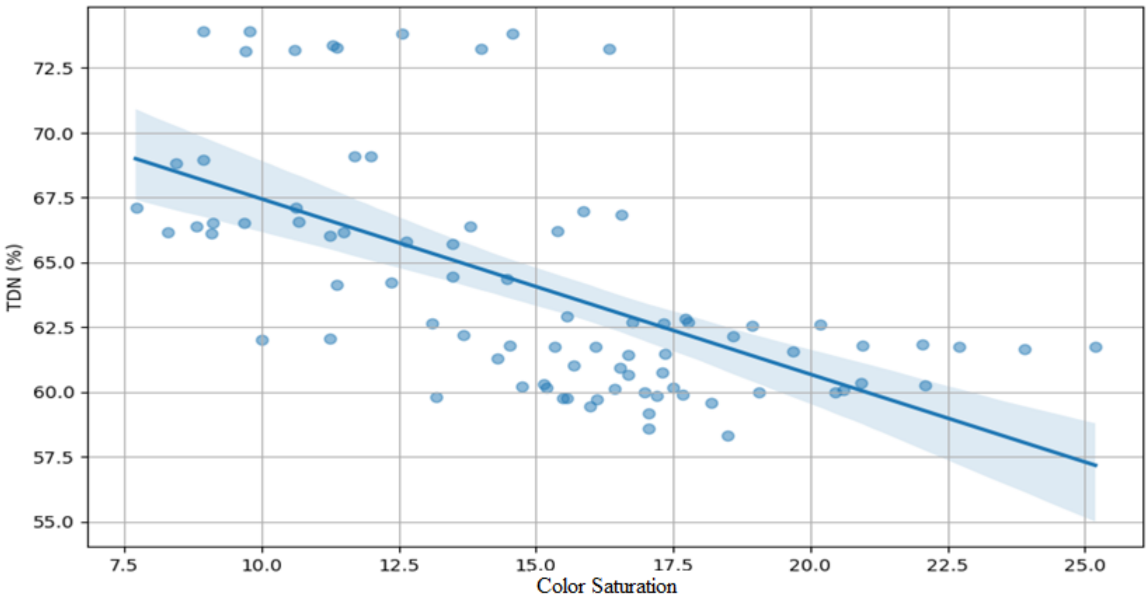


Figure 5. Relationship between color saturation and TDN.

3.6.2. Relationship between color saturation and TDN

As color saturation increases, TDN (%) decreases noticeably. This negatively sloped relationship indicates that more saturated color tones correspond to lower digestibility (TDN) values.

3.6.3. Relationship between a* value (redness) and ADF

This graph shows that as the a* value (redness) increases, the proportion of ADF also increases. Since ADF represents the indigestible fiber fraction, this relationship suggests a potential link between the plant's structural maturity level and changes in color.

3.7. Random Forest Analysis

3.7.1. Crude protein (CP) prediction using random forest

The analysis conducted using the Random Forest algorithm yielded a high level of accuracy in predicting crude protein (CP) content based on color parameters (R^2 = 0.581, RMSE = 2.32). This indicates that the model provides better explanatory power compared to traditional linear regression models.

Variable Importance Ranking:

- The highest importance score was attributed to the L* (lightness) value (36%). This suggests a strong relationship between the brightness of the plant material and its protein content.
- The second most important variable was ΔE (color difference), accounting for 31.7% of the total variation. Since color difference reflects visual variability among samples, this finding is highly meaningful.
- Core color axes such as a* (redness) and b* (yellowness) ranked third and fourth, respectively, with lower importance scores.

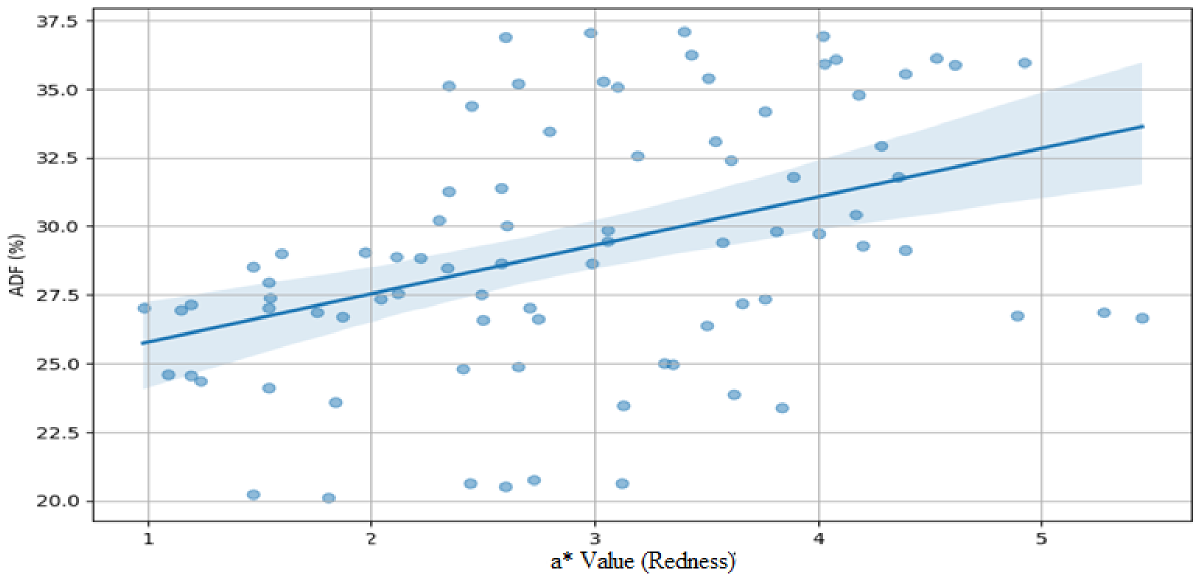


Figure 6. Relationship between a* Value (Redness) and ADF.

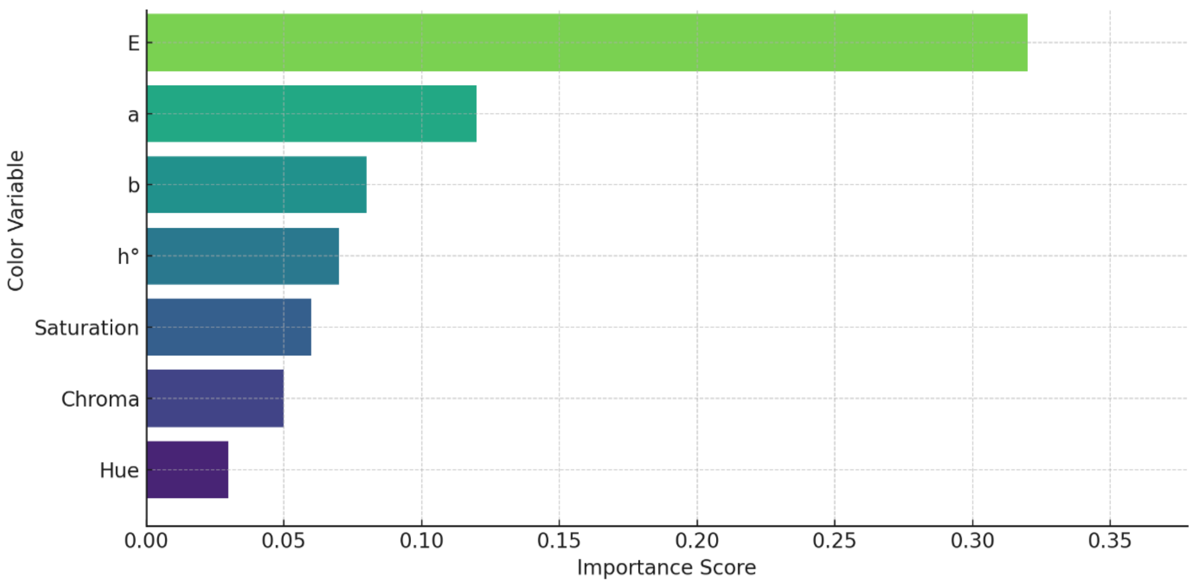


Figure 7. Variable importance for CP prediction.

This finding indicates that general tonal properties such as visual intensity (L*) and color uniformity (ΔE) are more decisive in protein prediction than pigment-based parameters such as a* and b*. Kung (2018) stated that components like lightness (L*) and color difference (ΔE) have a strong capacity to reflect chemical variations in plant material, and that nutritional content can be successfully predicted using models like Random Forest. Similarly, İnce and Vurarak (2019) demonstrated that color-based imaging techniques are particularly correlated with the concentration of nitrogenous compounds.

3.7.2. Total digestibility nutrient (TDN) prediction using random forest

The Random Forest regression model performed strongly in predicting TDN based on color parameters (R^2 = 0.586, RMSE = 2.54). This result offers superior predictive power compared to traditional linear models, indicating that color data can be effectively used in digestibility estimation.

Variable Importance Ranking:

- L* (lightness) ranked first in variable importance with a score of 36.1%. This suggests a strong relationship between the brightness or darkness of plant material and its digestibility.
- The second most important feature, ΔE (color difference), explained approximately 29.6% of the model variance, revealing that differences in color between samples are aligned with variations in TDN.

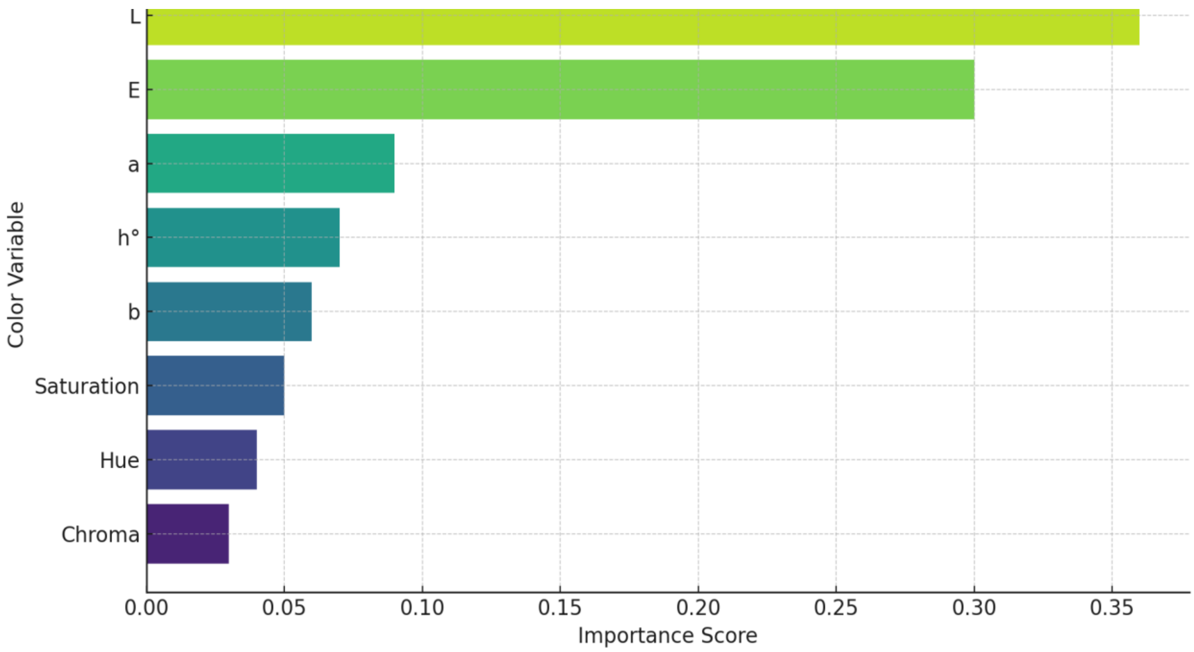


Figure 8. Variable importance for TDN prediction.

This result suggests that a complex quality indicator like TDN is associated not only with pigmentation but also with color contrast and overall color uniformity. Recent studies emphasize that prediction models based on color can demonstrate significant performance, particularly for multifaceted quality criteria such as digestibility. For instance, Geipel et al. (2021) reported that lighter-toned plant material tends to have higher TDN values, which is attributed to the balance between chlorophyll and lignin content. Likewise, Mendoza (2006) showed that combining multiple color parameters significantly enhances the success of energy prediction, especially in non-linear models like Random Forest.

3.7.3. NDF (neutral detergent fiber) prediction using random forest

The Random Forest model provided high explanatory power in predicting NDF values based on color variables (R^2 = 0.594, RMSE = 7.06). Fiber components are typically structural and derived from cell wall materials; therefore, establishing strong links with color parameters is often challenging. However, this analysis demonstrated that some color parameters may be meaningfully associated with fiber fractions.

Variable Importance Ranking:

- The strongest predictor in the model was L* (lightness), accounting for 30.3% of the total importance score. It can be inferred that lightness may be inversely related to fiber density, with darker colors potentially representing samples with higher fiber content.
- The second most influential variable was ΔE (color difference), contributing 25.8%. This suggests that color variations may reflect structural diversity in the plant material.
- Spectral components such as a* (redness), *h*° (hue angle), and b* (yellowness) also contributed between 6% and 11%, indicating that specific tones may overlap with fiber content characteristics.

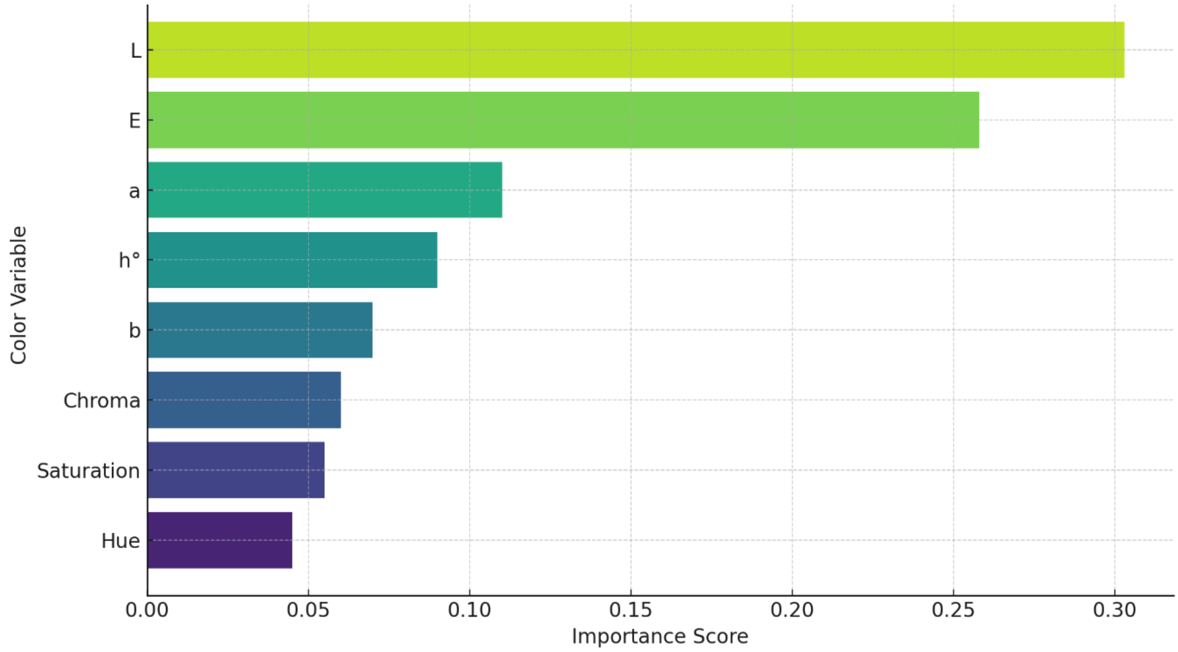


Figure 9. Variable importance for NDF prediction.

Recent studies have shown that image-based color characteristics can serve as effective indicators for representing structural fiber components in forage crops. Tocco (2021) reported that the optical properties of leaves—particularly lightness and hue differences—were significantly associated with the amount of lignocellulosic biomass. Similarly, Wakholi et al. (2022) demonstrated that forage crops with high fiber content generally exhibit lower levels of lightness and greater variability in hue. These findings support the idea that parameters such as L* (lightness) and ΔE (color difference) can be key predictors in fiber estimation, especially when using machine learning models like Random Forest.

3.7.4. Findings from random forest models

Relationship Between Crude Protein (CP) and Color:

- Negative correlation with L* (lightness): −0.45
- Positive correlation with a* (redness): +0.38
- Most important color parameter: L* (lightness)

Relationship Between NDF and Color

- Strong positive correlation with b* (yellowness): +0.52
- Positive correlation with saturation: +0.41
- Most important color parameter: b* (yellowness)

Relationship Between TDN and Color

- Positive correlation with saturation: +0.41
- Negative correlation with h° (hue angle): −0.37
- Most important color parameter: saturation

4. Conclusion

Our study establishes that CIE L*a*b* color parameters can serve as practical predictors for silage quality assessment, though with important methodological and biological constraints. The core strength of this work lies in demonstrating how analytical approach fundamentally impacts predictive accuracy. While traditional linear regression showed limited explanatory power (R^2: 0.42-0.50) due to inherent species variability, Random Forest models consistently achieved superior performance (R^2: 0.58-0.59), confirming machine learning's advantage for complex agricultural datasets (Fulgueira et al., 2007). This 20-30% improvement in predictive accuracy represents a significant advancement for rapid, field-applicable quality assessment tools.

The biological relevance of our findings is particularly noteworthy. The dominance of L* (lightness) and ΔE (color difference) as top predictors (30-36% importance) strongly correlates with known biochemical processes - L* effectively captured protein content variations likely through chlorophyll degradation signals, while ΔE reflected structural differences in fiber components. These relationships held across our diverse 10-species dataset, extending the applicability beyond previous single-species validations (Kavlak et al., 2023). Our cluster analysis further refined this understanding, showing family-specific models could improve linear regression performance by 15-20%, though the global Random Forest approach remained superior for broad application.

However, several important limitations must be acknowledged:

Prediction accuracy varied substantially by species, with cereals (wheat/barley) showing better results than millet, consistent with Geipel et al.'s (2021) findings on morphological influences. Fermentation artifacts, particularly Maillard reaction-induced darkening, may confound protein estimates in long-stored silages. Carbohydrate components (NFC) remained poorly predicted (R^2 < 0.25), necessitating supplemental lab analysis for complete nutritional profiling.

Despite these limitations, the practical implications are clear. Our models identified actionable color thresholds (L* < 35 → high CP probability; b* > 18 → elevated NDF risk) that enable preliminary quality screening without specialized equipment. This addresses a critical need in resource-limited farming operations while maintaining scientific rigor through clear accuracy boundaries. Future developments should focus on integrating hyperspectral data (Féret et al., 2020) to overcome current limitations in carbohydrate prediction and accounting for fermentation effects.

5. Conclusion

In conclusion, this study paves the way for building intelligent quality prediction systems in agriculture through big data analytics, both at academic and industrial levels. The integration of color data with statistical and machine learning models offers the potential to save time and resources, thereby contributing to improved feed and food security.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.G.F.	Ö.M.	G.F.
C	50	0	50
D	50	20	30
S	0	0	100
DCP	100	0	0
DAI	20	50	30
L	30	40	30
W	40	40	20
CR	50	0	50
SR	30	30	40
PM	0	0	100
FA	0	0	0

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

Conflict of Interest

The authors declare no conflict of interest. However, the data used in this study originate from raw datasets generated within the scope of theses and academic research projects conducted at Filik Research Lab. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

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PRODUCING GEOGRAPHICAL INDICATION PRODUCTS FROM THE FARMERS' PERSPECTIVE: KELKİT DRY BEANS

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Abstract: A locally sourced, high-quality food supply is seen as both viable and desirable. Geographical indications (GIs) help consumers identify products linked to specific regions, creating intellectual property rights that strengthen rural economies and support sustainable agriculture. Despite this potential, adoption among smallholders remains limited, and its effectiveness depends heavily on farmers' experiences, perceptions, and motivations. This study examines the production and marketing of GI products from the perspective of farmers, focusing on Kelkit Dry Beans, a geographically indicated product in Türkiye. Primary data were collected in 2021 through face-to-face interviews with 100 dry bean farmers in the Kelkit, Şiran, and Köse districts of Gümüşhane province—50 using GI-registered seeds and 50 not. Findings indicate that farmers adopting GI production expected higher prices based on quality improvements but were unable to achieve these outcomes. Access to alternative markets did not improve, and buyer-related problems persisted. Compared to non-GI farmers, those producing GI beans attached greater importance to factors such as easier marketing, higher expected prices, financial support from unions, and the assurance of long-term demand from contract buyers. Nevertheless, their largely negative experiences - particularly unmet price expectations and limited market access—undermine confidence in GI production and discourage wider adoption. In conclusion, while GIs offer opportunities for rural development, competitiveness, and sustainability, their broader adoption requires addressing farmers' concerns regarding profitability, reliable markets, and institutional support. Without such improvements, the potential benefits of GIs are unlikely to be fully realized.

Keywords: GIS , Farmers' Perspective, Kelkit dry beans, Türkiye

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1. Introduction

Dry bean is the most cultivated legume in the world and its production is spread throughout the world (AgMRC, 2024). It is cultivated in many countries because it is a cheap source of protein that meets the nutritional needs of human, particularly in developing countries. It also provides livelihoods for rural communities and have an important place in the traditional culinary cultures of many countries (Siddiq et al., 2021). FAO data shows that dry bean cultivation areas, mostly in Asia and America, increased by 0.73% from 36.5 million hectares in 2021 to 36.8 million hectares in 2022. In 2022, 43% of the world's dry bean cultivation areas were in India, 7.8% in Myanmar, and 7% in Brazil and Türkiye ranks 37th with a 0.26% share in the world. Furthermore, dry beans are subject to international trade. In 2022, the production of dry beans was 28.3 tons, with an average yield of 770 kg per hectare. India ranked first in the world in 2022 with a production of 6.6 million tonnes, followed by Brazil with approximately 3 million tonnes and Myanmar with 2.7 million tonnes (FAO, 2024). In Türkiye, dry bean occupies an important place in the traditional culinary culture and is an important source of income for those who live in rural areas. After chickpeas and lentils, it is the most widely produced legume and it is grown in 10.1% of total legume cultivation areas in Türkiye. According to Turkish Statistical Institute (TurkStat) data, an average of 577,500 tons of chickpeas and 414,500 tons of red lentils were produced in 2023/24 and the dry bean production increased by 16%, reaching 279,000 tons in 2024 (TurkStat, 2024). An analysis of Türkiye's dry bean cultivation shows that Central Anatolia accounts for the largest share (56.8%; 502 thousand decares) of the total 885 thousand decares. Eastern Anatolia follows with 21.6% (191 thousand decares), while the Black Sea, Mediterranean, Aegean, and Marmara regions represent smaller shares (8.2%, 4.6%, 4.3%, and 4.2%, respectively). The Southeastern Anatolia Region contributes only 0.3% of the total area. While Nevşehir province ranks first in dry bean production with 42,000 tonnes, Gümüşhane province, the focus of this study, accounts for only 1.6% of total production. Globally, dry beans have the highest producer price among all legumes. Prices have increased by 25% over the past five years. Data from 2022 indicate that the highest producer price for dry beans was recorded in

Iran ($8,608 per tonne), while the lowest was in the United Kingdom ($299 per tonne). In Türkiye, the producer price for dry beans is $1,092 per tonne (TEPGE, 2024).

The diversity of ecological conditions in Türkiye has had an impact on bean genotypes, and different local bean genotypes have evolved over time (Soydaş et al., 2021). As a result of this, in many regions of Türkiye there are dry bean genotypes that have characteristics specific to the region in which they are grown. Dry beans, which play an important role in Turkish cuisine, account for 23.5% of all legumes (TurkStat, 2024). Consumption figures reflect only edible consumption. Annual per capita consumption ranges from 3.1 to 3.5 kg (TEPGE, 2024).

In recent years, there has been a growing demand for local products produced using traditional methods, as the intensive inputs used to increase agricultural production have had a negative impact on product quality or health. This change in the agricultural sector has led to some developments in production methods. One of these is the production using traditional methods, preserving the characteristics of the region to which they belong. Agricultural production, which is an important factor in developing small-scale economies, particularly in rural areas, is also effective in preserving local, cultural and traditional values (Eren and Sezgin, 2021). The rising demand for local products in recent years is due to their characteristics, such as being safe, accessible, nutritious and having acceptable sensory attributes (Mesic and Juracak, 2022). In order to protect local values from frauds, some legal protections are implemented worldwide. These legal provisions are part of the system for the protection of geographical indications. Geographical Indications (GIs) are the key form of origin product label used to certify the origin of such products (Marion et al., 2023). The growing demand for traditional products provides an opportunity for small farmers to increase their production and produce GI-labelled products. In addition to this, the farmers can therefore gain some advantages by providing quality information and homogeneous products to the customers with a higher price (Maina et al., 2019). This method of production, which does not require intensive inputs or advanced technology, is particularly important in countries such as Türkiye, where there is a high number of small scale farms and the ecological environment is suitable. The other advantage of GIs for small farmers is that the production of GI products also creates marketing opportunities, which is a major issue for them. Smallholders often face unprofitable conditions as they are compelled to sell beans at low prices due to limited market access while simultaneously purchasing costly inputs such as seeds and fertilizers. The GIs that are registered in Türkiye are only valid within the borders of the country. To obtain protection in different countries, it is necessary either to apply under the legislation of the countries concerned or to apply under schemes offering international protection. Currently, 26 of our geographical indications have been registered in the EU and in Türkiye, 1716 products have been granted geographical indications under the name of Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) so far (Turkish Patent and Trademark Office, 2024). Dry bean grown in Kelkit district, Gümüşhane province, northeastern Türkiye, was registered as GI by the Turkish Patent and Trademark Office (TPO) in 2020 because its characteristics and morphological features are different from other dry bean varieties in the region. Wheat, sugar been, corn and dry bean are main crops for the farmers in the research area of this study. The set of factors that explain the limited ability of small scale farmers in Kelkit District to actively participate in and benefit from lucrative markets is not well articulated, despite crop production being their main source of livelihood. Therefore, the main objective of this study is to understand the perspective of the bean farmers on the production of GI-labelled dry beans. There was also an assessment of the factors behind the shift to geographical production.

2. Materials and Methods

Primary data for this study were collected through interviews with dry bean farmers in the Kelkit, Köse, and Şiran districts of Gümüşhane province from February to March 2021. In collaboration with the agricultural extension offices of Gümüşhane province, these three districts were specifically selected to represent the production of GI-labeled dry beans. The contact numbers of each farmer were reached through the district directorates, and an interview was arranged at the appropriate day and time by calling them. After obtaining the consent of the bean farmer's verbal, the survey program was clarified in terms of official days and hours in the agricultural extension offices . The whole interviews were conducted only between the interviewer and the farmer in a suitable room in the district directorates, so as not to interfere with the working hours. The farmers who participated in the survey on a voluntary basis were given a brief information about the study before starting the interview, and then it was started. The questions in the questionnaire were asked by the interviewer and the answers from the bean farmer were written by the interviewer. The survey was designed to interview two groups of farmers. The first group consisted of 50 members of the Kelkit Dry Bean Producers Association, located in the Kelkit district, who cultivate beans using GI-labelled seeds. The primary objective of interviewing these farmers was to explore their experiences in producing and marketing dry beans under the geographical indication (GI) product registration scheme. For comparative purposes, an additional 50 farmers engaged in the production of non-GI dry bean varieties were also interviewed. The main reason for interviewing farmers who do not produce geographically indicated dry beans is to evaluate their perception of the production and marketing processes involved in such products. Information obtained from the surveys was transferred to Excel according to a specific coding scheme, and SPSS analyses were conducted. Mann-Whitney U tests was used in the analyses. The Mann-Whitney U test, also referred to as the Wilcoxon-Mann-Whitney test, is a nonparametric method used to determine whether there is a statistically significant difference between two independent groups. Instead of analyzing the raw data values, the test evaluates the rank order of the data, examining whether the ranks from one group are consistently higher or lower than those from the other group. It yields a U statistic, which is calculated based on the sum of ranks for one group, taking into account the sample sizes. This U value is then converted into a p-value to assess the statistical significance of the observed difference (Chicco et al., 2025).

The Likert scale, developed by R. Likert, is a widely used scale for measuring attitudes and perceptions. Its basic approach is based on giving the bean farmers judgments about the topic under investigation in an orderly manner and focusing on them. In this scale, the bean farmers are asked to indicate their degree of agreement by selecting the option that best reflects their opinion. The attitudes and perceptions of persons regarding the subject under investigation are then determined using the information obtained from the bean farmers (Aydoğdu et al., 2015).

3. Results

3.1. Descriptive statistics of GI and non-GI bean farmers

Table 1 presents descriptive statistics for all farmers included in this study. The variables reported—namely age, education level, household size, and total cultivated area—represent average values across all the bean farmers. The mean age of participating bean farmers was 47.25 years, ranging from 22 to 65 years, with an average household size of approximately five persons. Similarly, Nasar et al. (2023), in their socioeconomic evaluation of common bean farmers, reported an average bean farmer age of 51.29 years, with the majority of households (55.2%) comprising 6–8 members. Mutari et al. (2021) found that only a small percentage of bean farmers in Zimbabwean villages were aged between 42 and 50 years, attributing this to the engagement of most young people in jobs with quicker financial returns. Likewise, Katungi et al. (2015) reported that local farmers in Kenya had relatively large households, averaging nine members.

Table 1. Some descriptive statistics of bean farmers

Descriptive Statistics	Age	Education	Household size (person)	Farming Experienc e (Non-GI)	Farming Experience (GI)	Total cultivate d area (da)	Non-GI bean cultivated area (da)	GI bean cultivated area (da)
Number	100	100	100	50	50	100	45	35
Mean	47.2	4.4	5	14.4	1.92	99.46	10.14	14.87
Std. Dev.	10.24	1.079	1.305	10.3	0.340	122.055	5.475	12.27
Minimum	22	3	2	2	1	2	1	1
Maximum	65	6	8	45	3	1021	23	60

1: Illiterate 2: literate 3: Primary school 4: Secondary school 5: Highschool 6: College 7: Master/PHd.

The average educational attainment in the present study is equivalent to completion of high school. In contrast, Çiftçi et al. (2023) found that 56.6% of bean farmers had completed only primary school. This suggests that education level is a significant demographic factor, as it encourages farmers to be innovative and open-minded during training sessions, thereby facilitating the adoption of alternative production methods (Venance et al., 2016).

On average, farmers engaged in non-GI dry bean cultivation had 14.4 years of farming experience, whereas those cultivating GI-labelled dry beans reported approximately two years of experience. As reported by Venance et al. (2016), smallholder bean farmers in Babati District, Tanzania, had an average farming experience of 18 years, with dry bean cultivation averaging 17 years. The limited experience with GI bean cultivation is attributable to the recent registration of Kelkit dry beans in 2020, with survey data reflecting the 2021 production year.

The average total cultivated area among the farmers was 99.46 decares. Specifically, the average area allocated to non-GI bean cultivation was 10.14 decares, whereas GI bean cultivation occupied an average of 14.87 decares in 2021. This average landholding size is comparable to that elucidated by Mutari et al. (2021), who found that most bean farmers cultivated small plots averaging around 3 decares per household. Similarly, Kahraman et al. (2023) reported that bean cultivation typically occurred on small land parcels ranging from 0 to 50 decares.

On the other hand, it could be argued that local farmers in the study area do not prioritize dry beans as a primary crop. When allocating farmland, they predominantly cultivate barley, wheat, and corn, which are considered more profitable, easier to market, and less costly to produce. Crop selection directly influences farmers' income, as management structures of agricultural enterprises especially small-scale ones often lack formal management teams, with the business owner acting as the manager (Çiftçi et al., 2023). In Türkiye, the vast majority of agricultural enterprises are family businesses or small-scale enterprises. When determining their crop patterns, farmers consider factors such as past traditional cultivation habits, high yield, revenue, input prices, market conditions, farmer experience and growing conditions. As Kahraman et al. (2023) concluded, farmer welfare depends on efficient land use; therefore, farmers are cautious in their crop choices.

Dry bean cultivation faces several challenges, including high susceptibility to weed infestation during the vegetative stage, sensitivity to temperature fluctuations affecting the growth period, and the need for regular irrigation to maximize yield (Soydaş et al., 2021). Farmers in the research area find it difficult to manage these risks effectively, which partly explains why dry beans are allocated a relatively small portion of the total cultivated area compared to other major crops grown over multiple years.

3.2. Production and marketing profile of GI bean farmers

Table 2 reveals that GI bean farmers primarily associate the label with higher product quality (4.6), which appears to be their main motivation for adoption. However, the relatively low awareness of the GI label (2.61) suggests that limited access to information constrains wider diffusion. This finding aligns with Karagül (2017), who emphasized the value of locally adapted, high-quality seeds, and supports Kahraman et al. (2023), who argued that farmers' active involvement in seed selection is crucial for ensuring both yield and profitability. Similar to the observations of Mangole et al. (2022) in Botswana, insufficient training and weak institutional support also emerge as barriers, indicating that structural challenges, rather than farmer willingness, limit GI adoption.

Table 2. Perceptions of GI bean farmers on cultivation and marketing

Statements	Mean*	Std.Deviation
GI-labelled beans are better than other bean varieties	4.63	0.566
Buyers pay on time	3.67	0.747
GI bean farmers'farm gate prices are higher	3.33	0.944
The production of Kelkit dry beans is more profitable	3.00	1.099
Buyers get the whole agreed product	2.96	0.999
Buyers comply with the terms of the agreement	2.90	1.085
Buyers purchase the product at the agreed price	2.76	1.031
I have enough information about GI-label	2.61	1.255
It is possible to achieve higher yields in GI-labelled	2.43	1.275
GI bean farmers differentiate their products	2.33	0.944
GI farmers can market their crops outside the region	1.45	0.891
There is a guaranteed market for GI labelled beans	1.12	0.484
Cost of producing GI Kelkit sugar beans is lower	1.12	0.389

* 1= strongly disagree, 5= strongly agree.

Regarding buyer relations, the bean farmers expressed moderate trust in timely payments (3.6), but lower scores for adherence to quantity, price, and contract terms (2.7–2.9) point to uncertainties in market reliability. Profitability perceptions were ambivalent (3.0–3.3), while the belief that production costs are not lower (1.1) reflects concerns about economic sustainability. Moreover, very low scores on market accessibility (1.1), financial capacity (1.4), and product differentiation (2.3) underscore systemic weaknesses in marketing and competitiveness. Collectively, these findings suggest that while the GI label holds symbolic value for quality, its economic potential remains underutilized due to knowledge gaps, market inefficiencies, and institutional shortcomings.

3.3. Factors influencing the transition of GI beans for GI and non-GI farmers

This section examines the motivational factors influencing GI farmers' decisions to transition to GI bean cultivation. Farmers who began producing dry beans using GI-certified seeds became members of the Kelkit Dry Bean Producers Association, which was established in 2019. These farmers started cultivating Kelkit dry beans during the 2020 and 2021 production periods. The association's membership plays a crucial role in increasing the production of Kelkit dry beans, which are registered to preserve local seed heritage. One of the primary challenges facing the association, which currently has only 50 members, is its limited membership size. To identify the factors that encourage farmers to join the association and to adopt GI-labelled seed production, Table 3 presents a list of potential motivators rated on a scale from 1 (not effective at all) to 5 (extremely effective). As shown in the Table 3, the most influential factors in the decision to switch to GI production were: the perception that dry beans produced with local seeds are of higher quality compared to other beans variety (4.10), and the farmer's past production experience (4.10). These responses from farmers who had started growing GI-labelled beans showed that the majority believed that bean quality and sufficient production experience were the most important factors in persuading them to change their production methods through GI labelled production. The findings also indicate that local farmers consider the use of certified seeds to be moderately effective (3.1) in revitalizing Kelkit dry bean production. On average, the bean farmers rated the statement that geographically indicated dry beans would be easier to market at 2.5. Based on this result, it can be said that farmers producing GIs encounter marketing difficulties. However, when the same statement was presented to the non GI-farmers, it was found to be quite effective in terms of better market expectations (3.9). This issue is about the timing of the surveys, which coincided with the pandemic. It is understood that GI does not affect product marketing during pandemic season. Farmers have been unable to use many existing channels to sell their products because of the lockdowns imposed due to the pandemic. As stated in Muroyiwa's (2024) research on the factors influencing the choice of maize and bean marketing channels for smallholder farmers in Lesotho, farmers grow beans not only to support their livelihoods, but also to sell their crops using effective marketing tools. This is why the farmers in this study could not use different marketing channels, such as retailers, wholesalers and vendors, and the Kelkit dry bean producers association could not purchase dry beans from GI farmers at that time. As a result, farmers were forced to either store their crops or sell them directly to consumers at low prices in local markets. This difficult situation also caused farmers to reduce the area allocated to growing dry beans the following season.

Table 3. Motivational factors influencing farmers' transition to GI dry bean production.

Statements	GI farmers		Non-GI farmers		Mann-Whitney U	Sig.
	Mean (M)	Std.Dev.	Mean*	Std.Dev.		
Experienced in dry bean farming	4.10	0.586	3.70	0.788	921.500	0.010**
Higher product quality	4.10	0.823	3.48	1.16	846.500	0.001**
Engagement in contract farming	-	-	3.58	0.859	-	-
If there is no loss of productivity	-	-	3.56	0.907	-	-
GI-labelled seeds will be used to expand Kelkit bean production	3.12	1.201	3.08	0.922	1169.500	0.683
Selling GI products at higher prices	2.90	1.065	3.78	0.840	639.000	0.000**
Easier marketing	2.53	1.082	3.90	0.677	393.500	0.000**
Producing health products	2.27	1.169	2.62	1.02	989.500	0.088*
Consultancy services available	2.18	1.409	3.06	1.30	802.500	0.001**
Production that's eco-friendly	2.16	1.124	2.62	0.966	904.500	0.020**
Demand from Dry Bean Association	1.80	0.979	-	-		
Government financial support	1.37	0.883	3.48	1.07	264.000	0.000**
Association or contract buyer support	1.22	0.468	3.54	1.07	189.500	0.000**
Access loans at good rates	1.10	0.368	2.22	1.37	678.500	0.000**

(M) 1= not effective at all; 5= extremely effective.
**Significant at the 5% level; *Significant at the 10% level.

Other factors such as support through consultancy services (2.1), producing health beneficial products (2.2), and lower environmental impact (2.1) were considered somewhat effective. Conversely, Table 3 shows that certain factors were not considered effective at all, including: access to credit at reasonable interest rates (1.1), financial support from the association or from buyers under contract (1.2), demand from the association (1.8), and government financial support for production with registered seeds (1.3). Looking at these results, it can be concluded that the production of GI-labelled dry beans in the research area should focus on improving new local varieties like GI-labelled seeds that are suitable for farmers' needs. The local authorities, the association and the NGOs must convince all the farmers in the region to switch to GI-labelled production methods. The number of association members needs to be increased, and financial support for inputs needs to be provided hereby the local authorities will act as a bridge between the association and the farmers.

The same factors were also presented to non-GI bean farmers (shown in the second column of Table 3). Among them, factors such as: easier market access (3.9), higher selling prices (3.7), contracted farming (3.5), higher yields (3.5), and financial support from buyers and the government (3.5) were found to be effective motivators for transitioning to GI production. Other factors such as quality (3.4), consultancy services (3.0), and revitalization of local production (3.0) were considered somewhat effective. Meanwhile, selling GI products at higher prices (2.9) and easier marketing (2.5) were found to be only slightly effective. Finally, factors like access to low-interest loans (2.2), environmentally friendly production methods (2.6), and producing health-beneficial products (2.6) were perceived as less influential.

The dry bean is one of the main legume crop in the research area. There is evidence of GI-labelled beans being cultivated, though in relatively low numbers. GI-labelled dry beans are a new alternative to non-GI dry beans, but this has not yet been fully explored or understood by local farmers in the research area. Therefore, when setting cultivation objectives for GI-labelled beans in the Kelkit, Köse and Şiran districts, it is important to consider developing market guarantee models, providing financial support and implementing contract farming. The findings in Table 3 show that the quality of dry beans, farming experience, access to markets, high yields and high prices are important factors in farmers' transition to GI-labelled production. Therefore, the attributes of the GI-labelled Kelkit dry bean variety need to be clearly defined through training programmes for local farmers and the number of members of the association must be increased through all the stakeholders in the region. It is concluded from Table 3 that examining the motivational factors that may affect the transition of the non-GI farmers to GI-labelled production reveals a statistically significant difference compared to the GI farmers. Non-GI farmers have different priorities and place greater importance on motivational factors such as easier product marketing, achieving higher product prices, receiving financial support from the Kelkit dry bean producer association or buyers with contracted farming, and receiving government financial support. Higher product quality is the main motivation for GI farmers to make the transition. It is also understood that these farmers' aim to obtain higher prices for their products by obtaining higher product quality through the geographically indicated production process.

4. Conclusion and Recommendations

This study presents significant findings regarding farmers' perspectives on geographically indicated (GI) production. The primary focus of the study is the experiences of farmers engaged in GI production, illustrated through the example of dry bean cultivation and marketing processes. It was observed that farmers who expected higher prices compared to traditional products by improving their product quality were unable to meet these expectations after transitioning to GI production. Furthermore, it was determined that farmers producing GI-labelled dry beans have not experienced improvements in access to alternative markets and have encountered challenges related to buyers.

The second key aspect of the study highlights the motivational factors that may influence farmers who do not produce geographically indicated (GI) certified dry beans to transition to GI production. The analyses indicate that these farmers place greater importance on motivational factors, including easier marketing, higher prices, financial support from producer associations or contract buyers, and government assistance, compared to those currently engaged in GI production. It is assessed that the negative experiences of farmers currently producing GI-labelled dry beans have an impact on this situation.

The findings of this study reveal that farmers who are not involved in the geographically indicated production process are influenced by the experiences of those who participate, and therefore approach participation with caution. The example of dry bean cultivation demonstrates that positive outcomes are necessary to encourage farmers to engage in the GI production process. In this context, the experiences of current GI bean farmers significantly influence non-GI bean farmers, who are cautious about GI production due to unmet expectations and market challenges. Coordinated efforts by producer organisations, government agencies, buyers and researchers, such as improved marketing support, contract farming, financial incentives and awareness programmes, are essential to encourage broader participation and promote sustainable rural development.

Author Contributions

The percentages of the authors' contributions are presented below. All author reviewed and approved the final version of the manuscript.

	N.D.	H.A.
C	30	70
D		100
S		100
DCP	100	
DAI		100
L		100
W	100	
CR	50	50
SR	100	
PM		
FA	100	

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors would like to acknowledge that this research was conducted in accordance with the principles of the Declaration of Helsinki. The Gümüşhane University Ethics Committee (approval date: January 15, 2021, protocol code: 2021/1).

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IMPACT OF CELLULOSE AND NANOCRYSTALLINE CELLULOSE ON *IN VITRO* SHOOT REGENERATION AND BIOCHEMICAL COMPOSITION OF *Echinacea purpurea*

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Abstract: This study aimed to investigate the effects of cellulose (C) and nanocrystalline cellulose (CNC) on *in vitro* shoot regeneration and biochemical composition of *Echinacea purpurea,* a medicinally valuable species. For this purpose, different concentrations (1.5, 3.0, and 6.0 gL^{-1}) of C and CNC were supplemented into Murashige and Skoog (MS) medium, and their effects on shoot and root development as well as total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) were evaluated. The results revealed that cellulose-based treatments exerted dose-dependent effects on both growth and secondary metabolite production. The highest shoot formation efficiency was obtained with 6 gL^{-1} C (95.0%), whereas the greatest number of shoots per explant was recorded at 3 gL^{-1} C (2.85 shoots/explant). In terms of biochemical parameters, the highest TPC and TFC levels were observed in both shoot (40.27 mg GAE g^{-1} DW and 9.06 mg QE g^{-1} DW, respectively) and root tissues (48.11 mg GAE g^{-1} DW and 12.01 mg QE g^{-1} DW, respectively) treated with 6 gL^{-1} CNC. Regarding TAC, the highest antioxidant activity was found only in shoot tissues treated with 6 gL^{-1} CNC (55.56%). These findings suggest that C and CNC have considerable potential as both structural support materials and biochemical elicitors in the *in vitro* propagation of *Echinacea purpurea.* The results offer new insights into the development of integrated strategies aimed at simultaneously optimizing biomass yield and phytochemical content in medicinal plant biotechnology.

Keywords: *Echinacea purpurea*, Nanocrystalline cellulose, Cellulose, *In vitro* culture, Shoot regeneration, Biochemical compounds

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1. Introduction

Plant tissue culture is a widely applied biotechnological platform for producing high-quality, genetically uniform, and disease-free plant material. In addition to its applications in micropropagation, it plays a key role in enhancing secondary metabolite biosynthesis, genetic transformation, and stress physiology under controlled *in vitro* conditions (Erkoyuncu and Yorgancılar, 2016; Ozyigit et al., 2023).

Recent developments have focused on incorporating bioactive and structural additives to improve the efficiency of *in vitro* culture systems. Among these, cellulose and its derivatives—such as microcrystalline cellulose (MCC), nanocrystalline cellulose (CNC), nanocellulose (NC), and nanofibers—have received growing attention due to their physicochemical stability, biocompatibility, biodegradability, and surface functionality (Miao and Hamad, 2013; Liu et al., 2015; Sichanova et al., 2022). These materials are abundant in nature and widely used in industrial and biomedical fields, but their application in plant biotechnology is still emerging.

When integrated into plant tissue culture media, cellulose-based materials can act as elicitors, triggering defense-related pathways and stimulating the accumulation of secondary metabolites, including phenolic compounds. At suitable concentrations, they may also enhance shoot proliferation, fresh and dry biomass accumulation. For example, CNC and MCC treatments have been reported to improve shoot regeneration and phenolic content in Artemisia absinthium cultures (Khosravi et al., 2024). Nanocellulose has also been shown to improve liquid culture systems by mitigating hyperhydricity, improving medium structure, and promoting phytochemical yield (Grzegorczyk-Karolak et al., 2017; Grzegorczyk-Karolak et al., 2024). Moreover, its use can reduce reliance on agar, a costly gelling agent that may interfere with nutrient uptake (Debergh, 1983; Pati et al., 2011).

Despite the promise of cellulose-based elicitors, their use in medicinal plants remains limited. No study has yet evaluated their role in *Echinacea purpurea*, a pharmacologically valuable species widely used in phytopharmaceuticals due to its richness in caffeic acid derivatives, especially cichoric and chlorogenic acids (Burlou Nagy et al., 2022). However, producing biomass-rich, chemically consistent material under conventional *in vitro* protocols remains a challenge for this species.

This study investigates the effects of nanocrystalline cellulose (CNC) and conventional cellulose (C) at varying concentrations on shoot and root development in *E. purpurea* nodal cultures. It also examines their impact on key biochemical traits including total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC). This work aims to provide new insights into the dual role of cellulose-based materials as structural supports and elicitors under *in vitro* conditions.

2. Materials and Methods

2.1. Seed Sterilization and *In Vitro* Culture Establishment

Seeds of *E. purpurea*, obtained from cultivated plants at Selçuk University (Türkiye), were surface sterilized according to the protocol described by Erkoyuncu and Yorgancilar (2021). Sterilized seeds were aseptically sown on MS (Murashige and Skoog, 1962) basal medium without plant growth regulators (PGRs) and maintained under controlled conditions for eight weeks. Healthy and sterile seedlings were then selected, and 1–1.5 cm-long nodal segments were excised for use as explants.

These explants were cultured on MS medium solidified with 7 gL^{-1} agar (control) or supplemented with cellulose (C) or nanocrystalline cellulose (CNC) at concentrations of 1.5, 3.0, and 6.0 gL^{-1} (Khosravi et al., 2024). Cellulose (Sigma-Aldrich, USA) and nanocrystalline cellulose (CNC; Nanografi, Türkiye), characterized as a spray-dried white powder with an average particle size of 10–20 nm in width, 300–900 nm in length, and 92% crystallinity (XRD), were used as solidifying agents. To promote shoot induction, all media were enriched with 0.5 mgL^{-1} 6-benzylaminopurine (BAP) and 0.1 mgL^{-1} α-naphthaleneacetic acid (NAA), according to Erkoyuncu (2019).

All cultures were incubated in a growth chamber (Sanyo MLR-351H) at 24 ± 2 °C, 65% relative humidity, under a 16/8 h light/dark photoperiod with a light intensity of 5 $\mu mol\ m^{-2}\ s^{-1}$. After five weeks of culture, growth parameters were recorded for each explant, including shoot induction frequency (%), number of shoots per explant, shoot length (cm), shoot fresh weight (mg), and shoot dry weight (mg). For rooted shoots, additional parameters such as root induction frequency (%), root length (cm), root fresh weight (mg), and root dry weight (mg) were also measured.

2.2. Sample Preparation and Extraction

After a 5-week culture period, shoot and root tissues from *in vitro*-grown plantlets were separated and air-dried at room temperature for 48 hours. The dried tissues were then finely powdered using a sterile porcelain mortar and pestle. For extraction, 0.2 g of each powdered sample was suspended in 25 mL of 80% HPLC-grade methanol and subjected to ultrasonic treatment for 30 minutes. The resulting extracts were filtered through 0.45 µm membrane filters and stored at 4 °C for subsequent biochemical analyses.

2.3. Determination of Total Phenolic Content (TPC)

Total phenolic content was quantified using the Folin–Ciocalteu method (Wu et al., 2006), with gallic acid as the reference compound. A 100 µL aliquot of each extract was mixed with 1 mL of diluted Folin–Ciocalteu reagent (1:4) and incubated for 5 minutes, followed by the addition of 0.5 mL of 20% Na_2CO_3. After a 30-minute incubation in the dark at room temperature, absorbance was recorded at 760 nm. Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE g^{-1} DW), based on a gallic acid calibration curve (25–500 ppm, R^2 = 0.99).

2.4. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was assessed using the aluminum chloride colorimetric method (Chang et al., 2002), with quercetin as the standard. Each reaction contained 500 µL of extract, 1.5 mL of methanol, 2.8 mL of distilled water, 0.1 mL of 1 M potassium acetate, and 0.1 mL of 10% $AlCl_3$. After 30 minutes at room temperature, absorbance was measured at 415 nm. TFC values were calculated from a quercetin calibration curve (25–1000 ppm, R^2 = 0.98) and expressed as mg quercetin equivalents per gram dry weight (mg QE g^{-1} DW).

2.5. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was measured using the DPPH radical scavenging assay (Erenler et al., 2015). For each sample, 300 µL of extract was mixed with 100 µL of 0.1 mM DPPH solution and incubated for 30 minutes in the dark at room temperature. Absorbance was read at 517 nm, and the DPPH scavenging activity was calculated using the equation 1.

$$\text{DPPH (\%)} = ((Aa - As) / Aa) \times 100 \quad (1)$$

Aa: the absorbance of the control (DPPH + methanol),
As: the absorbance of the sample (DPPH + extract).

2.6. Statistical Analysis

The experiment was conducted in a factorial arrangement based on a completely randomized design. The two experimental factors were cellulose type (C and CNC) and concentration (1.5, 3.0, and 6.0 gL^{-1}), with an additional MS + agar control group included. Each treatment consisted of three biological replicates, with ten Magenta vessels per replicate. In each vessel, five nodal explants were cultured. Biochemical analyses were performed in triplicate for each replicate.

Prior to statistical analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Two-way analysis of variance (ANOVA) was conducted to assess the effects of treatment, tissue type (shoot vs. root), and their interaction. Significant differences among means were evaluated using Tukey's HSD test. All univariate statistical analyses were performed using JMP Pro 17 (SAS Institute Inc., USA).

In addition, multivariate analyses—including correlation matrices and hierarchical clustering with heatmap visualization—were performed using R software (version 4.3.1). All results are presented as means ± standard error (SE), and differences were considered statistically significant at P<0.01, P<0.05.

3. Results

3.1. Effects of Cellulose and Nanocrystalline Cellulose on Growth Parameters

The shoot and root development of *E. purpurea* nodal explants cultured on MS medium supplemented with varying concentrations of cellulose (C) and nanocrystalline cellulose (CNC) was assessed based on several growth parameters. Shoot formation efficiency (%SFE), shoot number per explant (S/E), shoot fresh weight (SFW), and shoot dry weight (SDW) were significantly influenced by the treatments (P<0.01 or P<0.05), whereas shoot length (SL) did not differ significantly among the groups (Table 1).

Table 1. Influence of cellulose (C) and nanocrystalline cellulose (CNC) on shoot formation efficiency and biomass-related parameters of *E. purpurea* nodal cultures

	gL^{-1}	%SFE*	S/E (number)	SL (cm)	SFW (g)	SDW (g)
Control		80.0 ± 3.73ab	1.95 ± 0.25ab	2.86 ± 0.47	0.093 ± 0.01c	0.013 ± 0.02bc
	1.5	45.0 ± 3.73c	0.8 ± 0.18b	1.75 ± 0.4	0.115 ± 0.14b	0.018± 0.01a
C	3	85.0 ± 4.45ab	2.85 ± 0.36a	2.4 ± 0.42	0.083 ± 0.11c	0.012 ± 0.03bc
	6	95.0 ± 3.73a	1.35 ± 0.27ab	1.73 ± 0.21	0.039 ± 0.03d	0.008 ± 0.011c
	1.5	70.0 ± 3.73ac	0.8 ± 0.11b	3.22 ± 0.64	0.130 ± 0.04a	0.018 ± 0.012a
CNC	3	65.0 ± 4.56bc	1.05 ± 0.18b	2.76 ± 0.54	0.109 ± 0.05b	0.016 ± 0.02ab
	6	45.0 ± 3.73c	0.9 ± 0.14b	2.35 ± 0.73	0.102 ± 0.09b	0.015 ± 0.06ab
		**	*	n.s.	**	**

*Shoot formation efficiency (%SFE), shoot number per explant (S/E), shoot length (SL), shoot fresh weight (SFW), and shoot dry weight (SDW). Statistical significance: **P<0.01, *P<0.05, n.s. = not significant.

The highest %SFE (95.0%) was observed in the medium containing 6 gL^{-1} cellulose, while the lowest values (45.0%) were recorded in treatments with 1.5 gL^{-1} cellulose and 6 gL^{-1} CNC. The greatest shoot number per explant (2.85) was obtained with 3 gL^{-1} cellulose. Explants cultured with 1.5 gL^{-1} CNC exhibited higher shoot fresh and dry weights despite non-significant differences in SL. A significant reduction in SFW was observed at 6 gL^{-1} cellulose (P<0.05).

For rooting parameters, only root formation frequency (%RFS) exhibited a statistically significant difference among treatments (P<0.01) (Table 2). The highest %RFS value (50.64%) was recorded in the 6 gL^{-1} cellulose group, which was significantly greater than the control group (15.24%). Root length (RL), root fresh weight (RFW), and root dry weight (RDW) did not show significant differences among treatments, although the highest RFW value (0.387 g) was noted in the 1.5 gL^{-1} cellulose group.

Table 2. Influence of cellulose (C) and nanocrystalline cellulose (CNC) on root formation efficiency and biomass-related parameters of *E. purpurea* nodal cultures

	gL^{-1}	%RFS*	RL* (cm)	RFW* (g $plant^{-1}$)	RDW*(g $plant^{-1}$)
Control		15.24 ± 5.75c	5.13 ± 1.68	0.027 ± 0.01	0.005 ± 0.002
	1.5	13.33 ± 4.21c	4.52 ± 2.13	0.387 ± 0.12	0.028 ± 0.01
C	3	6.07 ± 1.85d	3.99 ± 1.82	0.253 ± 0.11	0.018 ± 0.01
	6	50.64 ± 5.89a	9.77 ± 2.21	0.130 ± 0.02	0.020 ± 0.004
	1.5	29.67 ± 2.27b	4.04 ± 1.26	0.054 ± 0.02	0.006 ± 0.002
CNC	3	15.71 ± 5.19c	5.04 ± 1.63	0.229 ± 0.07	0.021 ± 0.006
	6	13.0 ± 4.01c	1.46 ± 0.67	0.106 ± 0.05	0.014 ± 0.006
		**	n.s.	n.s.	n.s.

*Rooted shoot frequency (%RFS), root length (RL), root fresh weight (RFW), and root dry weight (RDW). Statistical significance: **P<0.01, *P<0.05, n.s. = not significant.

3.2. The Effect of Cellulose (C) and Nanocrystalline Cellulose (CNC) on Total Phenolic Content, Flavonoid Content, and Antioxidant Capacity

The total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) of *E. purpurea* shoot and root tissues were significantly influenced by cellulose-based treatments under *in vitro* conditions (Table 3). TPC and TFC levels showed statistically significant differences in both shoot and root tissues (P<0.01). TAC values were significantly different only in shoot tissues (P<0.05), while no significant differences were observed in root tissues (P>0.05). Notably, the 6 gL^{-1} CNC treatment consistently resulted in the highest TPC, TFC, and TAC values across both shoot and root tissues compared to all other treatments, highlighting its superior elicitor effect. In contrast, lower CNC concentrations and cellulose treatments produced variable or moderate effects, particularly in root tissues where TAC remained statistically unchanged.

In shoot tissues, the highest TPC (40.27 ± 0.07 mg GAE g^{-1} DW) and TFC (9.06 ± 0.24 mg QE g^{-1} DW) were recorded in the treatment containing 6 gL^{-1} CNC. The same concentration of CNC also resulted in the highest TPC (48.11 ± 1.24 mg GAE g^{-1} DW) and TFC (12.01 ± 0.79 mg QE g^{-1} DW) values in root tissues. These values were significantly higher than those of the control and other treatment groups (P<0.01). Regarding TAC, the highest values in shoot tissues were observed in both the 6 gL^{-1} C and CNC treatments. However, no statistically significant differences were detected in root tissue TAC among the treatment groups.

Table 3. Effect of cellulose (C) and nanocrystalline cellulose (CNC) on TPC, TFC, and TAC in shoot and root tissues of *E. purpurea.*

	gL^{-1}	TPC*		TFC*		TAC*	
		Shoot	Root	Shoot	Root	Shoot	Root
Control		22.92 ± 0.07bc	30.99 ± 0.75bc	8.16 ± 0.11a	2.72 ± 0.2c	76.81 ± 0.35bc	74.38 ± 0.4
	1.5	24.65 ± 0.31b	25.31 ± 0.9c	8.22 ± 0.17a	3.07 ± 0.16c	77.33 ± 0.36b	81.03 ± 0.57
C	3	20.81 ± 0.12c	36.14 ± 1.01b	6.87 ± 0.05b	5.51 ± 0.08bc	74.14 ± 0.23c	80.95 ± 0.66
	6	16.83 ± 0.59d	34.34 ± 2.06bc	4.68 ± 0.12c	4.85 ± 0.64bc	80.6 ± 0.31a	73.45 ± 3.62
	1.5	22.82 ± 0.81bc	36.27 ± 1.2b	8.86 ± 0.14a	4.67 ± 0.07bc	77.5 ± 0.3b	82.84 ± 0.1
CNC	3	25.47 ± 0.53b	36.01 ± 0.9b	8.47 ± 0.14a	6.61 ± 0.06b	77.24 ± 0.34b	80.0 ± 1.08
	6	40.27 ± 0.07a	48.11 ± 1.24a	9.06 ± 0.24a	12.01 ± 0.79a	80.52 ± 0.43a	78.53 ± 0.52
		**	**	**	**	*	n.s.

*Total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) in shoot and root tissues. Statistical significance: **P<0.01, *P<0.05, n.s. = not significant.

3.3. Multivariate Analysis of Growth and Biochemical Relationships

3.3.1. Correlation Analysis between Growth and Biochemical Parameters

A Pearson correlation matrix was constructed to evaluate the relationships among growth and biochemical traits under different cellulose-based treatments (Figure 1). Significant correlations were found between several morphological parameters and secondary metabolite contents.

Shoot fresh weight (SFW) showed a strong positive correlation with both root fresh weight (RFW; r = 0.91, P<0.01) and shoot dry weight (SDW; r = 0.91, P<0.01). RFW and root dry weight (RDW) were also highly correlated (r = 0.94, P<0.01). %SFE had a moderate positive correlation with S/E (r = 0.57, P<0.01). In contrast, %SFE was negatively correlated with shoot TPC (r = –0.63, P<0.01), shoot TFC (r = –0.64, P<0.01), and shoot TAC (r = –0.34, P<0.05). Among biochemical parameters, a strong positive correlation was observed between shoot TPC and shoot TFC (r = 0.79, P<0.01), and a weaker correlation with shoot TAC (r = 0.35). Root TPC was also positively correlated with root TFC (r = 0.84, P<0.01). Shoot length (SL) and root length (RL) did not show significant correlations with biochemical parameters. RL was moderately correlated with RFW (r = 0.59) and weakly with SDW (r = 0.22).

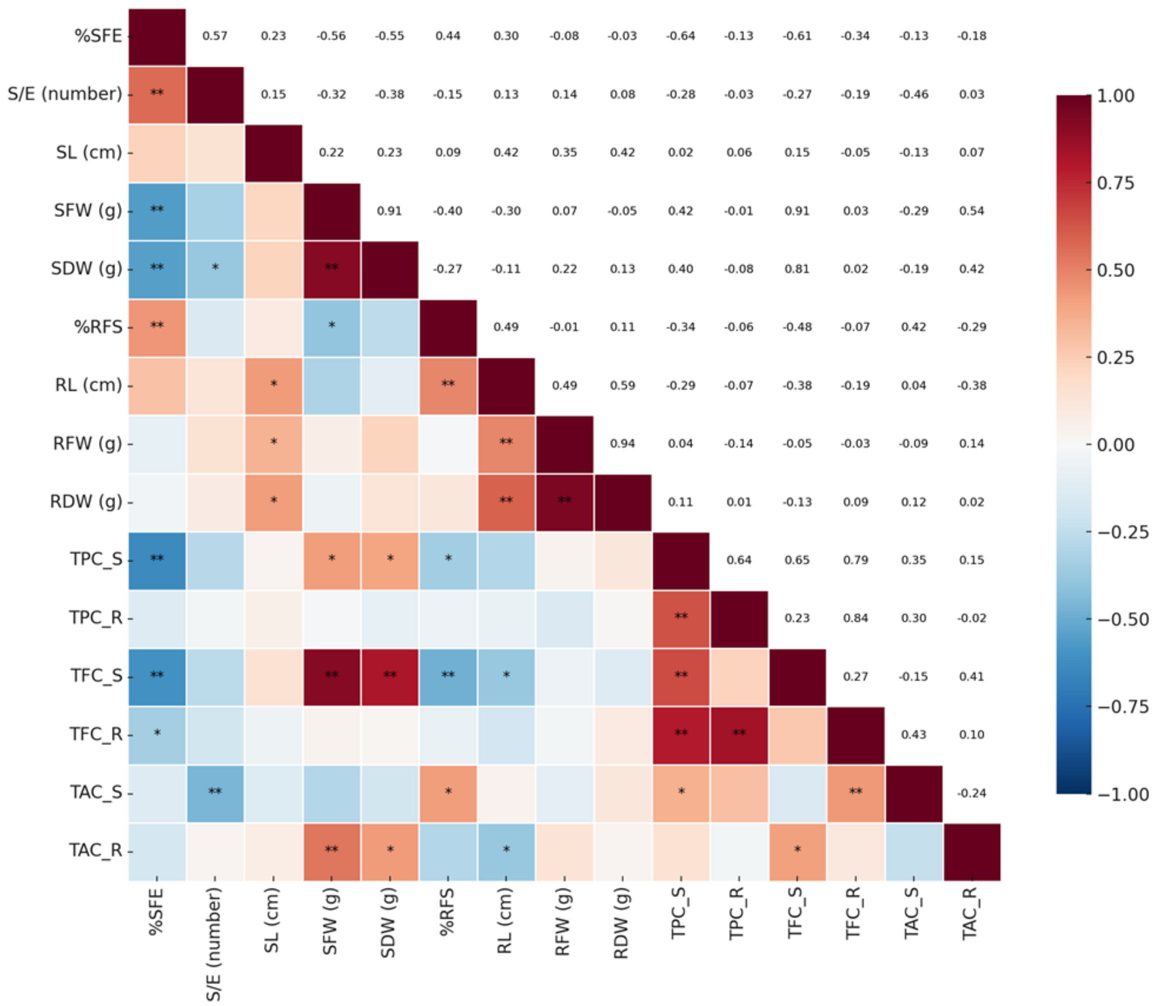

Figure 1. Heatmap showing Pearson correlation coefficients among morphological and biochemical parameters of *E. purpurea* cultured *in vitro* under different cellulose (C) and nanocrystalline cellulose (CNC) treatments. Color intensity indicates the strength and direction of the correlation: positive (red) and negative (blue) (*P<0.05, **P<0.01).

3.3.2. Heatmap Analysis of Treatment Groups and Parameters

To evaluate the overall responses to cellulose-based treatments, hierarchical clustering analysis was performed using z-score normalized data, and the results were visualized as a heatmap (Figure 2). The top dendrogram displays the clustering among treatment groups, while the left dendrogram shows the clustering of measured parameters. Red shades indicate relatively higher values, while blue shades indicate lower values. The 6 gL^{-1} CNC and C treatments were associated with higher values in several growth-related parameters, especially those related to root development, including %RFS, RL, RFW, and RDW. The 1.5 gL^{-1} CNC treatment displayed higher values in antioxidant-related parameters such as TPC (S), TFC (S), and TAC (S). The control group was clustered around neutral values (white), showing moderate responses across most traits. At the parameter level, SFW and SDW were closely clustered, as were TPC, TFC, and TAC. These clusters represent parameters that exhibited similar response trends across treatments.

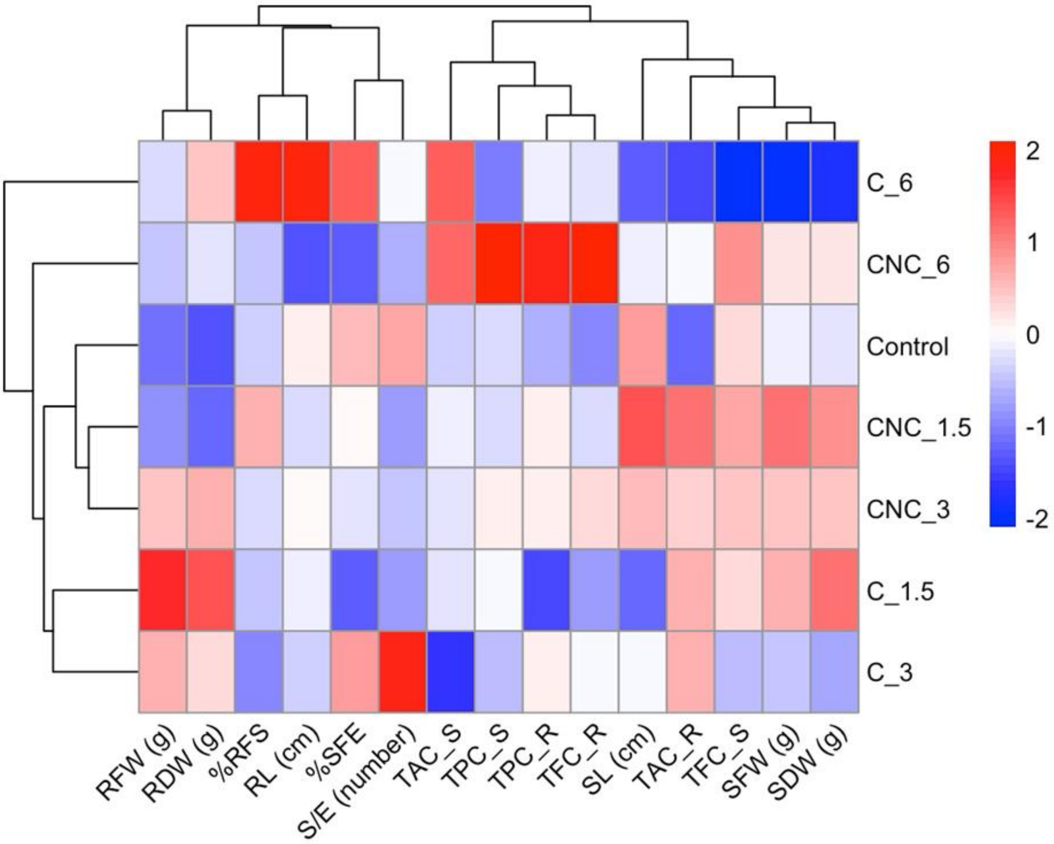

Figure 2. Hierarchical clustering heatmap representing the distribution of morphological and biochemical parameters of *E. purpurea* under different cellulose (C) and nanocrystalline cellulose (CNC) treatments.

4. Discussion

Cellulose-based treatments exhibited dose-dependent effects on shoot and root development in *E. purpurea* nodal cultures. Although the highest shoot formation efficiency (%SFE) was observed at 6 gL^{-1} cellulose, this concentration also resulted in a significant reduction in shoot biomass. Similarly, 6 gL^{-1} CNC was associated with both low %SFE and reduced shoot fresh and dry weights. These results are consistent with the hormesis model, in which low doses of certain nanomaterials enhance growth, while higher concentrations can suppress physiological performance. A comparable pattern was reported in *Artemisia absinthium* by Khosravi et al. (2024) where MCC and NCC at 1.5 gL^{-1} promoted shoot biomass, but higher concentrations (3–6 gL^{-1}) reversed this effect.

The influence of PGRs was ruled out in our study, as all media contained equal levels of BAP and NAA. This allows the effects observed to be attributed to the physicochemical characteristics of cellulose and CNC. Notably, the highest shoot number per explant was recorded at 3 gL^{-1} cellulose, suggesting an optimal range for shoot proliferation. A similar observation was made by Grzegorczyk-Karolak et al. (2024) in *S. viridis*, where liquid media containing nanocellulose supported higher shoot multiplication rates than conventional agar-based media, likely due to improved nutrient availability and medium porosity.

In terms of root development, only root formation frequency (%RFS) responded significantly to treatments. The 6 gL^{-1} cellulose group showed the highest %RFS, followed by 1.5 gL^{-1} CNC. However, root induction sharply declined at 6 gL^{-1} CNC. This dose-dependent trend aligns with findings in *Stevia rebaudiana*, where silver nanofibers stimulated rooting at 50 mgL^{-1} but suppressed it at 100 mgL^{-1} (Sichanova et al., 2022). Similarly, carbon nanofibers improved root growth in *Sorghum bicolor* and *Cicer arietinum* at low doses (0.5–1.0 ppm) but inhibited it at higher concentrations (≥10 ppm) (Dubey et al., 2024). Interestingly, *A. absinthium* did not exhibit significant root development changes in response to MCC or NCC, suggesting species-specific sensitivity (Khosravi et al., 2024).

The enhanced root responsiveness observed in *E. purpurea* may be attributed to species-specific differences in endogenous hormone regulation, metabolic baseline, or cellular antioxidant status. In particular, *E. purpurea* may possess a greater capacity to perceive or transduce elicitor-derived signals, resulting in more pronounced morphogenic responses under cellulose-based treatments.

The biochemical responses to cellulose and CNC treatments were equally noteworthy. In this study, the most significant increases in total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) were observed in shoot and root tissues treated with 6 gL^{-1} CNC. While Khosravi et al. (2024) reported that MCC and NCC applications in *A. absinthium* did not significantly alter TPC or TFC, Salvia-based systems responded more strongly. For example, in *S. viridis*, the use of nanocellulose led to a two-fold increase in polyphenols and up to a 6.5-fold increase in phenylpropanoid accumulation, including verbascoside (18.2 mg/g DW) (Grzegorczyk-Karolak et al., 2024). In contrast, *Hibiscus sabdariffa* showed a 21.4% decrease in TPC when treated with CNF alone, but a 39.76% increase in TAC when combined with IAA and GA_3 (Mirheidari et al., 2022). These findings highlight both compound-dependent and species-dependent differences in secondary metabolism. Since all treatments in our study received equal amounts of plant growth regulators, the observed increases in biochemical traits are likely attributable to the intrinsic physicochemical properties of CNC and cellulose.

Multivariate analyses provided further insight into the physiological complexity of the observed responses. Correlation analysis revealed strong positive associations between shoot and root biomass traits (e.g., SFW and RFW; SDW and RDW), indicating a coordinated regulation of resource allocation (Figure 1) (Mohiuddin et al., 2021). Interestingly, shoot formation efficiency (%SFE) showed negative correlations with antioxidant traits such as TPC and TFC, implying a potential trade-off between morphogenic output and secondary metabolite accumulation (Ivanisevic et al., 2011). Heatmap-based clustering also revealed dose- and compound-specific response profiles, separating treatments based on their distinctive biochemical and growth outcomes (Fathy et al., 2023) (Figure 2). These findings support the notion that cellulose-based elicitors induce complex and interdependent responses that may be fine-tuned to optimize either biomass or phytochemical yield, depending on the desired application.

In particular, the high crystallinity, surface area, and reactive hydroxyl groups of CNC may facilitate enhanced nutrient diffusion, redox buffering, or cell wall interaction, which in turn may stimulate secondary metabolism (Islam et al., 2018). Moreover, previous studies have indicated that nanomaterials can activate defense-related signaling pathways and upregulate key enzymes such as phenylalanine ammonia-lyase (PAL), which is central to the phenylpropanoid pathway (Kralova and Jampilek, 2021; Bayat et al., 2024). In addition, CNC may induce transient oxidative stress signals that enhance antioxidant enzyme activity (e.g., SOD, CAT, APX), which are closely associated with the accumulation of phenolics and flavonoids as part of the plant's adaptive response (Mirheidari et al., 2022; Bayat et al., 2024; Dubey et al., 2024). These combined structural and signaling effects suggest that CNC not only serves as a physical additive but also acts as a biochemical elicitor of secondary metabolism (Kralova and Jampilek, 2021). Although not directly measured in the present study, it is plausible that similar mechanisms contributed to the observed increases in phenolic and flavonoid contents. However, further studies are needed to clarify the underlying mechanisms through transcriptomic or metabolomic approaches.

5. Conclusion

This study demonstrated that cellulose (C) and nanocrystalline cellulose (CNC) significantly influence in vitro shoot regeneration and secondary metabolite production in *E. purpurea* in a dose-dependent manner. Among the tested treatments, 3 gL^{-1} cellulose was identified as the most effective concentration for maximizing shoot proliferation, whereas 6 gL^{-1} CNC markedly enhanced total phenolic and flavonoid content, particularly in root tissues. High doses of both materials tended to reduce biomass, supporting the hormetic response model. Rooting responses also varied with concentration and compound type, highlighting the importance of species-specific and dose-specific optimization.

Biochemical analyses revealed that CNC is a potent elicitor of antioxidant metabolism, potentially due to its unique physicochemical properties. Furthermore, multivariate analysis underscored complex physiological interactions, suggesting a trade-off between growth and secondary metabolite accumulation. The strong correlations between biomass traits and coordinated clustering of phenolic parameters reinforce the need to balance morphogenic and biochemical objectives in tissue culture systems.

Overall, this research provides novel insights into the dual role of cellulose-based materials as physical supports and biochemical elicitors in plant biotechnology. The findings offer promising perspectives for optimizing *in vitro* propagation strategies aimed at both high biomass yield and enhanced phytochemical content in medicinal plant species such as *E. purpurea.* Beyond the experimental findings, the study also highlights that CNC may serve as a sustainable and commercially applicable additive in plant tissue culture, reducing dependency on costly gelling agents and contributing to environmentally friendly large-scale micropropagation systems.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	M.T.E.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were. conducted, ethical committee approval was not required for this study.

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