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Growing possibilities of pitaya (*Hylocereus spp.*) under protected cultivation in subtropical conditions

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ABSTRACT

Global climate change has recently increased interest in some tropical fruit species in the Mediterranean and Aegean regions of Türkiye. Among these tropical species, pitaya (dragon fruit) has gained popularity due to its tolerance to drought, ability to bear fruit one year after planting, suitability for soilless cultivation, and significant export potential. The objective of thise study is to investigate pitaya cultivation under protected cultivation in subtropical conditions. The research was conducted between 2020 and 2022 in the Manavgat district of Antalya on White Jaina and Red Jaina pitaya cultivars. The morphological parameters of the plants were evaluated as well as their physical properties and yield characteristics. The findings have revealed that the duration from flowering to harvest ranged between 44 and 45 days on average for both cultivars. The average fruit weight was measured as 264.60 g and 344.00 g for White Jaina and Red Jaina, respectively, and the difference was found to be statistically significant (P<0.05). Depending on the cultivar, the fruit circumference was measured as 20.58 cm and 21.99 cm, the fruit length as 13.07 cm and 12.22 cm, and the soluble solids content as 10.98% and 12.35%, respectively. By the end of the second year of the study, the yield per hectare was calculated as 1.74 tons ha^{-1} for White Jaina and 1.65 tons ha^{-1} for Red Jaina. The findings have indicated that both pitaya cultivars could be cultivated under protected cultivation conditions in Manavgat, which has a colder winter climate than the Alanya and Gazipaşa districts of Antalya.

1. Introduction

Pitaya, commonly covering several cactus genera (*Cereus spp*., *Opuntia spp*., and *Hylocereus spp*.), is primarily cultivated for the fruits of the Hylocereus spp. genus for commercial purposes. Among these, the most widely grown species are *H. polyrhizus*, *H. megalanthus*, and *H. undatus* (Luders and McMahon 2006). While the origin of pitaya is considered to be tropical forest regions, its commercial cultivation has been reported in Mexico, South America, Thailand, and Vietnam (Borchetia et al. 2022). Although data regarding pitaya production are not provided in FAQ statistics, producer countries provide this information through their official sources. Globally, the highest production is achieved in Vietnam, with 30000 hectares of cultivation area and 640000 tons of production. Vietnam also holds a prominent position as both the leading producer and exporter at the global level (Fernandes et al. 2017; Gezici 2019).

Pitaya is widely used in the food, cosmetics, and pharmaceutical industries for its nutritional content and health benefits. This fruit is a rich source of betalains, polyphenolic compounds, carotenoids, and antioxidants (Kiranmai 2022). It also contains high total phenols, flavonoids, and vitamin C (Fernandes et al. 2017). Among pitaya cultivars, those with red flesh are particularly rich in antioxidants such as beta-carotene, lycopene, vitamin E, phosphorus, and calcium, which contribute to healthy bone, teeth, and skin development. Additionally, its black seeds are an important source of essential fatty acids (Gonzaga 2017; Borchetia et al. 2022). Pitaya has been reported to help digestion, exhibit anti-diabetic properties, reduce blood pressure, neutralize toxins, especially heavy metals and the treatment of asthma and coughs. Furthermore, it is known to help prevent various types of cancer, particularly colon cancer (Prisa 2022).

Pitaya could adapt to subtropical regions. While pitaya plants are typically cultivated in open fields under tropical climates, it has also been grown under nets or greenhouses in certain microclimates in subtropical regions. For instance, in subtropical areas such as Israel and California, pitaya have been grown under nets to protect the plants from the harmful effects of sunlight (Mizrahi et al. 2002). Pitaya is sensitive to day temperature changes during the period from fruit set to harvest. In greenhouses, temperatures during November can range from a minimum of -2°C to a maximum of +15°C; however, higher temperatures with limited fluctuations are essential for the flowering and fruit set of the pitaya (Prisa 2022).

Studies on pitaya cultivation have been conducted across various geographical regions and environmental conditions. Some of these studies are summarized below:

In *Hylocereus undatus*, it was reported that approximately 21 days are required from bud formation to flowering, and about 35 days from pollination to fruit harvest (Costa et al. 2014). Menezes et al. (2015) found that the duration from flowering to harvest for *H. undatus* took 41 days. It was also found that the average fruit weight was 442.47 g, fruit length was 93.73 mm, fruit diameter was 83.41 mm, pulp firmness was 6.16 N, and the total soluble solids content was 19.58%. Budhathoki et al. (2023) reported that, in *Hylocereus polyrhizu,* pitayas were randomly collected from various regions in Nepal, and fruit weight was found to have ranged from 20.12 to 300 g, fruit length ranged from 0.48 to 9.84 cm, fruit diameter ranged from 0.35 to 8.42 cm, and soluble solids content ranged from 11.7% to 14.70%. Gübbük et al. (2017) observed that flowering in the 'Bloody Mary' and 'Cosmic Charlie' cultivars began in May-June and continued until September, requiring 35-40 days from flowering to fruit harvest. For the 'Bloody Mary' cultivar, fruit yield per plant was 8-10 fruits in the first year and over 15 fruits in the second year. In contrast, the 'Cosmic Charlie' cultivar yielded 4-5 fruits per plant in the first year and 8-10 fruits in the second year. Fruit weight was recorded as 400-600 g for 'Bloody Mary' and 600-700 g for 'Cosmic Charlie.' Zimmerman et al. (2017) examined a total of 25 pitaya cultivars in the U.S. Virgin Islands. It was found that cultivars such as 'Dark Star,' 'Delight,' 'Makisupa,' 'Halley's Comet,' 'Physical Graffiti,' and 'Purple Haze' had a high production potential. In the Philippines, pitaya harvest begins in May, approximately 30-35 days after flowering, with peak yields occurring between July and October and continuing until November (Rodeo et al. 2018). In *Hylocereus undatus* (white) and *Hylocereus polyrhizus* (red) species, the maximum fruit length was reported as 9.51 cm, fruit diameter as 7.19 cm, and fruit weight as 265.86 g (Parmar and Karetha 2020). For the yellow pitaya species (*Selenicereus megalanthus*), flowering begins in spring and continues until autumn, while fruit harvest starts in summer and ends in winter. The period from flower formation to fruit harvest ranges from 147 to 166 days, while the period from pollination to fruit harvest is between 96 and 110 days (Rabelo et al. 2020). In red pitaya (*Hylocereus polyrhizus*), the harvest season extends from April to September, with an annual yield of 20.1 tons per hectare (Then et al. 2020). In *H. undatus* (white) and *Hylocereus* (red-fleshed) species, 8% of flower buds form in July, 77% by late August, and the remaining 15% in September. The fruits of *Hylocereus* species reach harvest maturity approximately 40-45 days after pollination, with the fruit ripening period extending from September to early December. The average fruit weight was recorded as 273 g for *H. undatus* and 315 g for *Hylocereus* (Trivellini et al. 2020).

In Türkiye, tropical fruit cultivation has not yet reached the desired level when considering the production area and volume. However, Gübbük et al. (2017) reported that some tropical fruit species gave positive results in open fields and greenhouse conditions in specific microclimate locations such as Gazipaşa and Alanya. However, the climate in the Manavgat location is colder compared to Alanya and Gazipaşa. The aim of the study was to determine whether pitaya can be grown in greenhouses in cool subtropical climates.

2. Materials and Methods

2.1. Plant material and cultivation conditions

The study was conducted at the Ilıca campus of Akdeniz University's Manavgat Vocational School (Manavgat, Antalya, Türkiye; 36°48'31.08" N and 31°23'16.47" E, at an altitude of 24 m above sea level) between November 2020 and December 2022. The greenhouse structure is made of iron, top height and height from the gutter were 4.20 m and 3.00 m, respectively. The greenhouse was covered with plastic and ventilated from the sides and top. The White Jaina and Red Jaina cultivars were used as the plant material. In the trellising system, galvanized steel poles measuring 150 cm in length were installed, and circular rings with a diameter of 70 cm were mounted on top of the poles. Fifty centimeters of each pole were embedded into the soil for stabilization. The plants were planted on November 6, 2020, with a spacing of 1.8 m between plants and 3.0 m between rows, with four plants arranged around each trellising system (Figure 1).

A double-line drip irrigation system was installed for each row. To protect the plants from high-temperature stress during the summer months, 40% shading net was used in the greenhouse (Figure 1). Cultural practices were performed following the methodology outlined by Gübbük et al. (2017).

Temperature and humidity were recorded hourly using a mini meteorological station during the growing period. During the vegetation period, the minimum temperature ranged between 10.57°C and 24.63°C, the average temperature ranged between 14.02°C and 28.71°C, and the maximum temperature ranged between 20.21°C and 34.06°C. Relative humidity values varied from a minimum of 40.72% to 51.79%, an average of 56.84% to 71.18%, and a maximum of 71.24% to 85.14% respectively.

Pollination was performed manually three days per week between 22:00 and 24:00. The details regarding the shading net application and pollination process are given in Figure1.

Figure 1. From left to right: Images showing the trellising system, shading net application, and pollination process.

2.2. Observation of morphological characteristics in plants

After planting, all lateral shoots were removed until the plants reached 100 cm in height. When the plant reached 100 cm, the top part was cut to encourage lateral shoot formation (Figure 2). The number of days that plants took to reach 100 cm height, and the first flowering and the number of days that took from flowering to harvest were determined for each species.

2.3. Investigation criteria of fruit physical characteristics

Fruit weight was measured by using a precision balance in grams. The circumference and length of the fruits were measured in centimeters using a measuring tape.

2.4. Fruit quality characteristics

2.4.1. Flesh firmness

Flesh firmness was determined using a Loyka GY-3 handheld penetrometer with an 8 mm diameter tip. Measurements were taken from three different points on the surface of each fruit and expressed in N.

2.4.2. Soluble solid content

The soluble solid content of the fruits was measured as a percentage (%) using a digital refractometer. The measurements were conducted on juice extracted from the fruits using a fruit juicer.

2.4.3. Fruit peel color (L, a, b*)*

The peel color of the fruits was determined by measuring the L, a*, and b* values at three equidistant points around the circumference of each fruit. L indicates lightness (white/black), a* represents the red/green axis, and b* represents the yellow/blue axis. A 3NH-NR60CP colorimeter was used for these measurements.

2.5. Yield

Yield was calculated in tons per hectare (ton ha^{-1}) by multiplying the average fruit weight, the average number of fruits per plant, and the number of plants per hectare (approximately 1850 plants per hectare).

2.6. Data evaluation and statistical analyses

The experiment was laid out in a completely randomized design and 36 plants were used for each cultivar. A t-test was employed to determine differences between the cultivars, and the data were analyzed using the Minitab 17 statistical software package.

3. Results and Discussion

3.1. Plant's morphological characteristics

The results regarding the plant's morphological characteristics of the pitaya cultivars are given in Table 1. The days from planting to reach 100 cm, days from planting to first flowering, and days from flowering to harvest were not found to be statistically significant between the cultivars (P>0.05). Days from planting to reaching 100 cm in height was found to be 163.9 days on average for the White Jaina cultivar and 182.1 days for the Red Jaina cultivar respectively. The first flowering after planting occurred on June 19, 2022 for the White Jaina cultivar, and May 25, 2022 for the Red Jaina cultivar. However, flowering predominantly occurred in July and August for both cultivars. The days from planting to the first flowering was recorded as 619.40 days for White Jaina and 621.20 days for Red Jaina respectively. The duration from flowering to harvest was 44.18 days for White Jaina and 45.10 days for Red Jaina.

Figure 2. Removal of lateral shoots and apical pruning in pitaya plants.

Table 1. The results of plant morphological traits in pitaya cultivars

Trait	White Jaina (Mean)	Red Jaina (Mean)	P Value
Time to reach 100 cm height (days)	163.9	182.1	>0.05
Time to first flowering (days)	243.5	258.3	>0.05
Time from flowering to harvest (days)	40.2	41.1	>0.05

No statistically significant differences were found between the cultivars for the examined morphological traits (P>0.05).

Based on our findings, it could be claimed that flowering in both pitaya cultivars began at the end of May, with a peak in flowering occurring in July and August and continued until September. The results were found to be similar with some of the previous studies. Gübbük et al. (2017) reported that flowering in pitaya began in May-June and continued until September. Trivellini et al. (2020) carried out another study on pitaya under protected cultivation in Pisa, Italy. The researchers reported that 8% of the total flower buds were formed in July, 77% in late August, and the remaining 15% in September. Rodeo et al. 2018 found that the fruit harvest started in May, reached its peak between July and October, and continued until November in the Philippines.

In this study, the number of days from flowering to harvest were found to be around 44-45 days for both cultivars. The number of days from flowering to harvest was reported as 35-40 days in Türkiye (Gübbük et al. 2017), 30-35 days in the Philippines (Rodeo et al. 2018), 40-45 days in Italy (Trivellini et al. 2020), and 35, 41, and 96-110 days in three different studies conducted in Brazil (Costa et al. 2014; Menezes et al. 2015; Rabelo et al. 2020).

3.2. Fruit physical characteristics

3.2.1. Fruit weight

The average fruit weight was found to be statistically significant (P<0.05) for both cultivars (Figure 3). The fruit weight was recorded as 264.60 g for the White Jaina cultivar and 344.00 g for the Red Jaina cultivar respectively. Parmar and Karetha (2020) reported that the fruit weight was 265.86 g for S1 (*Hylocereus undatus*) and 260.27 g for S2 (*Hylocereus polyrhizus*). Trivellini et al. (2020) reported as 273 g for Clone 1 (*H. Undatus*) (white-fleshed cultivar) and 315 g for Clone 2 (*H. undatus* × *H. polyrhizus*) (red-purple-fleshed cultivar). Our results were found to be similar to previous studies. Menezes et al. (2015) and Magalhães et al. (2019) reported higher fruit weight values than that of our study as 442.47 g (*Hylocereus undatus*), and 436.84 g (*Hylocereus polyrhizus*) respectively. This difference is believed to have resulted from ecological and varietal differences.

3.2.2. Fruit circumference and length

Average fruit circumference and fruit length differences were found to be statistically insignificant for both cultivars (P>0.05) (Figure 4). The fruit circumference was determined to be 20.58 cm for the White Jaina cultivar and 21.99 cm for the Red Jaina cultivar respectively. On the other hand, the fruit length was 13.07 cm for the White Jaina and 12.22 cm for Red Jaina. As reported in previous studies, the fruit width was 7.19 cm for *Hylocereus undatus* (Parmar and Karetha 2020), 8.34 cm for *Hylocereus undatus* (Menezes et al. 2015), and 10.66 cm for *Hylocereus polyrhizus* (Magalhães et al. 2019). Fruit length was recorded as 9.51 cm for *Hylocereus undatus* by Parmar and Karetha (2020) 9.87 cm for *Hylocereus undatus* by Menezes et al. (2015), and 8.45 cm for *Hylocereus polyrhizus* by Magalhães et al. (2019). The values for fruit circumference and length were consistent with some studies, while some of them were found to be different from our study. The variations observed in fruit weight might be attributed to differences in ecological conditions and the cultivars used.

Figure 3. Fruit weight (g) values were determined for White Jaina and Red Jaina cultivars.

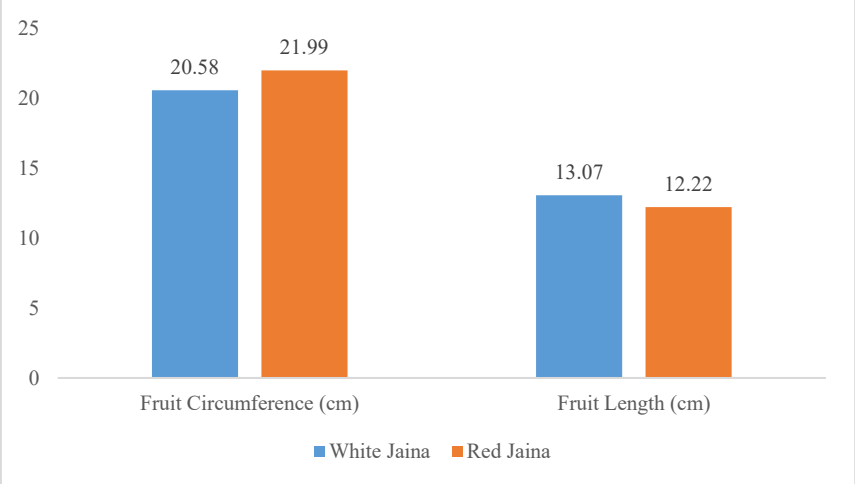

Figure 4. Fruit circumference and fruit length values were determined for White Jaina and Red Jaina cultivars.

3.3. Fruit quality characteristics

3.3.1. Fruit flesh firmness

The differences in fruit flesh firmness between pitaya cultivars were found to be statistically insignificant ($P>0.05$) (Figure 5). The fruit flesh firmness was determined to be 1.11 N for the White Jaina cultivars and 1.13 N for the Red Jaina cultivars. Menezes et al. (2015) reported a pulp firmness of 6.16 N for Hylocereus undatus and noted that firmness significantly decreases during ripening due to water loss. Centurión et al. (2008) observed a firmness of 6.3 N in fruits harvested 31 days after flowering. In Hawaii, Wall and Khan (2008) harvested mature fruits 45 to 50 days after flowering and reported a firmness of 5.7 N. Enciso et al. (2011) also noted that firmness is a relatively underexplored variable in postharvest studies of pitaya.

3.3.2. Soluble solids content

The difference in soluble solids content between pitaya cultivars was found to be statistically significant ($P<0.05$) (Figure 6). The soluble solids content was determined as 10.98% for the White Jaina cultivar and 12.35% for the Red Jaina cultivar respectively.

The soluble solids content in pitaya has been reported with varying values across different studies and cultivars. Menezes et al. (2015) reported an soluble solids content of 19.58% in *Hylocereus undatus*, while Ortiz and Takahashi (2015) recorded a value of 12.2% for *Hylocereus undatus*. Magalhães et al. (2019) reported a maximum soluble solids content of 15.44% in white-fleshed cultivars of *H. undatus*. Brar et al. (2023) observed soluble solids content values ranging from 8.63% to 9.31% in three dragon fruit species grown under subtropical conditions in northwestern India—one white-fleshed (*H. undatus*, DG-I) and two red-fleshed (*H. polyrhizus*, DG-II; *H. costaricensis*, DG-III). Öziyci et al. (2024) reported the highest soluble solids content as 13.70% for the white-fleshed Costa Rica White cultivar, and the lowest as 8.50% for the red-fleshed Philippines Purple cultivar. Our findings are in agreement with those reported by Öziyci et al. (2024).

3.3.3. Fruit skin color (L, a*, b*)

The differences in the fruit skin color parameters (L, a*, b*) between pitaya cultivars were found to be statistically insignificant ($P>0.05$) (Figure 7). The L value for the fruit skin color of pitaya cultivars was found to have ranged between 32.30 and 35.30, the a* value between 19.90 and 21.96, and the b* value between 7.46 and 8.15. The L and a* values obtained in this study were lower than those reported by Menezes et al. (2015), who reported an L value of 51.23 and an a* value of 35.50 for the species *Hylocereus undatus*. Similarly, Öziyci et al. (2024) reported L values ranging from 22.65 to 57.44, a* values from 0.51 to 40.72, and b* values from-5.55 to 11.84 across two white and six red pitaya cultivars.

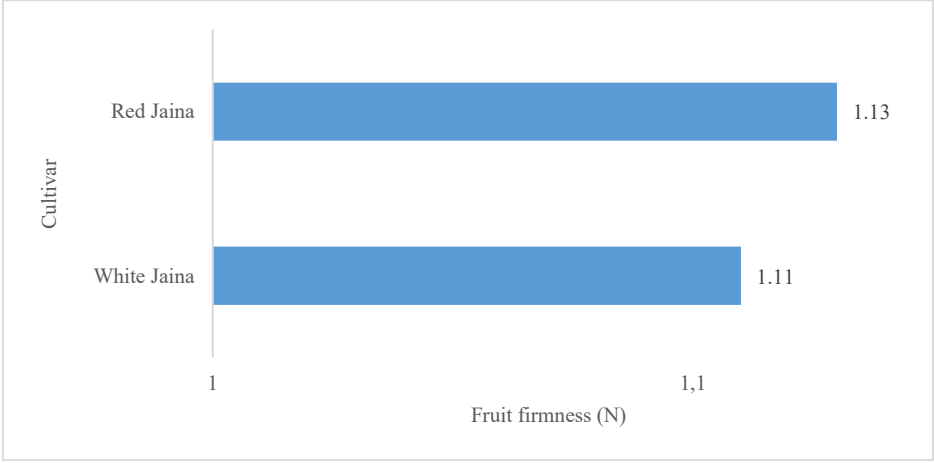

Figure 5. Fruit flesh firmness (N) values were determined for White Jaina and Red Jaina cultivars.

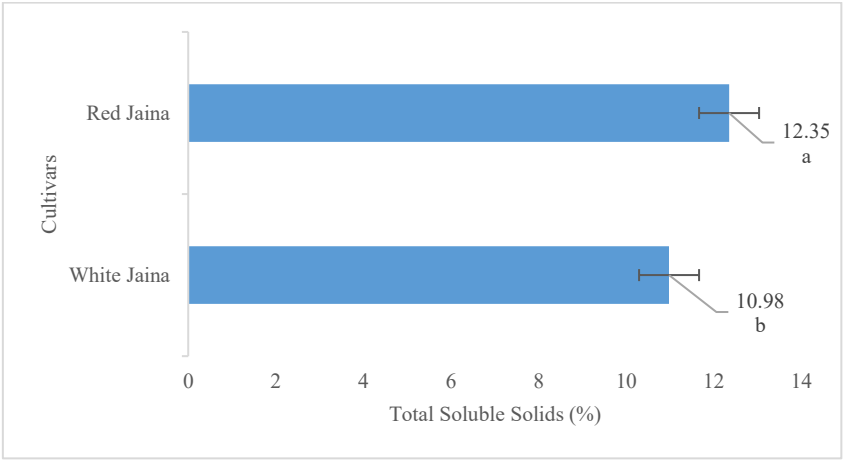

Figure 6. Soluble solids content (%) determined for White Jaina and Red Jaina cultivars.

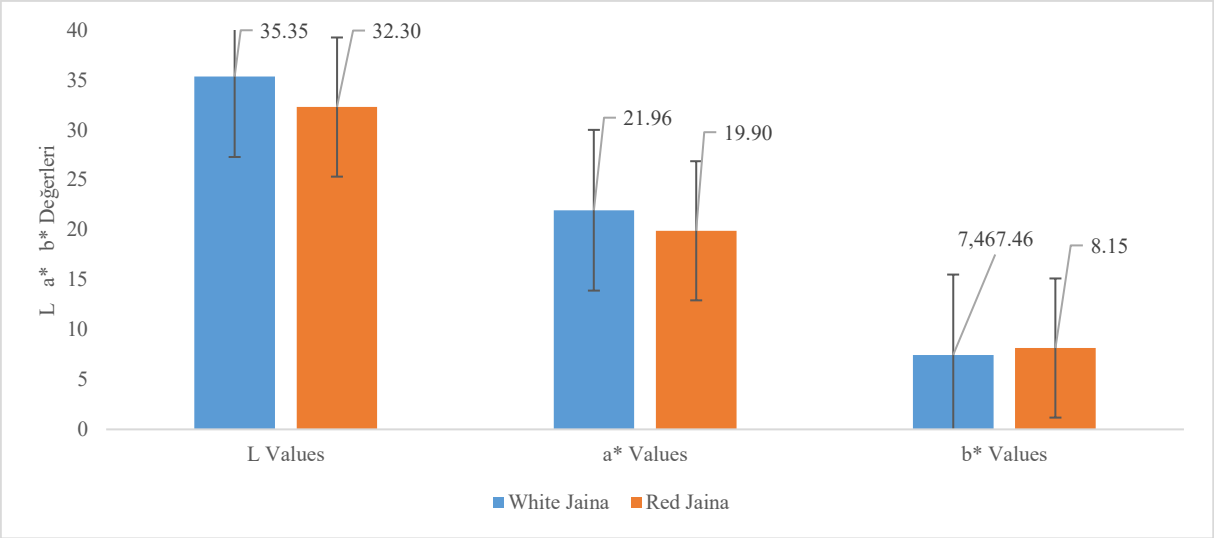

Figure 7. L, a*, b* values determined for the White Jaina and Red Jaina cultivars.

3.4. Yield

The differences observed in yield per hectare among pitaya varieties were found to be statistically insignificant (P>0.05). The yield per hectare of the White Jaina variety was determined to be 1.74 t ha^{-1}, while that of the Red Jaina variety was 1.65 t ha^{-1}. A study conducted in Brazil showed that the yield values obtained in the first year were consistent with the current study; however, it was observed that the yield increased substantially over the years (Alves et al. 2021). Nevertheless, the results obtained in this study were lower than those of many other studies, as they reflect data from the initial years (Sibut et al. 2023).

4. Conclusion

In conclusion, both cultivars are recommended for cool subtropical areas when the yield and some investigation criteria are considered. Furthermore, when we consider the water shortage problem in the Mediterranean climate, pitaya can be seen as an alternative crop in this region.

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Chemical profile and repellent effects of two essential oils on *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae)

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ABSTRACT

The confused flour beetle (*Tribolium confusum* Jacquelin du Val; Coleoptera: Tenebrionidae) is one of the most common pests of stored food products worldwide. Synthetic insecticides are commonly used to control this pest; however, their long-term application poses health risks and environmental concerns. The aim of this study was to evaluate the repellent activity of essential oils (EOs) from *Lavandula angustifolia* Miller and *Origanum vulgare* L. (Lamiaceae) against *T. confusum*. In this study, EOs from *L. angustifolia* and *O. vulgare* were extracted by hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS). The repellent activity of the oils against *T. confusum* adults was evaluated using a two-choice bioassay. The data were statistically analyzed using one-way ANOVA followed by Tukey's HSD test ($P<0.05$). The EO of *O. vulgare* was rich in carvacrol (96.7%), with minor components including thymene (2.01%), thymol (0.72%), and endo-borneol (0.53%). In contrast, *L. angustifolia* EO contained linalyl acetate (24.02%), linalool (15.79%), (+)-2-bornanone (10.74%), and eucalyptol (8.34%). Repellency was significantly higher in *O. vulgare* EO compared to *L. angustifolia* EO. Repellency was tested at concentrations of 1%, 2%, 4%, and 8% using a two-choice filter paper assay. The repellent activity of both oils increased significantly with concentration ($P<0.05$). *O. vulgare* EO showed the highest repellency, reaching 70% at 8% concentration after 1 hour of exposure. In contrast, *L. angustifolia* EO achieved a maximum repellency of 52% at the same concentration. At lower concentrations (1-2%), both oils showed limited efficacy. The results demonstrate that *O. vulgare* EO has promising repellent activity and could be considered a potential botanical insecticide for integrated pest management (IPM) of *T. confusum*.

1. Introduction

The rapid increase in the global population has posed substantial challenges in meeting essential nutritional demands. Cereals, which represent a significant proportion of agricultural commodities in Türkiye, also maintain a critical role in global food production systems (Dörtok and Aksoy 2018; Teke 2019; Küçüktopçu et al. 2023; Yetkin and Atakan 2022; Henteş and Işıkber 2024). The confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), is a persistent and economically important pest of stored cereals and processed cereal products, known for causing extensive damage to a wide variety of commodities (Walter 1990; Demirel et al. 2009; Kumar 2017; Yağcı et al. 2023). The adult measures approximately 1/4 inch in length, displays a characteristic reddish-brown coloration, and is distinguished by antennae terminating in a well-defined, four-segmented club (Walter 1990; Demirel et al. 2009).

Tribolium confusum exhibits a broad global distribution, facilitated by its ability to reproduce within a wide temperature range (19-37.5°C) and survive under very low relative humidity levels (greater than 1%) (Rees 2004). Its economic impact is considerable, attributed to both its global prevalence and its increasing resistance to various insecticides (Kavallieratos et al. 2011; Kavallieratos et al. 2013; Athanassiou and Kavallieratos 2014; Kavallieratos et al. 2015). In addition to its economic significance, *T. confusum* poses potential public health risks through the secretion of defensive chemicals that can cause skin irritation, itching, and respiratory disorders (Mullen and Durden 2019; Kavallieratos et al. 2020).

Historically, methyl bromide was one of the primary fumigants used to control insect pests in stored agricultural products. However, due to its detrimental effects on the ozone layer, its use has been largely phased out in compliance with the Montreal Protocol (Protocol 1987). Following this regulatory transition, phosphine emerged as the most widely used fumigant globally. Despite its effectiveness, the extensive and prolonged use of phosphine has led to the development of resistance in various stored-product insect populations, including T. *confusum* (Afful et al. 2018).

In response to this challenge, several alternative fumigants have been investigated-such as sulfuryl fluoride, ethyl formate, hydrogen cyanide, and nitric oxide (Navarro and Qian 2007; Yan et al. 2025). However, some of these compounds present limitations in terms of efficacy, human safety, and environmental compatibility (Navarro and Qian 2007). These shortcomings have underscored the need for sustainable pest control strategies and have directed attention toward botanical insecticides,

particularly essential oils (EOs), which offer promising repellent and insecticidal properties with lower ecological risks.

The limitations associated with conventional fumigants have prompted the development of novel, less toxic, and environmentally sustainable pest management strategies. Plants employ a variety of defense strategies to protect themselves against herbivores and pathogens. One such strategy involves the production of secondary metabolites, which are synthesized within plant cells and exhibit insecticidal and behavior-modifying properties against a wide range of pests (Güncan and Durmuşoğlu 2004). These bioactive compounds are typically classified into groups such as alkaloids, glycosides, phenolics, terpenoids, tannins, and saponins (Shanker and Solanki 2000). In particular, EOs-complex mixtures of volatile organic compounds-primarily consist of terpenic and non-terpenic hydrocarbons and their derivatives (Baser 2009; Sağlam and Özder 2013; Alkan 2023). These botanical products not only provide a renewable and eco-friendly approach to pest control but may also contribute to delaying or preventing resistance development in insect populations.

Several studies have examined the biological activities of EOs derived from *Origanum* and *Lavandula* species, both of which are rich in volatile compounds with insecticidal and repellent properties. For instance, Demirel et al. (2009) evaluated the toxicity of EOs from various *Origanum* species against *T. confusum*, reporting relatively low lethality for *O. vulgare*, while rosemary and thyme oils demonstrated stronger insecticidal effects. More recently, Alkan (2023) investigated the repellent activity of *O. onites* EOs under laboratory conditions and reported a significant repellent effect on *T. confusum*, indicating its potential as a natural alternative for pest control.

While previous research has provided valuable insights into the insecticidal and repellent properties of *Lavandula* and *Origanum* EOs, continued exploration remains important to better understand species-specific effects and application potential. Differences in chemical composition, experimental conditions, and insect responses across studies suggest that further comparative evaluations could enhance our understanding of their practical use in pest management. In this context, the present study investigates the repellent activity of EOs extracted from *L. angustifolia* and *O. vulgare* against *T. confusum*, contributing to the growing body of knowledge on plant-based alternatives within integrated pest management (IPM) programs.

2. Materials and Methods

2.1. Insect rearing

This study was conducted in Adana, Türkiye. A laboratory colony of *T. confusum* was maintained on cracked wheat supplemented with 5% (w/w) brewer's yeast, following the protocol described by Athanassiou et al. (2016). Adults ranging from 7 to 28 days old were used in the bioassays. The cultures were reared in 1000 mL glass jars and maintained in a climate-controlled chamber at 25 ± 1°C and 60 ± 5% relative humidity (R.H.), under continuous darkness.

2.2. Plant material and EOs *extraction*

The plant materials used in this study were collected from Pozantı, Adana, Türkiye, in 2024. The aerial parts of *O. vulgare* and the flowers of *L. angustifolia* were harvested during their full flowering stage. Leaves of *O. vulgare* and flowers of *L. angustifolia* were air-dried in the dark at ambient room temperature prior to extraction. EOs were obtained through hydrodistillation using a Clevenger-type apparatus. For each extraction, 500 g of plant material was distilled with 5000 mL of distilled water for 6 hours.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the EOs was analyzed using gas chromatography-mass spectrometry (GC-MS). Separation was carried out using a DB-Wax capillary column (60 m × 0.25 mm i.d. × 0.25 µm film thickness; J&W Scientific, Folsom, USA). Helium was used as the carrier gas, at a constant flow rate of 1.1 ml per min. The oven temperature was initially set at 60°C (held for 6 min), then increased at a rate of 1°C per min to 220°C and held for an additional 4 min. The injector, interface, and ion source temperatures were all maintained at 220°C. One microliter of each EO, diluted in 1 mL of dichloromethane, was injected in split mode (50:1 split ratio). Electron impact ionization was conducted at 70 eV. Compound identification was based on comparison with mass spectra in commercial libraries (Wiley 275 Library) and authentic standards. Relative percentages of components were calculated from peak areas in the chromatograms.

2.4. Repellency Bioassays

The repellent activity of the EOs was evaluated using the area preference method, as described by Devi et al. (2020) and Hu et al. (2019). Filter paper discs (6 cm diameter) were cut in half. One half was treated with 400 µL of the EOs solution diluted in 1% ethanol and distilled water, at concentrations of 2%, 4%, or 8% (v/v). The other half was treated with 400 µL of 1% ethanol (control).

After allowing solvent evaporation (8-10 minutes), the treated and control halves were rejoined and placed in sterile petri dishes (90 mm diameter). Ten unsexed adult beetles were released into the center of each dish, which was then sealed with adhesive tape. All tests were performed in a controlled environment at 26 ± 2°C and 70 ± 5% R.H. Insect distribution was recorded at 1, 24, and 48 hours post-exposure. The percentage repellency (PR) was calculated using the formula adapted from McDonald et al. (1970):

$$\text{Percentage Repellency (PR)} = \left[\frac{Nc-Nt}{Nc+Nt}\right] \times 100 \qquad [1]$$

Where;
Nc= number of insects on the control half
Nt= number of insects on the treated half.

2.5. Statistical analysis

All experiments were conducted with a minimum of four replicates. Results are presented as mean ± standard deviation (SD). Statistical comparisons between treatments were performed using one-way analysis of variance (ANOVA), using SPSS v20.0 (IBM Corp., Armonk, NY, USA). A significance threshold of $P<0.05$ was adopted for all analyses.

3. Results

The chemical composition of the EOs was determined through GC–MS analysis (Figure 1). EOs from *O. vulgare* consisted of four major compounds, whereas *L. angustifolia* contained a total of 33 identified constituents. In *L. angustifolia*

EO, the dominant components were linalyl acetate (24.02%), linalool (15.79%), (+)-2-bornanone (10.74%), eucalyptol (8.34%), endo-borneol (7.04%), 3,7-octadiene-2,6-diol, 2,6-dimethyl- (6.72%), and trans-linalool oxide (furanoid) (5.10%) (Figure 1). In contrast, the *O. vulgare* EO was overwhelmingly comprised of carvacrol (96.7%), followed by thymene (2.01%), thymol (0.72%), and endo-borneol (0.53%) (Figure 1).

The repellent activity of both EOs against *T. confusum* was evaluated at varying concentrations and time intervals (1, 24, and 48 hours) (Tables 1 and 2). Repellency increased significantly with both concentration and exposure time (P<0.05). For *L. angustifoli*a, at 1 hour, repellency ranged from 5.0% at 1% concentration to 52.0% at 8%. After 24 hours, repellency values were 10.0%, 30.6%, 31.5%, and 49.5% for 1%, 2%, 4%, and 8% concentrations, respectively. At 48 hours, repellency declined slightly but remained highest at 8% concentration (41.5%).

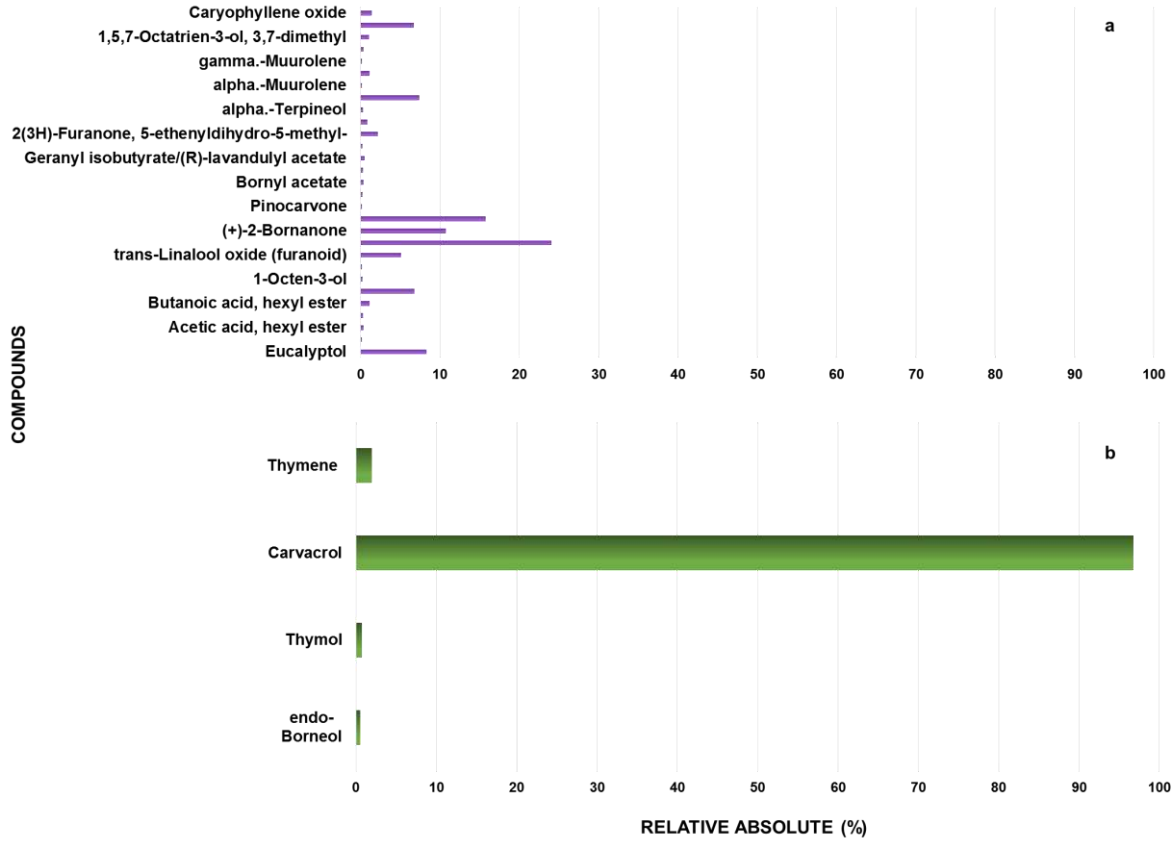

Figure 1. Chemical composition of EOs extracted from *L. angustifolia* (a) and *O. vulgare* (b), analyzed by gas chromatography coupled with mass spectrometry (GC-MS), obtained in Adana, Türkiye.

Table 1. Percentage repellency (mean ± SE) of different concentrations of *L. angustifolia* EOs against *Tribolium confusum* during bioassays conducted in Adana, Türkiye

Concentrations	1h	24h	48h
L.angustifolia 1%	5.0 ± 5.77b*	10.0 ± 5.77b	10.0 ± 6.72c
L.angustifolia 2%	40.0 ± 4.63a	30.0 ± 6.55ab	25.0 ± 3.27b
L.angustifolia 4%	41.8 ± 5.4a	31.5 ± 4.46ab	37.0 ± 2.77ab
L.angustifolia 8%	52.0 ± 1.78a	49.5 ± 4.11a	41.5 ± 2.85a

*Values are presented as means ± standard error (SE) of four replicates. Means within a column followed by different letters are significantly different according to Tukey's test (P<0.05).

Table 2. Percentage repellency (mean ± SE) of varying concentrations of *O. vulgare* EOs on *Tribolium confusum*, evaluated in Adana, Türkiye

Concentrations	1h	24h	48h
O.vulgare 1%	15 ± 2.89c*	10 ± 12.90c	5 ± 6.72c
O.vulgare 2%	35 ± 5.00b	30 ± 5.77bc	30 ± 5.77b
O.vulgare 4%	55 ± 2.89a	50 ± 5.77ab	45 ± 5.00ab
O.vulgare 8%	70 ± 5.77a	65 ± 5.00a	65 ± 5.00a

*Values are presented as means ± standard error (SE) of four replicates. Means within a column followed by different letters are significantly different according to Tukey's test (P<0.05).

Similarly, *O. vulgare* showed higher repellency across all time points. At 1 hour, repellency ranged from 15.0% (1%) to 70.5% (8%). After 24 hours, values were 12.0%, 30.5%, 50.5%, and 65.0% for increasing concentrations, respectively. By 48 hours, repellency remained at 65.0% at 8% concentration, while lower concentrations showed reduced effectiveness (e.g., 5.6% at 1%).

One-way ANOVA followed by Tukey's HSD test revealed statistically significant differences among concentrations for both oils. For *L. angustifolia*, repellency varied significantly over time (F_{1h}= 12,321; df_{1h}= 3, 15; $P_{1h} < 0.001$; F_{24h}= 7, 780; df_{24h}= 3, 15; $P_{24h} < 0.001$; F_{48h}= 11,959; df_{48h}= 3, 15; $P_{48h} < 0.001$). Similarly, *O. vulgare* showed highly significant differences (F_{1h}= 30,556; df_{1h}= 3, 15; $P_{1h} < 0.001$; F_{24h}= 8,871; df_{24h}= 3, 15; $P_{24h} < 0.001$; F_{48h}= 19,909; df_{48h}= 3, 15; $P_{48h} < 0.001$).

In general, repellency was highest at 1 hour post-exposure and declined over time for *L. angustifolia*, while *O. vulgare* maintained strong activity for up to 48 hours. At all durations, the 8% concentration induced significantly higher repellency than the lower doses, with *O. vulgare* consistently outperforming *L. angustifolia*.

4. Discussion and Conclusion

This study clearly demonstrates the differing repellent effects of *O. vulgare* and *L. angustifolia* EOs against *T. confusum,* largely attributable to their distinct chemical compositions. *O. vulgare* EO showed a significantly higher repellency, which is likely due to its high carvacrol content-a monoterpenoid phenol known for its neurotoxic effects on insects (Enan 2001; Jukic et al. 2007; Tong and Coats 2010; Anderson and Coats 2012; Evergetis et al. 2018; Giatropoulos et al. 2023). In contrast, *L. angustifolia* EO, dominated by linalyl acetate and other oxygenated monoterpenes, exhibited weaker repellency.

EOs exert diverse biological effects such as toxicity, repellency, and feeding inhibition, primarily due to their rich mixtures of monoterpenes and phenolic compounds (Regnault-Roger et al. 2021). In our chemical analysis, *O. vulgare* EO contained 96.7% carvacrol, consistent with Kim et al. (2010), who reported 67.2% carvacrol as the main repellent and insecticidal agent against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Similarly, our findings align with Alkan (2023), where *O. onites* EO showed 68% repellency at 0.25 µL cm^{-2} after 2 hours; in our study, *O. vulgare* EO achieved 65% repellency at 8% after 48 hours. This suggests that while initial repellency may be high, EO effectiveness tends to decrease over time due to their volatility.

For *L. angustifolia*, the major components identified-linalool and linalyl acetate-are consistent with previous work by Pokajewicz et al. (2021), who found these compounds accounted for over 80% of lavender oil samples. This consistency suggests a stable chemotype across different regions and explains the moderate repellency observed in our study.

EOs are gaining attention as eco-friendly alternatives for stored product pest control due to their low environmental persistence and reduced resistance risk (Chaudhari et al. 2021). Their volatile constituents function as semiochemicals that affect insect behavior via olfactory receptors (Mossa 2016), while compounds such as carvacrol and thymol disrupt neural signaling by inhibiting acetylcholinesterase (Jukic et al. 2007).

In conclusion, both EOs tested showed repellent effects against *T. confusum*, with *O. vulgare* being significantly more effective. The dominance of carvacrol likely contributes to its strong bioactivity. Going forward, efforts should focus on enhancing EO stability in storage environments, evaluating cost-effectiveness, and conducting field trials to support their integration into pest management programs.

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Optimization of different DNA extraction methods for the molecular detection of resistance genes

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ABSTRACT

Tomato spotted wilt virus (TSWV) and Pepper mild mottle virus (PMMoV) not only cause economic yield losses but also limit production for pepper plants. Resistant genes are the only reliable management strategies to control such viral diseases, but these viruses are able to overcome such resistance mechanisms. Therefore, novel resistance genes should be used to control the TSWV and PMMoV diseases. For molecular detection of resistance genes, DNA quality and purity are extremely vital to obtain proper results. This study aims to identify the best extraction method using resistance gene markers from different leaves of pepper plants. PCR analyzes revealed that fresh first real leaf gave reliable DNA quality with the CTAB DNA extraction method and then the Dellaporta extraction method which yielded lower concentrations. Although the commercial DNA extraction method offered convenient results within PCR analyzes, it is cost effective for molecular breeding such as marker assisted selection programs in developing countries. The study has clearly addressed optimized DNA concentration, using different extraction methods, leaf samples from pepper plants and their storage conditions for ultimate results in breeding programs.

1. Introduction

Pepper (*Capsicum annuum* L.) holds significant economic importance in both Türkiyeand worldwide in terms of production and consumption. According to production volume, China, Mexico, and Türkiye are the top three producers worldwide (FAO 2018). National statistics from TUIK indicate that Türkiye produced 2636905 tons of pepper in 2020 (TUIK 2021). The pepper is consumed fresh and is also processed in the food industry as dried, pickled, paste, and spice products. The interest in identifying capsaicinoids found in hot peppers has recently increased due to their diverse applications, such as in topical creams, defensive sprays, and alternative medical treatments (Christo and Cauley 2009).

Pepper is an annual plant cultivated in temperate, warm climates, and various cultivars are grown in open fields and greenhouses depending on the climatic conditions. There are many cultivated types in Türkiye including Capia, sivri, charleston, dolma, California wonder, Maraş pepper, chili peppers, ornamental peppers, pickling peppers, jalapeño, and cherry types. However, viral diseases are among the major factors that significantly impact both yield and quality in pepper cultivation areas (Anandakumar et al. 2008). There are no effective chemical control methods against viral diseases; therefore, developing resistant varieties and implementing vector control measures are essential. Tomato spotted wilt virus (TSWV) is transmitted by thrips species such as *Frankliniella occidentalis* and *Thrips tabaci*, it is a tospovirus that rapidly spreads in pepper production areas. On the other hand, Pepper mild mottle virus (PMMoV) is a *tobamovirus*, and it is seedborne and transmitted through mechanical manners.

Currently, advance biological and biotechnological methods are widely applied in agricultural production. Through molecular genetic analysis of plant DNA, it is possible to identify plant genotypes and construct genetic maps similar to human genetic studies. Marker-assisted selection (MAS) is particularly important in plant breeding to detect the presence of disease resistance genes. One of the key technologies enabling this is the polymerase chain reaction (PCR), discovered by Mullis (1986). The PCR allows for the amplification of specific regions of DNA from any organism with known genomic sequences (Mullis et al. 1986).

Molecular markers linked with resistance alleles such as *L3*, *L4*, and *Tsw* genes are commonly used in resistance breeding against PMMoV and TSWV diseases. The SCAC568 CAPS marker has been associated with resistance to TSWV, it is used in breeding programs to distinguish between resistant and susceptible genotypes at the co-dominant level (İkten 2019). Resistance to *tobamoviruses* is effectively identified by markers linked to *L3* and *L4* genes. Among various studies, the most reliable primers for identifying *L4* genes associated with PMMoV resistance are AP-7/AP-8 primers developed (Matsunaga et al. 2003; Fidan and Barut 2019).

Traditional breeding methods consider quality and quantity of DNA extracted from different pepper plant populations can often limit PCR efficiency. The initial step in a successful PCR

reaction is the isolation of total nucleic acids. Commercial DNA extraction kit has been used in 90% of resistance screening studies against TSWV in Turkish pepper varieties. While these commercial kits provide fast and clean DNA extraction, they are costly for large-scale sample processing. Among alternative methods, the CTAB method (Doyle and Doyle 1987) is widely used; however, the use of chloroform in this method poses serious health risks such as dizziness, fatigue, respiratory irritation, and long-term damage to liver and kidneys. Moreover, the phenological stage of the sampled plant, storage conditions, and sample quantity also influence DNA extraction qualities. Therefore, it is essential to employ low-cost, less hazardous chemicals during DNA isolation. This necessity has prompted us to search for reliable extraction protocols (Aka-Kaçar 2003; Aleksić et al. 2012).

The aim of this study was to optimize CTAB, Dellaporta, and commercial kit-based DNA extraction methods for the isolation of total nucleic acids intended for use for PCR applications, and to evaluate the impact of DNA quality on PCR efficiency using molecular marker analysis across different pepper varieties.

2. Materials and Methods

In this study, two different internationally developed protocols, modified CTAB and Dellaporta, and commercial kit, GeneMark Plant DNA Purification Kit (APS Lifetech, Netherland), were used to extract total DNA. In PCR gene screening, SCAC568 was used in the resistance analysis against TSWV, and AP-7/AP-8 markers were used in the resistance analysis against PMMoV respectively. For each gene analysis, 3 different commercial pepper varieties from 7 different segments were studied where these varieties were *L4* resistant commercial variety (A), *Tsw* gene containing variety (B) and susceptible pepper variety (C) to both TSWV and PMMoV.

For each commercial variety, 3 individual pepper seeds were sown in vials containing 1 volume (v) peat: 1 v perlite: 1 v vermiculite mixture. The vials were placed in acclimatization chambers in total darkness at 26ºC for 48 hours. After this period, the acclimatization chamber was adjusted to 16 hours of light and 8 hours of night period at 26ºC. For analyzes, 39 plants, with cotyledons and first 5 true leaves emerging after the cotyledons were collected and stored at -20ºC. The leaf samples were either used or stored over 90 days at -80ºC for DNA extractions. Detailed DNA isolation protocols and preparations of extraction solutions are provided below, and the obtained DNA samples were statistically analyzed by T test (Santos et al. 2010).

2.1. The CTAB extraction protocol

In the CTAB method, 5 ml CTAB + 5 µl β-Mercaptoethanol was mixed and used in the crushing stage of the samples. A 500 mg leaf sample was first crushed with 15 µl of CTAB solution. The amount was completed to 500 µl and transferred to microtubes. The tubes were incubated at 65 ºC for 2 hours at 350 rpm. Eppendorf tubes were cooled for 5 minutes, 500 µl of 24 chloroform: 1 isoamyl alcohol solution was added to them, they were turned upside down and centrifuged at 13000 rpm for 15 minutes, and the supernatant liquid was transferred to new microtubes. A 500 µl of 24 chloroform: 1 isoamyl alcohol was added to the microtubes again and the centrifugation process was repeated. The samples were transferred back to new tubes then supernatant liquid was drawn as cleanly and carefully as possible and transferred to new tubes. Isopropanol alcohol was added as much as the drawn volume and turned upside down. The microtubes containing the samples were kept at -20ºC overnight. The next day, the samples were centrifuged at 13000 rpm for 20 minutes. The supernatant was carefully poured off and cold 70% alcohol was added to the samples and centrifuged at 13000 rpm for 5 minutes and the supernatant liquid was poured off. 500 µl of ethanol was added to the samples again and the process was repeated. After the last liquid part was poured off, the samples were left to dry. After the samples dried, they were diluted with 100 µl of ddH_2O and kept at +4ºC for one day (Doyle and Doyle 1987).

2.2. The Dellaporta extraction protocol

In this method (Dellaporta et al. 1983), a 500 mg sample was ground in 1.2 ml of extraction buffer including 100 mM Tris, 50 mM EDTA, 500 mM NaCl, 10 mM β-Mercaptoethanol. A volume of 600 µl from the homogenized sample was transferred into 1.5 ml microtubes. Subsequently, 70 µl of 10% SDS was added, and the tubes were incubated at 65°C for 10 minutes. The tubes were inverted twice during incubation then samples were allowed to cool for 5 minutes. 200 µl of 5 M potassium acetate was added to each tube and the tubes were placed on ice for 10-30 minutes. During this period, the tubes were inverted twice then the samples were centrifuged at 10000 rpm for 10 minutes at 10 °C. From the supernatant of each centrifuged tube, 600 µl was carefully transferred into new tubes. An equal volume (600 µl) of ice-cold 96% ethanol was added, and the tubes were inverted a few times. The samples were placed overnight in a−20°C deep freezer. The next day, the samples were centrifuged at 10000 rpm for 10 minutes to precipitate the nucleic acids. The supernatant was carefully removed without disturbing the pellet. The remaining pellets in the tubes were left to air dry for 10-15 minutes. Finally, 200 µl of sterile distilled water was added onto dried pellets, and the tubes were incubated at 37°C for 15 minutes to completely dissolve the nucleic acids.

2.3. Commercial kit DNA extraction protocol

DNA isolations were performed from collected plant samples according to the protocol recommended by the manufacturer of the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit (Lot No: 2921857). After completion of the isolation procedures, the quality of the DNA was assessed by staining the samples with loading dye and preparing them in a 1.5% agarose gel made with TAE buffer solution. A volume of 6 µl from each sample was loaded into individual wells of the gel, and electrophoresis was carried out at 100 V for 45 minutes. The gel was stained with ethidium bromide and visualized under a UV transilluminator. To determine the purity and concentration of the nucleic acids following the isolations, spectrophotometric measurements were conducted for each sample, then DNA concentrations were adjusted to 50 ng μl^{-1}.

In the PCR reactions, a total volume of 50 µl reaction mixture was used, containing genomic DNA at a concentration of 50 ng μl^{-1}, 12 µl of ddH_2O, 24 µl of Ecotaq, and 2 µl each of forward and reverse primers (Table 1). The thermal cycling program in the PCR machine (Thermo Scientific, Germany) as follows: an initial denaturation at 95°C for 3 minutes then 30 cycles of denaturation at 95°C for 50 seconds (s.), annealing at 57/58°C for 45 s and extension at 72°C for 50 s with a final extension step at 72°C for 10 minutes.

For each plant sample, three different isolation methods and three different sampling conditions were applied, resulting in 12 distinct outcomes per sample. A total of 156 data were obtained

Table 1. The markers used to amplify TSWV and PMMoV resistance genes from isolated DNA samples in PCR analyses

Marker Name		Primer Sequence	Amplicon Size (Bp)	Annealing Temperature
SCAC568	Forward	5'GTGCCAGAGGAGGATTTAT3'	568	57°C
	Reverse	5'GCGAGGTGGACACTGATACT3'		
AP-07 / AP-08	Forward	5'CGTACTGTGGCTCAAAACTC3'	1400	58°C
	Reverse	5'ATTCGCACCGTTTAGCCCGT3'		

with each primer set based on 13 samples. Following the completion of the PCR reactions, the samples were subjected to electrophoresis in agarose gels, stained with ethidium bromide, and visualized under UV light, similar to the procedure used for DNA quality assessment.

3. Results

There is no existing chemical control which controls viral diseases therefore developing resistant cultivars against viral diseases in pepper is one of the most critical strategies for disease management. In advanced technology, marker-assisted selection (MAS) at the DNA level has emerged as a safer and more sustainable molecular method for resistance breeding. For MAS screening, high-quality nucleic acids are the first step toward success in molecular studies. Therefore, it is crucial in such studies, to obtain nucleic acids that are able to be used in a highly pure form both quickly and economically.

The selection of the most appropriate technique depends on several criteria, including target nucleic acid (DNA or RNA), source organism and type of plant material (leaf, seed, peel tissue, etc.), whether the leaf is young or mature, the timing of the study, the storage method, quantity, and location of the samples, as well as the desired outcomes (yield, purity, time required for purification, etc.). The applications following extraction also significantly impact the cost and success of the technique. For this purpose, three different methods, two different phenological stages, and two testing times were analyzed using two resistance markers in PCR studies.

When comparing the methods, the highest DNA concentration was obtained using the CTAB method yielding 391.23 ng μl^{-1}, followed by the Dellaporta and the commercial kit respectively. One of the key indicators affecting DNA quality is A260/A280 ratio which is used to assess nucleic acid purity. The absorbance ratio of approximately 1.8 indicates pure DNA, while a value around 2.0 suggests pure RNA. A 260/280 ratio greater than 1.8 indicates RNA contamination, while a 260/230 ratio below 1.8 suggests protein contamination. Any type of contamination may lead to several issues including poor outcomes or sample loss in molecular studies. Contamination also affects a shelf life of the sample causing quick degradation of nucleic acids (Yörek 2005). Spittle et al. (2010) reported that chemicals, purification method, plant materials and extraction environments cause contamination, thereby lowering the 260/280 ratio (Furda et al. 2014; Pierre et al. 2018). Considering all our general findings, it was determined that the commercial kit provided the most optimized DNA isolation method based on 260/280<1.8 and 260/230<1.8 ratios (Table 2). The highest DNA yield was achieved using the CTAB method from the first true leaf stage (Table 2).

As can be seen in Table 3, the commercial kit with an average of 144 ng μl^{-1} DNA obtained from the first true leaf, there was not only the highest quality but also the cleanest DNA extracted with absorbance A260/A280 1.860 and 260/230 1.981 ratios. Although, the highest DNA was obtained with an average of 325.42 ng μl^{-1} in the CTAB method, while it was desired to be 260/280>1.8, this ratio remained at 260/280 1.308 in the isolation made from fresh leaves. The lowest DNA with the dirtiest phenolic compounds and RNA were obtained from samples stored at -80°C with Dellaporta. The amount of DNA was high in the samples taken from the first true leaf in the Dellaporta and CTAB methods where DNA quality was high and ratios were close to optimum values. The amount and quality of DNA isolated from the 5th true old leaf and their storage at -80ºC decreased. All the obtained data was subjected to the T test (Table 3), there was no statistical difference between Dellaporta and CTAB methods in terms of DNA amounts, while there was a significant difference between the commercial kit and the other two extraction methods (Table 3). When the old leaves and the leaves stored at -80ºC were statistically examined, a significant difference was found between CTAB and Dellaporta methods (Table 3).

The optimization of DNA isolation was based on comparisons of concentration, purity, and band clarity. Additionally, the suitability of the extracted DNA for PCR applications was assessed using molecular markers linked to the *Tsw*, *L3*, and *L4* resistance genes. For PCR analyses, SCAC568 marker associated with the *Tsw* gene for TSWV resistance, and CR-20, CAMS451 markers associated with *L3* and *L4* genes for PMMoV resistance were utilized respectively. The PCR amplifications were performed under optimized conditions, and the presence or absence of resistance alleles in the genotypes was determined based on the resulting band patterns on agarose gels.

The results indicated that although all three methods successfully yielded DNA suitable for molecular studies, the modified CTAB method provided higher DNA concentrations and better-quality results compared to the others. The Dellaporta method yielded relatively lower concentrations but still produced DNA suitable for PCR. While the commercial kit offered convenience and consistent purity, it was found to be less cost-effective for large sample sizes (Martellossi et al. 2005).

Proteins absorb at 280 nm, the A260/A280 ratio is commonly used to assess nucleic acid purity. Pure DNA typically yields a ratio of approximately 1.8, whereas pure RNA gives a value around 2.0. The 260/280 ratio greater than 1.8 indicates RNA contamination, while a ratio lower than 1.8 at 260/230 suggests protein contamination (Shokere et al. 2009; Sedlackova et al. 2013). Any form of contamination can lead to numerous problems. In molecular studies, contamination may result in poor outcomes and even the loss of the sample. Furthermore, contamination negatively affects the storage stability of the sample, potentially causing a more rapid degradation of the nucleic acids obtained (Yörek 2005; Luciano et al. 2007; Visvikis et al. 1998). According to Spittle et al. (2010), Gallagher (2011) and Glasel (1995), chemicals used during extraction, the purification method, and the working environment or materials can all contribute to contamination, thereby decreasing the 260/230 ratio.

To assess the impact of DNA quantity and quality on molecular analyses, PCR assays were conducted using five commercial pepper cultivars that carry the *L4* and *Tsw* resistance

Table 2. Plant materials and DNA extraction methods are compared with absorbance ratios and provide the best DNA extraction methods

Methods		Plant Material								
		First real leaf			Old leaf			90 days stored leaf at -80°C		
DNA extraction method	No	Total DNA amount (ng µl^{-1})	260/230 Ratio	260/280 Ratio	Total DNA amount (ng µl^{-1})	260/230 Ratio	260/280 Ratio	Total DNA amount (ng µl^{-1})	260/230 Ratio	260/280 Ratio
Dellaporta	1	273.18	1.432	2.203	201.01	1.008	0.815	187.18	0.998	0.789
	2	247.23	1.682	1.987	113.32	0.948	0.997	99.14	0.902	0.824
	3	260.33	1.340	2.036	148.36	0.961	1.011	88.16	0.879	1.001
	4	261.28	1.548	2.502	217.28	1.089	0.903	151.99	0.856	0.789
	5	305.56	1.246	1.525	120.54	1.023	1.107	108.09	0.826	0.698
	6	236.58	1.585	1.966	139.78	0.893	0.963	90.25	0.905	0.891
Mean		**264,01**	**1.472**	**2.037**	**156.15**	**0.897**	**0.966**	**120.80**	**0.909**	**0.832**
CTAB	1	260.06	1.514	2.098	113.31	1.110	0.996	93.31	0.986	0.891
	2	307.36	0.958	1.628	120.11	0.893	1.004	80.56	0.754	0.904
	3	391.23	1.457	2.190	186.34	0.991	1.001	93.65	0.587	0.801
	4	248.60	1.482	1.963	119.05	1.594	1.028	102.45	0.759	0.831
	5	344.43	0.859	1.588	105.26	0.981	1.208	78.05	0.802	1.154
	6	400.85	1.580	1.869	213.62	0.944	0.919	102.30	0.659	0.958
Mean		**325.42**	**1.308**	**1.839**	**142.95**	**1.085**	**1.026**	**91.72**	**0.757**	**0.923**
Commercial Kit	1	121.12	1.845	2.090	98.45	1.113	1.112	76.42	1.001	1.003
	2	118.34	1.861	1.986	99.68	1.216	1.201	69.69	0.989	1.102
	3	178.87	1.885	2.032	100.24	1.227	1.138	70.12	1.015	1.003
	4	150.34	1.933	1.989	86.00	1.025	1.094	75.20	1.596	1.601
	5	130.25	1.650	1.799	102.33	1.079	1.064	80.03	1.036	1.102
	6	169.02	1.991	1.981	98.37	1.198	1.289	83.26	0.936	1.025
Mean		**144.65**	**1.981**	**1.860**	**97.51**	**1.143**	**1.145**	**75.78**	**1.096**	**1.156**

Table 3. Values of each DNA extraction method analyzed in T statistical test

Plant Tissue	Measurement	DNA Extraction Method	Compared Extraction Method	Statistical Deviation[¥]
First real leaf	Total DNA amount	Dellaporta	CTAB	NS
		Dellaporta	KIT	**
		CTAB	KIT	**

*Significant at the 0.05 probability level, **Significant at the 0.01 probability level, [¥]Differences between means of methods and DNA values were tested by *t* test, NS: none significant.

genes, along with the susceptible commercial variety which lacks both L4 and *Tsw* resistant genes.

PCR was performed using DNA extracted by three different methods from three different sample types, targeting the *Tsw* CAPS marker for resistance to Tomato Spotted Wilt Virus (TSWV) and the Ap7/Ap8 marker used for resistance to Pepper Mild Mottle Virus (PMMoV).

Despite reduced DNA quality and quantity, the L4 primer was successfully amplified in all samples. However, the Tsw CAPS marker for TSWV resistance only produced reliable amplification in the samples obtained from the first true leaves across all nine combinations. In contrast, DNA extracted from the 5 true leaf and stored at –80°C, using the CTAB, Dellaporta, and a commercial kit extraction method resulted in low products in PCR reactions where the Tsw CAPS marker did not perform as effectively. In this study, PCR was conducted on five resistant and susceptible commercial F1 hybrid pepper genotypes using DNA extracted to identify the presence of 2 genes, the *L4* gene confers resistance to Tobacco Mosaic Virus (TMV), Pepper Mild Mottle Virus (PMMoV), and the *Tsw* gene provides resistance to Tomato Spotted Wilt Virus (TSWV). Different DNA extraction methods with the resistance genes were evaluated for such devastating diseases caused by *Tobamoviruses* and TSWV in pepper. Upon examination of the PCR results, it was found that all three DNA isolation methods performed well when DNA was extracted from the first true leaves. As shown in Figure 1A commercial genotype lacked the *Tsw* gene, while the other five genotypes carried this resistance gene. After digestion with the *XbaI* enzyme, it was confirmed that these five genotypes were resistant (Figure 1B). However, when DNA was extracted from older leaves or from samples stored at -80°C, the *Tsw* gene failed to amplify reliably across all three methods. This suggests that DNA from the first true leaf must be used when analyzing the *Tsw* gene in pepper. Otherwise, the other commercial genotypes were genetically resistant but the resistant bands did not appear, while susceptible genotype bands were visible (Figure 1C). Despite the high DNA quantity, the presence of phenolic compounds negatively affected quality with 260/280 ratios ranging from 0.832 to 1.156, and 260/230 ratios between 0.757 and 1.085 values that deviate significantly from the desired thresholds. While a 260/280 ratio>1.8 indicates RNA contamination, a 260/230 ratio<1.8 is generally acceptable but the values from older or frozen leaves were far from ideal and the ratio was negatively affected (Figure 1D) in the PCR outcomes (Nadeem et al. 2017; Mitsouras and Faulhaber 2009; Bozkaya 2012; Şimşek et al. 2008).

Regarding PMMoV resistance, the *L4* gene was successfully detected using AP7-AP8 primers in PCR conducted with DNA extracted using all three methods at different time points. These results highlight the need to optimize conditions for specific gene markers, these results were consistent with results in Nadeem et al. (2017). In conclusion, when working with the *Tsw* gene in pepper, it is essential to use DNA extracted from the first true leaf under optimal conditions. For the *L4* gene associated with PMMoV resistance, the AP-7 and AP-8 primers amplified product in all tested conditions.

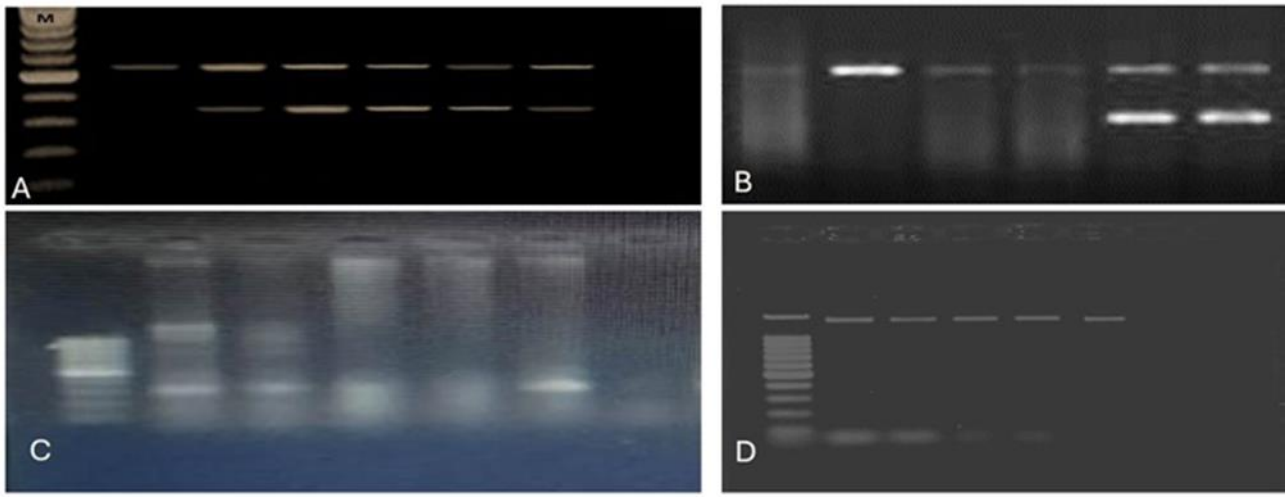

Figure 1. The gel visualizations of *Tsw* and *Ap7*/*Ap8* primers are used to asses DNA qualities obtained with different extraction methods in PCR. A) The CAPS primers have amplified products on agarose gel where first real leaf was used to extract DNA with commercial kit, B) An agarose gel view with old leaf DNA samples were extracted with Dellaporta, C) The AP primers have amplified products on agarose gel where 90 days old leaf samples wereused to extract DNA using CTAB, and D) The AP primers have amplified products on agarose gel where 90 days old leaf samples were used to extract DNA using commercial kit method.

Although potassium acetate yielded lower DNA concentrations than phenol-chloroform for protein removal during DNA isolation (Bozkaya 2012), it still provided DNA of sufficient quantity and quality, hence, the potassium acetate may be preferred over phenol-chloroform which poses health and environmental risks (Bozkaya 2012). Furthermore, careful handling and preservation of plant tissues positively influence the quality of the isolated DNA.

4. Discussion and Conclusion

For the successful isolation of total nucleic acids, DNA should be free from both organic and inorganic contaminants. As the isolation protocol progresses and the sample becomes purer, more delicate handling is required. For instance, the tubes should not be vortexed or exposed to high RPM centrifugation. DNA can be shared due to excessive agitation or damage from impacts and may degrade if exposed to high temperatures for extended periods.

If the DNA is not handled cleanly, PCR analyzes may be inhibited, resulting in failed amplification or low-quality band patterns. Another key factor affecting DNA quality is the developmental stage of the plant at the time of sampling. In breeding studies, especially for marker analysis, using the first true leaf that emerges after the cotyledon provides more reliable results. Whenever possible, DNA isolation should be performed immediately after sampling from fresh tissue. Even when young leaves are collected from older plants, DNA quality tends to decline with plant age.

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Photovoltaic and biogas based renewable energy applications in livestock farms in Karaman province

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ABSTRACT

This study evaluates the economic and environmental advantages of integrating renewable energy systems into livestock farms in Karaman province,Türkiye. A hybrid model combining biogas and photovoltaic (PV) systems was developed and simulated for a representative cattle farm. Biogas production potential was calculated based on local livestock waste data, and a rooftop PV installation was modeled using region-specific solar radiation values. The results demonstrate that such a hybrid system can produce both electrical and thermal energy sufficient to meet on-farm demands, while significantly reducing greenhouse gas emissions. Economic analysis reveals a feasible payback period, and sensitivity tests show resilience against energy price fluctuations. This study is one of the first to model an integrated PV and biogas hybrid energy system at the provincial level in Türkiye, demonstrating its economic and environmental viability.

1. Introduction

Increasing industrialisation with the Industrial Revolution has led to an increase in CO_2 emissions. The increase in CO_2 emissions has led to global warming as a result of air pollution and ozone depletion, while industrial waste has caused environmental pollution and serious damage to natural life. The United Nations Framework Convention on Climate Change, the Paris Agreement signed in 2015, which sets out legal responsibilities for mitigation, adaptation and financing of climate change, aims to limit the increase in global average temperature to 2°C above pre-industrial levels (United Nations 2024). According to the results of the greenhouse gas inventory, total greenhouse gas emissions decreased by 2.4% to 558.3 million tonnes of CO_2 equivalent in 2022 compared to the previous year (TÜİK 2024). As a result of these negative figures, the search for clean and renewable energy sources has begun. The global effort to raise awareness about clean energy is the result of the popularity of renewable energy sources such as solar, wind, biomass, geothermal, hydropower and wave in recent years. According to the International Energy Agency's "Renewable Energy 2023" report, global renewable energy capacity will reach 510 GW in 2023, an increase of almost 50 per cent and a new record. One third of the increase in global renewable capacity will come from solar power plants (IEA 2024a). In Türkiye, the installed power generation capacity reached 108 GW by the end of March 2024. with 29.6% hydro, 23.2% natural gas, 20.2% coal, 11.2% wind, 11.7% solar, 1.6% geothermal and 2.5% from other sources (ETKB 2024). In light of the data obtained, it can be seen that approximately 55% of the installed capacity in Türkiyeis provided by renewable energy sources.

Biomass is an energy source with high potential for heating and electricity generation. It can be produced worldwide from sewage treatment plants, landfills, animal wastes, agricultural wastes with no food or feed value, forest products other than industrial timber, and by-products from the processing of used tyres. Biogas is a sustainable energy carrier consisting mainly of 60% methane and 35-40% carbon dioxide (Ökten 2021).

When reviewing the literature, there are both provincial and regional studies on biogas production from cattle waste inTürkiye: Ağrı (Erhan 2019), Balıkesir (Salihoğlu et al. 2019), Bilecik (Yerel Kandemir and Açıkkalp 2019), Kırklareli (Kalaycı et al. 2019), Sivas (Polat Bulut and Topal Canbaz 2019), Erzincan (Kurnuç Seyhan and Badem 2021), Bitlis (Demir Yetiş et al. 2019), East Anatolian provinces (Çağlayan 2020), Kahramanmaraş (Ay and Kaya 2020), Kayseri (Nuralan Poyraz et al. 2020), Mersin (Gülşen Akbay and Kumbur 2020), Adana (Erkan Can 2021), Antalya (Atılgan et al. 2021), Central Anatolian provinces (Topal Canbaz and Polat Bulut 2021), Çorum (Seyitoğlu and Avcıoğlu 2021), Düzce (Kurt 2021), Eskişehir (Kaynarca et al. 2021), Mardin (Yenigün et al. 2021), Bingöl (Demir and Çulun 2022), Iğdır (Tırınk 2022), Aksaray (Et Yapılcan and Bakırtaş 2023), Şırnak (Gündüz and Bayrakdar Ateş 2024).

In this study on energy consumption in dairy farming, three different dairy farms were analysed for 30 weeks and it was found that 37% of the electricity consumption of the farms was for cooling, 31% for water heating, 19% for vacuum pump and 10% for lighting systems (Cucchiella et al. 2015). Velo et al. (2014) compared an independent battery-wind-diesel hybrid

system with a grid-only system in their economic study of electricity supply to a dairy farm in Spain. The farm's electricity demand was 63 kWh day^{-1} and the hybrid system designed to supply it consisted of a 20 kW wind turbine, a diesel generator and a battery. They calculated that in a location with an average wind speed of 7.39 m s^{-1}, the consumption costs would be reduced by 18% if 800 Ah batteries were used instead of 200 Ah.

A study on the design of modular hybrid renewable energy systems including biogas and solar PV for different dairy farms in the Konya, Erzurum and Izmir provinces of Türkiyeconcluded that the grid-connected system including PV and biomass is more feasible than the biomass system alone in terms of net present cost, return on investment, energy cost and annual value (Kirim et al. 2022). Bilgili (2018) developed a rooftop solar energy system designed to meet electrical energy with PV solar panels and reduce carbon dioxide in modern dairy farms in Çukurova conditions. In a solar energy system with an installed capacity of 330 kW, the payback period of the system was calculated to be 6 years and the economic life to be 20 years. The application of solar energy in a livestock farm in Konya was found to prevent 750 tonnes of CO_2 emissions per year (Orhan and Şahin 2022). It was calculated that the combined use of solar and animal biogas energy in Eskişehir-Sarıcakaya will reduce 22 thousand tonnes of CO_2 emissions compared to other energy sources (Kaynarca and Onay 2024). The modeling of hybrid power systems based on solar, wind and biogas to meet the electrical energy demand of a cattle farm in Afyonkarahisar has been estimated with Genetic Algorithm in different scenarios considering sustainable energy and environmental goals (Oz et al. 2023).

The aim of this study was to evaluate the applicability of PV and biogas systems in cattle farms in the Karaman province in terms of energy efficiency, environmental impact, and economic feasibility. Karaman was selected due to its favorable solar radiation levels and significant cattle population, making it a representative region for rural renewable energy applications. The biogas potential of cattle farming in the Karaman province of Türkiye was investigated and a rooftop SPP installation on the roof of a cattle farm, that produces its own energy with PV solar energy systems and uses the energy it produces, was simulated and analysed economically and environmentally. This study modeled the PV + biogas hybrid system in Karaman for the first time in the literature from an integrated economic and environmental perspective. While the literature often presents independent studies on either biogas production or photovoltaic systems, this study considers these two renewable energy sources as a hybrid system and presents an integrated solution. The distinguishing aspects of the study are as follows;

- Local focus: Unique data for Karaman Province based on local climate, sunshine duration and animal waste potential were used.
- Hybrid system approach: Photovoltaic and biogas systems weredesigned in an integrated manner and optimised for energy efficiency and environmental sustainability.
- Economic and environmental analysis: Both economic and carbon footprint analyses were included in the same study to more comprehensively assess the feasibility of hybrid systems.
- Scaling factor: The system was scaled according to the total cattle population in Karaman province, demonstrating its general applicability.

While many studies in the literature focus on a specific energy source (e.g. only biogas or PV systems), the combination of these two systems in this study offers a new perspective in terms of environmental impact and cost analysis.

2. Materials and Methods

The methodology was structured in the following steps (Figure 1):

i. Data Collection: Provincial animal population data (2022), climatic data (solar radiation, sunshine duration), and technical specifications of PV and biogas systems were compiled from official databases (TÜİK, MGM, EPİAŞ).

ii. Biogas Calculation: Manure production was calculated, followed by an estimation of biomethane potential and sizing of the biogas reactor using literature-based assumptions.

iii. PV System Simulation: A rooftop solar PV system was designed using PVsyst software based on local radiation data and farm dimensions.

iv. Economic and Environmental Analysis: Payback period, sensitivity scenarios, and carbon footprint comparisons were performed.

2.1. Biogas energy

Biogas is produced naturally as a result of anaerobic digestion, which is the biological conversion of organic carbon to carbon dioxide and methane (Eq. 1) (Yurtekin 2023). Figure 2 shows the production of biogas from animal waste (Chowdhury et al. 2020).

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \quad [1]$$

A typical biogas composition consists mainly of 55-70% methane (CH_4), 30-45% carbon dioxide (CO_2) and small amounts of hydrogen, hydrogen sulphide, carbon monoxide and nitrogen gases. Although the composition of biogas varies and has different characteristics depending on the raw material used, the type of plant and the environmental conditions, the calorific value of biogas containing 99% methane can generally be accepted as 37.3 MJ m^{-3} and the calorific value of biogas containing 65% methane as 24.0 MJ m^{-3} (EİE 2024). The biomethane potential (BMP) of waste that can be used in biogas processing plays a crucial role in determining the biogas production capacity of waste (Erkan 2020). Biogas yield refers to the amount of biogas obtained from a given amount of organic waste and indicates the production capacity of the plant. A high biogas yield provides more energy production and more efficient waste management. The methane volume fraction determines the percentage of methane gas in the biogas. High methane content increases the energy value of biogas and provides more efficient use in energy production processes (Kaya and Kılıç 2017). Biogas yields and methane volume ratios of biomass sources are shown in Table 1 (Akman 2023).

Table 2 shows the equations used in the biogas production plant during biogas production (Akman 2023).

2.2. Solar energy

Karaman has a continental climate with cold and snowy winters and hot and dry summers. The average annual maximum and minimum temperatures are 5.6-18.9°C. The average daily sunshine is 7.9 hours (MGM 2024). Figure 3 shows the solar energy potential of Karaman Province. It can be seen that the total annual solar radiation in Karaman is 1700-1800 kWh m^{-2} and the longest sunshine period is in July (EİGM 2024).

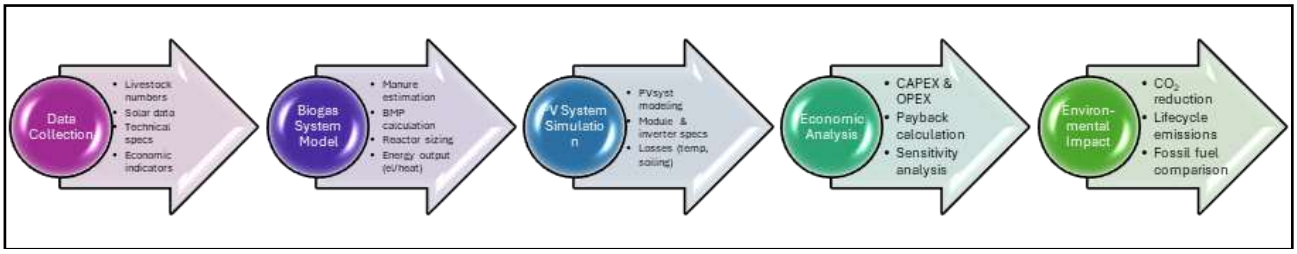

Figure 1. Methodological flowchart.

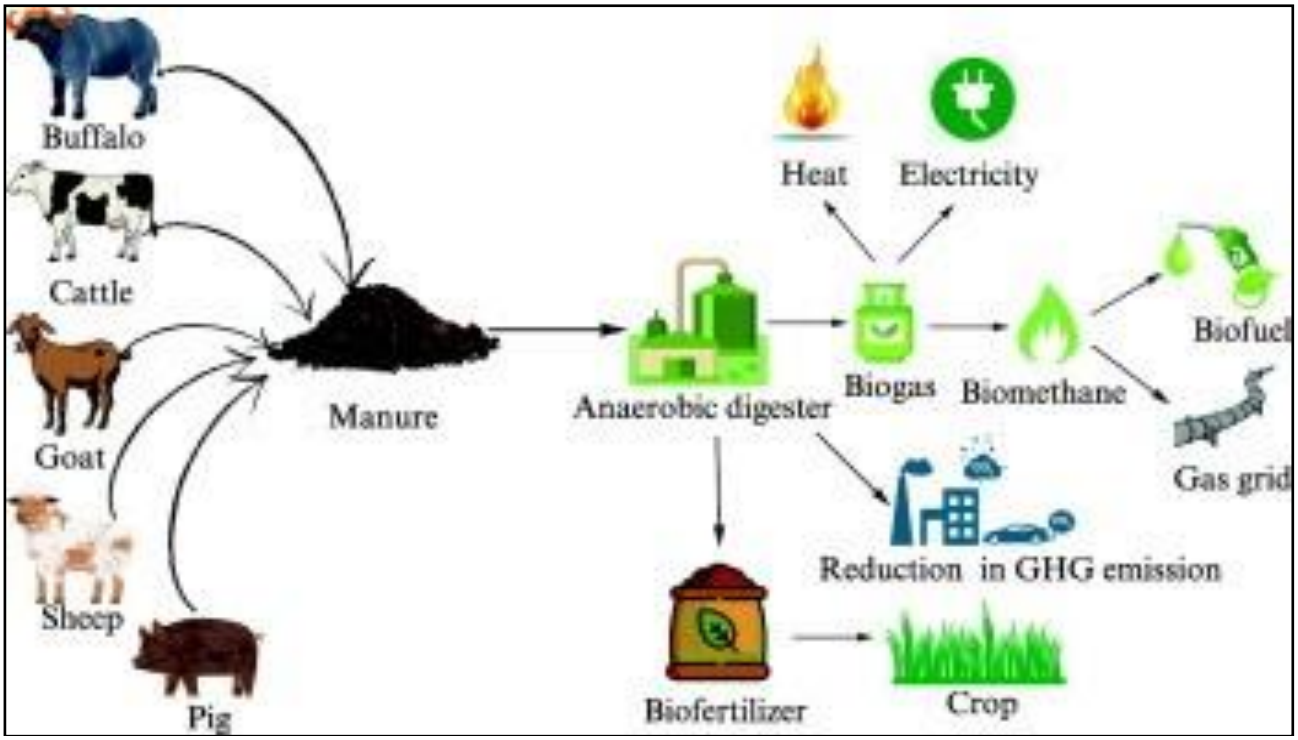

Figure 2. Biogas production from animal waste (Chowdhury et al. 2020).

Table 1. Biogas yields and methane volume fractions of biomass sources (Akman 2023)

Sources	Biogas yield (l kg^{-1})	Methane volume fraction (%)
Cattle manure	90-310	65
Poultry manure	310-620	60
Pig manure	340-550	65-70
Wheat straw	200-300	50-60
Rye straw	200-300	59
Barley straw	290-310	59
Maize stalks and waste	380-460	59
Flax, hemp	360	59
Grass	280-550	70
Vegetable waste	330-360	Variable
Variable agricultural waste	310-430	60-70
Fallen tree leaves	210-290	58
Algae	420-500	63
Waste water sludge	310-800	65-80

Table 2. Equations used in biogas energy systems (Akman 2023)

Equality Name	Equality	Equality No
Wet weight potential of animal manure	$WF = \sum NA \times \frac{LW \times FP \times CRM}{1000}$	2
Biomethane potential of the plant	$MP = \sum WP \times \frac{DM \times ODM \times MPR}{1000}$	3
Amount of diluted waste to be loaded into the reactors	$RW = WW \times \frac{DM}{DM_{pr}}$	4
Densities of waste mixtures	$\rho = 998 \times (1 - 0.004094 DM_{pr\%})^{-1}$	5
Active volume of pre-storage	$FTAV = UD \times \frac{\rho}{WW}$	6
Volume of pre-storage	$FTV = FTAV \times tfh_{ca} = 5.7 + 3.8v$	7
Waste volume	$Q_h = \frac{RW}{\rho}$	8
Active reactor volume	$RVa = Q_h \times HWT$	9
Nominal reactor volume	$RVn = RVa \times tr$	10
Electrical energy obtained from biomethane	$E_e = MP \times M_E \times \eta_e$	11
Thermal energy obtained from biomethane	$E_t = MP \times M_E \times \eta_t$	12
Installed power of the biogas cogeneration plant	$P = \frac{E_e}{t}$	13

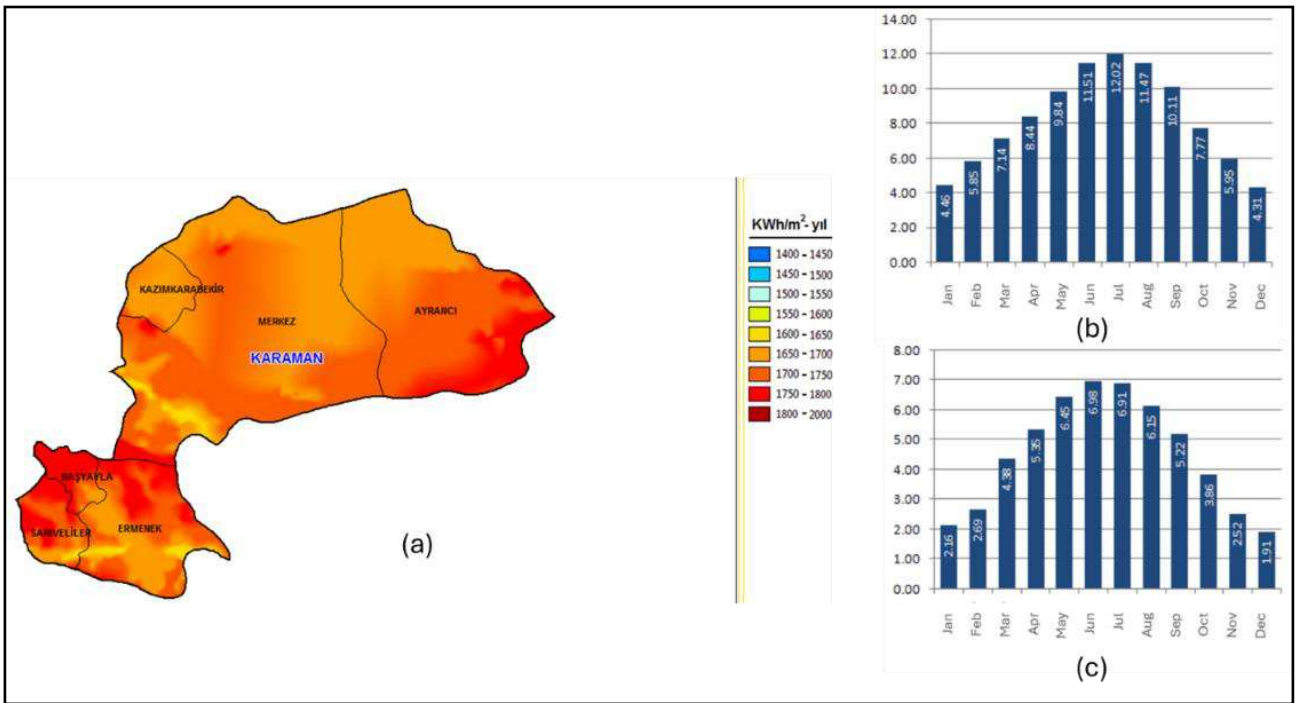

Figure 3. Karaman province: (a) Solar radiation map; (b) Sunshine hours; (c) Global radiation values (kWh $m^{-2}day^{-1}$) (EİGM 2024).

In this study, a PV solar power plant generating 300 kWe of electrical energy was modeled considering data such as the average electrical energy requirement of 0.6-1.2 kW for one cattle per day (Cucchiella et al. 2015), a barn with a barn area of 5000 m^2 and a roof area of 5300 m^2 for 250 cattle, regional climatic conditions and sunshine duration. SketchUp (SketchUp 2024) was used to model the system (Figure 4). Considering the existing environmental conditions at the project site and today's technology, it was preferred to use half-cell and multi-busbar large-cell PV modules with monocrystalline structure and higher efficiency. In order to make more efficient use of the available space in the system, PV modules with a power of 550 Wp, which can generate more power per unit area, were preferred. The PV modules used are Elin's ELNSM72M-550-HC-HV monocrystalline model, and 648 units were used. In the inverter group, our models were created by using the system parts that we

call series inverters. 3 Huawei's SUN2000-100KTLM1-400 V model 100 kW array inverters were placed in the system. The main advantage of using array inverters in partial systems is that in case of any malfunction or change, instead of using a central inverter and stopping the whole system, intervening in a small area on the array will both extend the life of the system and eliminate energy loss.

The energy of a photovoltaic system depends on two basic variables: electrical and thermal. While electricity is generated by the photovoltaic effect, solar cells also heat up due to the thermal energy generated by solar radiation. Since the thermal energy generated on PV surfaces cannot be used for any useful purpose, it is lost to the environment as heat loss (Joshi et al. 2009a).

The equations used in PV systems are given in Table 3 (Joshi et al. 2009a; b).

3. Results

The total number of cattle in Karaman province was estimated to be 63515 in 2022 (Karaman Agriculture and Forestry 2024). In this study, based on the examples in the literature and established examples (Erhan 2019; Nuralan Poyraz et al. 2020; Kurnuç Seyhan and Badem 2021), the electrical efficiency of the electrical unit of the reactor is 40%, the thermal energy conversion efficiency is 42%, the annual operation time of the cogeneration system is 8200 hours, the number of daily loads is 2, the process dry matter content is 10%, the pre-storage tolerance coefficient is 1.15, the reactor tolerance coefficient is 1.15, and the hydraulic standby time is 25 days. Accordingly, the annual wet manure is calculated to be 828824.4 tonnes and the biomethane potential produced in the plant is calculated to be 20.4 million m^3. As a result of the calculation made for the sizing of the forebay, the daily amount of diluted waste to be

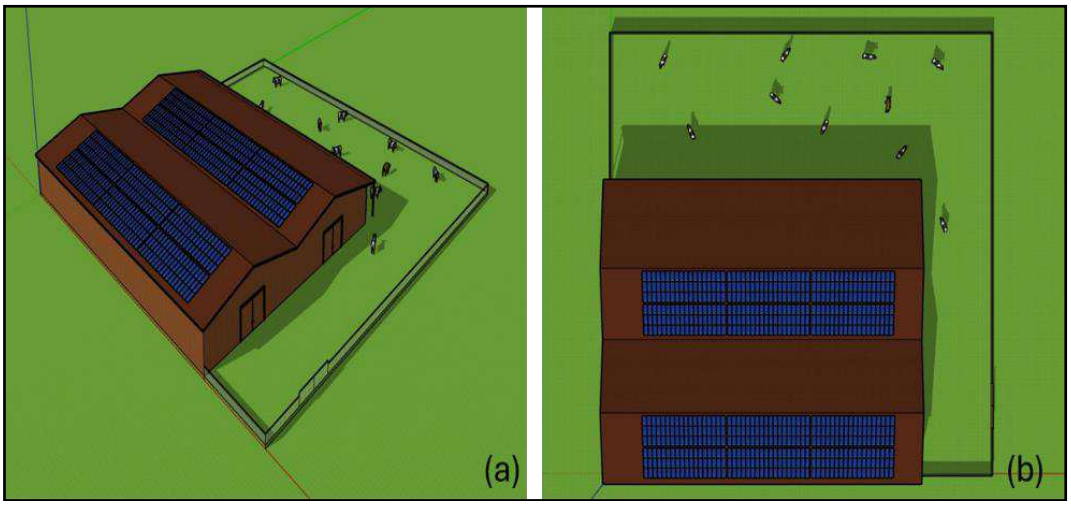

Figure 4. Karaman SPP: (a) 3D model; (b) 3D model top view.

Table 3. Equations used in PV solar energy systems (Joshi et al. 2009a; b)

Equality Name	Equality	Equality No
Maximum output power	$P_{max} = V_{max} I_{max}$	14
Open circuit voltage under atmospheric conditions	$V'_{oc} = V_{oc} \frac{100 + (T_{cell} - 25)(\tau_{ec})}{100}$	15
Open circuit current under atmospheric conditions	$I'_{sc} = I_{sc} \frac{\sigma_T}{1000}$	16
Filling factor	$FF = \frac{V_{max} I_{max}}{V'_{oc} I'_{sc}}$	17
PV system output energy	$E_o = \frac{P_{max}}{FF} + \dot{Q}$	18
Thermal energy	$\dot{Q} = h_{ca} A (T_{cell} + T_{amb})$ $h_{ca} = 5.7 + 3.8v$	19
Energy efficiency	$\eta = \frac{\frac{P_{max}}{FF} + \dot{Q}}{\sigma_T A}$	20
Performance ratio	$PR = \frac{Real\ energy\ output}{Theoretical\ energy\ output}$	21

loaded into the reactor was found to be 3167.8 tonnes, the active volume of the forebay was found to be 6088.1 m^3 and the required volume of the forebay was found to be 6998.4 m^3. The daily waste input to the reactor is 3044.3 m^3. The required active reactor volume for the biogas plant should be 76103.2 m^3 and the net volume should be 87518.4 m^3.

The amount of electricity to be produced by burning the biomethane produced in the plant is 78.73 GWh per year and the amount of thermal energy to be produced is 321.73 GWh per year. The installed capacity of the CHP plant is 9.57 MWe. The economic equivalent of the electrical energy and thermal energy to be produced by the plant annually is ₺224.1 billion and ₺820.4 billion respectively (EPİAŞ 2024).

In the design of the farm with a capacity of 250 cattle, 648 photovoltaic (PV) modules with a power of 550 Wp were used in the PVsyst programme (Mermoud and Wittmer 2014), and an annual energy production of 577 MWh was achieved. The largest losses in PV system efficiency were due to temperature (4.91%) and pollution (3%) (Figure 5).

The annual electrical energy demand of a cattle farm depends on parameters such as heating, ventilation, lighting, milking, milk tank, feeding (Çiçekli 2019). The annual energy demand of the farm where this study was conducted was calculated to be 233.4 MWh. In this case, the energy produced was equal to the energy required. The initial investment cost of installing a PV solar power plant on an existing barn roof is ₺ 6.4 million, depending on parameters such as panels, inverters, cables. The payback period can be calculated as 5.21 years by adding operational maintenance, staff salaries and the average inflation rate (20%) added to the electricity price each year to the initial investment cost (Energy Agency 2024).

The sensitivity analysis was carried out to assess the impact of changes in energy prices on the cost-benefit balance (CBA) of the system. In the analysis, the average unit price of electricity for 2024 is ₺ 0.9 kWh^{-1} (EPİAŞ 2024) and scenarios of 20% increase and 20% decrease in this price are analyzed. The economic returns of the biogas plant and the PV system were evaluated separately (Table 4).

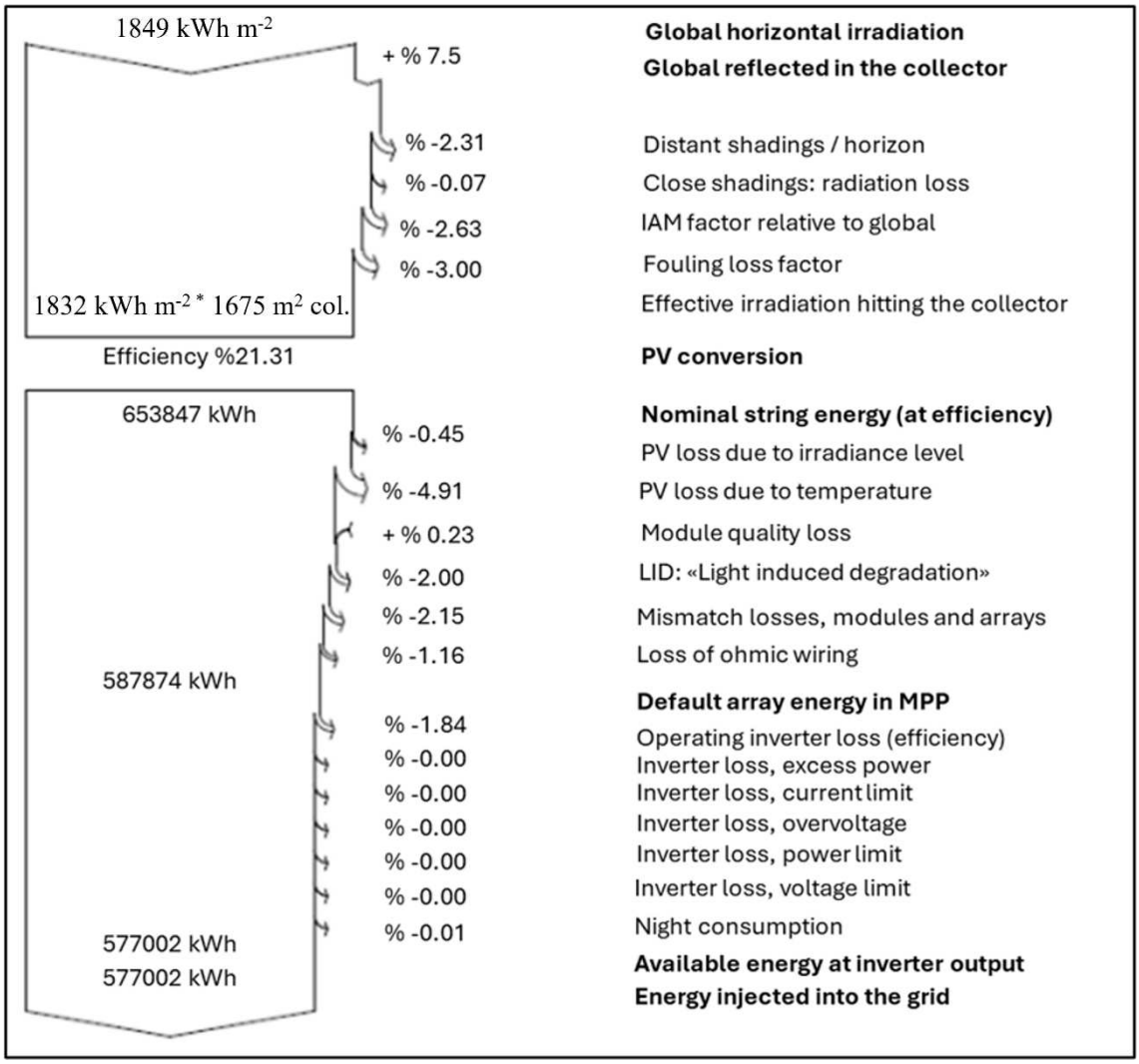

Figure 5. Grossman diagram.

Table 4. Sensitivity analysis for hybrid system CBA

Parameters	-20% price decrease	Current status (0,9 ₺)	+20% price increase
Annual revenue of PV system (million ₺)	0.415	0.519	0.623
Annual revenue of biogas plant (million ₺)	70.85	88.56	106.27
Annual revenue of hybrid system (million ₺)	71.27	89.08	106.89
Payback period (years)	7.22	6.5	5.8

A 20% price increase reduces the payback period of the hybrid system from 6.5 to 5.8 years. A 20% price decrease increases the payback period to 7.22 years, reducing the economic viability. The sensitivity analysis shows that the PV system is less vulnerable than the biogas system. This suggests that the biogas system has a greater financial impact. The hybrid system, which integrates both energy sources, provides an economic return independent of energy price fluctuations. This analysis shows that the hybrid system is able to protect its financial and environmental benefits against possible fluctuations in the energy market. In this context, hybrid systems offer an important opportunity for energy security and economic sustainability.

Carbon footprint is the total amount of greenhouse gases emitted directly or indirectly into the atmosphere by an individual, organization or product. This measurement is usually expressed in tonnes of carbon dioxide equivalent (tCO_2e). Renewable energy systems, particularly solar energy systems, have a much lower carbon footprint than fossil fuels. Solar energy systems do not directly emit CO_2 during electricity generation and therefore do not contribute to increasing atmospheric greenhouse gas concentrations (IEA 2024b). For example, a coal-fired power plant emits approximately 820 kg of CO_2 per megawatt hour (MWh) of energy produced (IPCC 2024). In contrast, solar PV systems emit only 20-50 kg CO_2e/MWh over their life cycle (NREL 2024). Equation 22 can be used to calculate the amount of CO_2 that would be emitted into the atmosphere if a fossil fuel system were to produce the same amount of electricity (IPCC 2024).

$$CO_2\ emission\ (kg\ CO_2) = Electricity\ production\ (kWh) \times Emission\ factor\ \left(\frac{kg\ CO_2}{kWh}\right) \quad [22]$$

According to this calculation, 473.14 tonnes of CO_2 would be emitted annually if a fossil fuel system were to generate the same amount of electricity. However, with the PV solar power system, this amount is greatly reduced and only 11.54-28.85 tonnes of CO_2 are emitted, taking into account the lifecycle emissions. In other words, when PV solar power systems are used for production, 94-98% less carbon dioxide is emitted compared to fossil fuels. As a result, the PV solar power plant significantly reduces carbon emissions compared to energy systems powered by fossil fuels. This contributes significantly to environmental sustainability by reducing the carbon footprint of the farm. In the long term, such investments in renewable energy play a vital role in combating climate change and offer economic as well as environmental benefits.

When calculating the carbon emissions of biogas plants, the amount of CO_2 produced as a result of biogas combustion should be taken into account (Eq. 23) (IPCC 2024).

$$Total\ CO_2\ emissions\ \left(\frac{kg}{year}\right) = Biomethane\ potential\ \left(\frac{m^3}{year}\right) \times 2.75\left(\frac{kg\ CO_2}{m^3\ CH_4}\right) \quad [23]$$

The total CO_2 emission of the biogas production plant is 55.4 thousand tonnes per year. If the electricity produced in this plant was generated using natural gas (Eq. 22), the CO_2 emissions would be 334.4 thousand tonnes per year. It emits 83% less CO_2 per year than a natural gas power plant.

4. Discussion and Conclusions

In this study, renewable energy applications on livestock farms in the Karaman Province were investigated in detail. The results clearly demonstrate the advantages of hybrid renewable energy systems in terms of both economic and environmental benefits. The biogas and PV systems used in the study increased the energy independence of the farms while significantly reducing the carbon footprint.

The annual amount of wet manure used for biogas production in this study was 828824 tonnes and the biomethane production potential was calculated to be 20.4 million m3. The combustion of this biomethane in the CHP unit produced 78.73 GWh of electricity and 321.73 GWh of thermal energy. In addition, 648, 550 Wp PV modules were designed using PVsyst software for a farm with a capacity of 250 cattle. This system produced 577 MWh of energy per year, meeting all the farm's energy needs, with losses of 4.91% due to temperature and 3% due to pollution.

In the economic analysis of the system, the initial investment cost of the PV solar power plant was determined to be TL 6.4 million and the payback period was calculated to be 5.21 years. In the analysis of the environmental impact of the renewable energy systems, the PV system reduces CO_2 emissions by 94-98% compared to fossil fuels, while the biogas plant provides 83% lower carbon emissions compared to the natural gas plant. As a scaling factor, the energy production and environmental benefits of the plant were confirmed by the number of cattle in Karaman province (0.394%).

The findings of the present study demonstrate the considerable potential of renewable energy systems, not only in terms of energy production, but also with regard to environmental sustainability and economic feasibility. These results can serve as an incentive for the more widespread use of renewable energy sources.

In addition to the technical and economic evaluations, policy support mechanisms could enhance the practicality of such systems. For instance, incentives under the Ministry of Agriculture and Forestry's sustainable agriculture programs could help promote the adoption of PV and biogas systems in livestock farms. Local governments could also support such transitions through infrastructure and permitting facilitation.

This study has several limitations. The calculations were based on static livestock population data from 2022, which may vary annually. Panel pollution and temperature effects were assumed to be constant, and inflation rates used in economic estimates are subject to change. Future studies should incorporate dynamic data and scenario-based modelling for more robust conclusions.

Future studies could investigate the use of optimization algorithms for the integrated management of biogas plants and photovoltaic (PV) systems. Furthermore, the effects of different organic waste mixtures could be analyzed to increase the efficiency of biogas production plants. Active cooling systems for temperature management or thermal energy recovery methods can be investigated to improve the performance of PV systems. Extensive field studies can be conducted to assess the potential of hybrid systems in different geographical regions. Finally, extending the economic analysis with scenarios depending on the fluctuations in energy prices would be useful to demonstrate the feasibility of the system under different market conditions.

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Nomenclatures

A : PV module surface area
CRM : Collectability rate of manure
DM : Dry matter content of the waste
DMpr : Dry matter ratio to be used in the process
Ee : Amount of electricity generated from the plant (kWh year^{-1})
Et : Thermal energy produced from the plant (kWh year^{-1})
FP : Daily fertiliser production (kg day^{-1})
FTAV : Front tank active volume (m^3)
FTV : Front tank volume (m^3)
h_{ca} : Convection and radiation heat transfer coefficient between the solar cell and the atmosphere
HWT : Hydraulic waiting time (days)
I_{sc} : Short circuit current
LW : Live weight
Me : Energy content of methane gas (kWh m^{-3})
MP : Amount of methane produced from the plant (m^{3}CH$_4$ year^{-1})
MPR : Specific methane production rate of the waste (m^{3}CH$_4$ kg^{-1} ODM)
NA : Number of animals
ODM : Organic dry matter content of the waste
P : Installed power of cogeneration system (kWe)
$\dot{Q}$: Thermal energy
Qh : Waste volume to be loaded to the reactor in one day (m^3 day^{-1})
RVa : Reactor active volume (m^3)
RVn : Reactor net volume (m^3)
RW : Amount of reconstituted waste to be loaded in one day
T_{amb} : Ambient temperature
T_{cell} : PV module cell temperature
t : Annual operation time of the cogeneration system (hours)
tf : Front tank tolerance coefficient (1.1-1.15)
tr : Reactor tolerance coefficient (1.1-1.15)
UD : Number of uploads per day
v : Wind speed (m s^{-1})
V_{oc} : Open circuit voltage
WF : Wet fertiliser amount (kg day^{-1})
WP : Waste potential (tonnes year^{-1})
WW : Wet weight of incoming waste in one day
σ_T : Total solar radiation
ρ : Density of the mixture (kg m^{-3})
τ_{ec} : Temperature dependent voltage coefficient of variation
η_e : Electricity conversion efficiency of the cogeneration system
η_t : Thermal energy conversion efficiency of cogeneration system

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Development and evaluation of machine learning models for predicting chicken meat production

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ABSTRACT

Chicken meat production plays a vital role in the global food industry by providing a cost-effective and sustainable protein source for a rapidly growing population. Given strategic importance, accurately estimating production levels is essential for improving operational efficiency, optimizing resource use, and responding to market demand. In recent years, data-oriented methods have become integral to modern agriculture, with machine learning models emerging as powerful tools for modeling agricultural outputs. This study aims to develop and compare predictive models for chicken meat production in Türkiye using four machine learning algorithms: Linear Regression (LR), Random Forest (RF), k-Nearest Neighbors (k-NN), and Extreme Gradient Boosting (XGBoost). The models were trained on a comprehensive dataset spanning 62 years (1961–2022), incorporating meteorological, agricultural, and economic variables. Key predictors such as feed use, weight gain, and environmental factors were included. The dataset was carefully prepared to ensure robust model training and validation. Model performance was evaluated using multiple metrics, including the coefficient of determination (R^2), MAE, MSE, and RMSE. Results indicated that the Linear Regression model achieved the highest R^2 value and the lowest error rates among all algorithms. These findings underscore the potential of AI-based approaches to enhance decision-making and resource management in the poultry sector. With further integration of diverse data sources and advanced learning techniques, this framework can contribute to the development of more efficient, adaptive, and sustainable poultry production systems.

1. Introduction

Chicken meat has become one of the most widely consumed sources of animal protein worldwide due to its low cost, short production cycles, and high feed conversion efficiency (FAO 2013). The growing global population and shifting dietary habits are further increasing the demand for this economical and efficient source of protein. However, the increasing demand has made not only the quantity of production but also its sustainability a critical concern. Today, ensuring sustainability in the poultry sector necessitates optimizing production processes, utilizing resources efficiently, and responding promptly to market demands (Mottet and Tempio 2017). In this context, the accurate and timely prediction of chicken meat production is of critical importance for both internal decision-making within the industry and the development of national food security strategies.

Predictive modeling has become a fundamental component of modern agricultural practices, providing data-driven insights that significantly contribute to the optimization of production systems and improvement of resource management. Among these, machine learning (ML) offers significant advantages over traditional statistical methods by identifying complex patterns within large datasets (Ahmed and Hussain 2022). Machine learning (ML) has been successfully applied across various agricultural domains, including yield prediction, early detection of plant diseases, and livestock management (Gorczyca and Gebremedhin 2020). However, the use of these methods in the context of chicken meat production remains limited, presenting a significant opportunity for innovation. Chicken meat production is influenced by a wide range of interacting variables, including feed intake, growth rate, climatic conditions, and economic dynamics (Vandana et al. 2021; Istiak and Khaliduzzaman 2022; Liu et al. 2024). The nonlinear and interdependent relationships among these factors make accurate prediction particularly challenging. Nevertheless, studies that integrate meteorological and agricultural data have demonstrated that ML algorithms are highly suitable for addressing this complexity, offering a powerful alternative to traditional modeling techniques (Yıldız et al. 2024).

This study aims to develop prediction models for chicken meat production using four machine learning algorithms—Linear Regression (LR), Random Forest (RF), k-Nearest Neighbors (k-NN), and Extreme Gradient Boosting (XGBoost)—and to evaluate their predictive performance. The models were trained on a comprehensive dataset spanning 62 years (1961-2022) from Türkiye. This dataset includes not only agricultural variables such as chicken meat production and the number of live chickens, but also meteorological indicators such as average temperature and precipitation, as well as economic indicators such as producer prices and gross domestic product. The performance of

the models was evaluated using widely accepted metrics, including the coefficient of determination (R^2), Mean Absolute Error (MAE), Mean Squared Error (MSE), and Root Mean Squared Error (RMSE). In this context, the study aims to contribute to sustainability goals in chicken meat production by leveraging historical and multidimensional data, and to provide a scientific foundation for the development of data-driven decision support systems.

2. Materials and Methods

2.1. Material

This study utilized a dataset comprised of 21 variables relevant to chicken meat production, encompassing agricultural, meteorological, demographic, and economic factors. The variables included chicken meat production (tonnes), live chicken count (1000 heads), chicken meat producer price ($ per tonne), population (units), rural population (units), urban population (units), agricultural area (1000 ha), pasture and meadow area (1000 ha), food price inflation (%), temperature (°C), rainfall (mm), gross national product (GNP million $), gross domestic product (GDP, million $), GDP per capita ($), barley production (tonnes), corn production (tonnes), barley price (tonnes $), corn price ($ per tonne), wheat price ($ per tonne), chicken import quantity (1000 heads), and chicken export quantity (1000 heads). The dataset spans the years 1961-2022 and was employed as the training set for developing predictive models.

Agricultural data, including chicken meat production, live chicken count, agricultural area, pasture and meadow area, barley production, and corn production, were retrieved from the Republic of Turkey Ministry of Agriculture and Forestry (TMAF 2024). Meteorological data, comprising temperature and rainfall, were sourced from the General Directorate of Meteorology under the Ministry of Environment, Urbanization, and Climate Change (GDM 2024). Demographic and economic data, such as chicken meat producer price, GNP, GDP, GDP per capita, barley and corn production and prices, wheat price, and chicken import and export quantities, were obtained from the United Nations Food and Agriculture Organization (FAO 2024a).

The statistical properties of these variables, including their mean, standard deviation, maximum, and minimum values, are summarized in Table 1, providing insights into the data distribution and variability within the dataset.

2.2. Data preparation

The dataset underwent a comprehensive preprocessing stage to ensure data quality and enhance model performance. Missing values were imputed using mean value calculations, while outliers were identified and removed based on predefined threshold values. To standardize the scale of all features and improve the accuracy of machine learning algorithms, feature normalization was applied. The data was then partitioned into training and test subsets, with 70% allocated to training and the remaining 30% reserved for testing.

2.3. Machine learning models

This study utilized four machine learning algorithms: Linear Regression (LR), Random Forest (RF), k-Nearest Neighbors (k-NN), and Extreme Gradient Boosting (XGBoost). To optimize the performance of these algorithms, hyperparameter tuning was conducted using the Grid Search method, which systematically evaluates combinations of hyperparameters to identify the optimal configuration for each model.

Table 1. Statistical properties for attributes

Attributes	Mean	Standard Deviation	Minimum	Maximum
Chicken meat production (tonnes)	709492	716027.9	60000	2417995
Live chicken count (1000 heads)	156150.4	122168.3	26116	391394
Chicken meat producer price ($ per tonne)	2022.367	327.8872	847.3	3271.8
Population (units)	56014.39	17363.83	28255	85279.55
Rural population (units)	21823.53	1349.229	19121.82	24723.16
Urban population (units)	33622.59	17580.66	9025.07	65453.23
Agricultural area (1000 ha)	38753.7	1176.671	36517	41223
Pasture and meadow area (1000 ha)	12415.62	1797.074	10000	14617
Food price inflation (%)	19.35789	12.1942	3.896166	77.86761
Temperature (°C)	12.05032	1.530993	9.58	15.1
Rainfall (mm)	606.1389	70.49718	460.69	793.8
GNP (million $)	349669.4	283621.1	904.19	941689.7
GDP (million $)	369093.9	294729.9	23609.87	957799
GDP per capita ($)	5286.164	3465.499	649.337	12507.8
Barley production (tonnes)	6258981	1868969	2900000	9551000
Corn production (tonnes)	2727806	1921058	800000	8500000
Barley price ($ per tonne)	202.8625	49.39973	100.1	370.3
Corn price ($ per tonne)	228.0594	44.37527	138	375.3
Wheat price ($ per tonne)	245.6938	55.0746	126.9	438.7
Chicken import quantity (1000 heads)	1495.148	1389.77	2	6150
Chicken export quantity (1000 heads)	8022.277	12665.12	1	62335

2.4. Performance evaluation

The predictive performance of the models was assessed separately on the training and test datasets. Model predictions were compared to the actual values in the test set, and performance was evaluated using four key metrics: the Coefficient of Determination (R^2), Mean Absolute Error (MAE), Mean Squared Error (MSE), and Root Mean Square Error (RMSE).

Coefficient of Determination (R^2): This metric indicates the proportion of variance in the dependent variable explained by the independent variables. R^2 values range from 0 to 1, with values closer to 1 reflecting a better fit of the model to the data.

Mean Absolute Error (MAE): MAE represents the average magnitude of absolute differences between predicted and actual values, offering a measure of the accuracy of predictions. A lower MAE indicates superior model performance.

Mean Squared Error (MSE): MSE calculates the average of the squared differences between predicted and actual values, penalizing larger errors more significantly. It provides a measure of the model's overall prediction error.

Root Mean Square Error (RMSE): RMSE, the square root of MSE, directly reflects the magnitude of prediction errors in the original data units, offering a clear and interpretable metric of accuracy.

The use of these diverse metrics allows for a comprehensive evaluation of model performance, capturing various aspects of prediction quality. The formulas used to calculate these metrics are outlined below to ensure clarity and reproducibility in the evaluation process.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (1)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|y_i - \hat{y}_i| \quad (2)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2 \quad (3)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \quad (4)$$

$(n) = Total\ number\ of\ observations$; $(yi) = (i) - th\ observation\ of\ true\ values$; $(\hat{y}_i) = (i) - th\ observation\ of\ predicted\ values$

2.5. Software

The analyses were conducted using the Python programming language, version 3.12.2 (Python Software Foundation 2024), leveraging libraries such as Pandas (1.3.0) for data manipulation, Numpy (version 1.21.0) for numerical operations, Matplotlib (version 3.4.2) for data visualization, and Scipy (version 1.10.0) for scientific computations (Yıldız et al. 2024).

3. Results and Discussion

The forecast models developed using LR, RF, k-NN and XGBoost algorithms were evaluated using agricultural, meteorological and economic data affecting chicken meat production. As a result of the analyses, the forecast accuracy of the models was compared with R^2, MAE, MSE and RMSE metrics. In the obtained findings, LR provided the highest accuracy with R^2= 0.9907 and the lowest error values (MAE= 51324.28; MSE= 5932206846.61; RMSE= 77020.82). RF showed high accuracy with R^2= 0.9855 but its error values (MAE= 63976.58; MSE= 9284599433.44; RMSE= 96356.63) were higher than LR. XGBoost provided similar accuracy with R^2= 0.9836 but fell behind RF in terms of performance with MAE= 60,364.73, MSE= 10468242376.31 and RMSE= 102,314.43. k-NN had the lowest accuracy rate with R^2= 0.9585 and showed the highest error values (MAE= 100259.51; MSE= 26524968103.15; RMSE= 162864.88). These results show that LR is superior to other algorithms in terms of error rates and accuracy (Table 2).

The agreement analysis between the predicted and actual values reveals that the estimates generated by the LR algorithm closely align with the y= x line at a 45-degree angle, as illustrated in the agreement graph (Figure 1). This strong alignment highlights the high accuracy of the LR predictions and the model's excellent fit to the dataset. Supported by low error metrics, including MAE, MSE, and RMSE, these results confirm that the LR algorithm effectively captures the underlying relationships within the data and is the most suitable model for this specific dataset.

In this study, the Linear Regression (LR) algorithm stood out with lower error rates and higher accuracy compared to the other models, which is consistent with findings from similar studies in the literature. For instance, in a study conducted by Yıldız et al. (2024) to predict honey production in Türkiye, meteorological and agricultural data were jointly evaluated, and the LR algorithm emerged as the most successful model with an R^2 value of 0.97. This result indicates that the LR algorithm can achieve high accuracy even in multivariate data structures across different production systems.

Studies conducted specifically in the poultry sector emphasize the effectiveness of machine learning algorithms in handling complex multivariate biological datasets. Yıldız et al. (2025) developed models integrating internal and external quality parameters to predict egg quality in Japanese quails, where the Random Forest and Gradient Boosting algorithms stood out with accuracy rates exceeding 97%. Similarly, in a study conducted by Sehirli and Arslan (2022), the aim was to classify egg quality without relying on Haugh Unit (HU) data; the logistic regression algorithm was identified as the most successful model, achieving an accuracy of 98.6% and an MCC value of 0.96. In the same study, the Random Forest algorithm was reported to exhibit high performance in the classification of egg quality based on HU data.

Table 2. Performance metrics of algorithms on the test set

Algorithms	R^2	MAE	MSE	RMSE
LR	0.9907	51324.28	5932206846.61	77020.82
RF	0.9855	63976.58	9284599433.44	96356.63
k-NN	0.9585	100259.51	26524968103.15	162864.88
XGBoost	0.9836	60364.73	10468242376.31	102314.43

R^2, coefficient of determination; MAE, Mean Absolute Error; MSE, Mean Squared Error; RMSE, Root Mean Square Error.

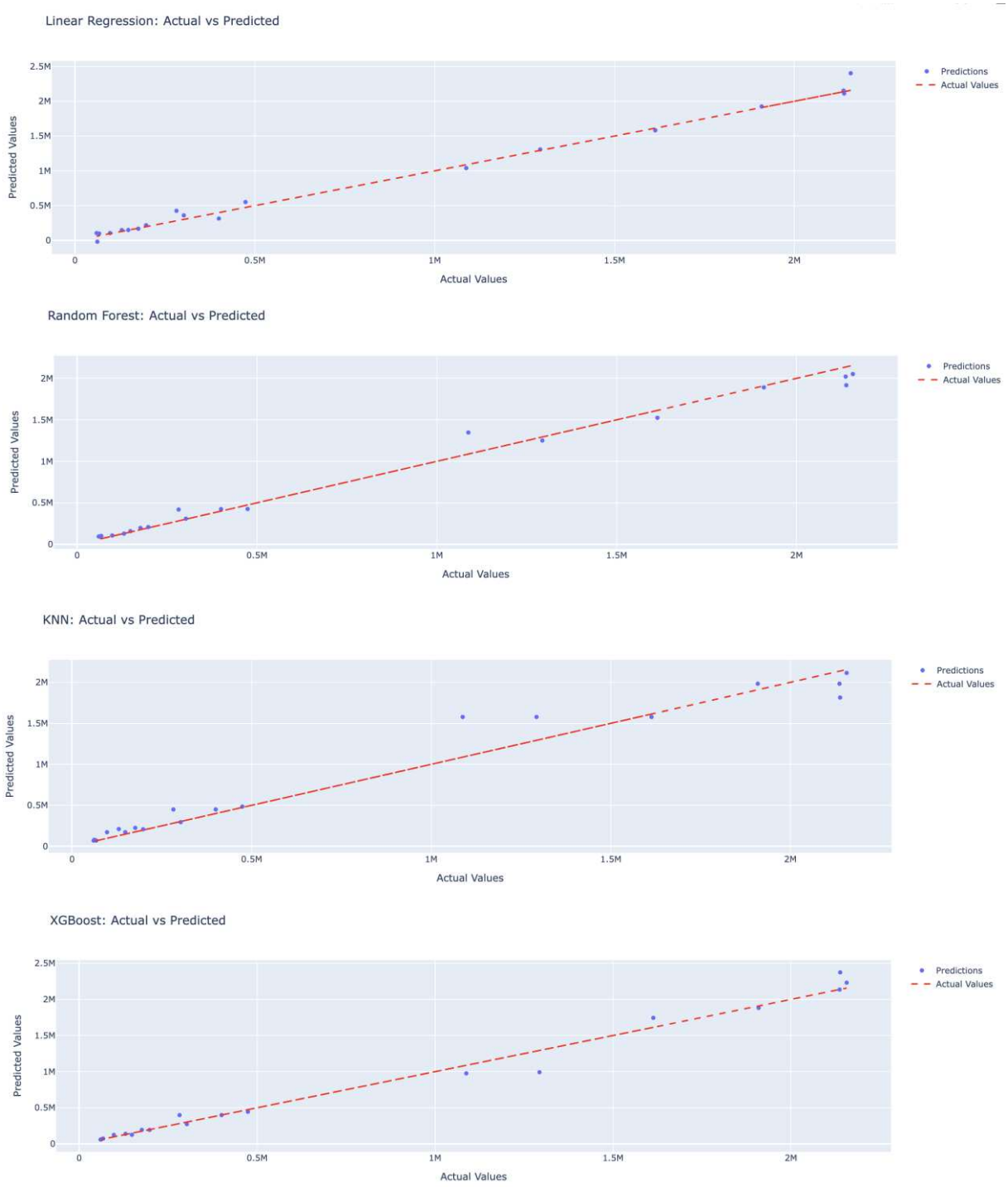

Figure 1. Analysis of the alignment between predicted and actual values.

The widespread integration of machine learning algorithms into animal production systems provides a broader context in which to interpret the results of the current study. For instance, Alsahaf et al. (2018) employed four different algorithms—Random Forest (RF), Extremely Randomized Trees (ET), Gradient Boosting Machine (GBM), and XGBoost (XGB)—to estimate the age at which pigs reached 120 kg slaughter weight, concluding that GBM and XGB yielded the lowest error metrics. Similarly, Srivastava et al. (2021) assessed the predictive capabilities of RF, XGB, and Support Vector Machine (SVM) in estimating carcass weight (CWT), marbling score (MS), backfat thickness (BFT), and eye muscle area (EMA) in Hanwoo cattle. Their findings indicated that EGB performed best for CWT and MS, whereas SVM achieved the lowest mean squared error for BFT and EMA.

In the literature, it has been shown that these algorithms have versatile application areas such as not only production estimation, but also optimization of operational processes and analysis of consumer behavior. Lyu et al. (2023) showed that real-time predictions provide a practical solution by optimizing

weighing methods in broiler production with a machine learning-based model. Chiras et al. (2023) emphasized the importance of machine learning methods in supporting strategic decision processes in the food sector by analyzing consumer behavior.

The feature importance analysis used in this research was conducted to determine which variables have a greater impact in the prediction models. In particular, the feature importance scores calculated using the RF algorithm revealed the main factors affecting chicken meat production. According to the results of this analysis, Gross Domestic Product (GDP), urban population and national income per capita (GDP per capita) stood out as the most important variables (Figure 2). While the increase in GDP provides economic stability and production capacity, thus enabling more investment in the sector, the increase in per capita income enables consumers to turn to more and higher quality protein sources. In addition, the growth of the urban population has increased the demand for chicken products by increasing fast-paced consumption habits. These findings highlight the critical impact of economic and demographic factors on chicken meat production (FAO 2013; FAO 2024b).

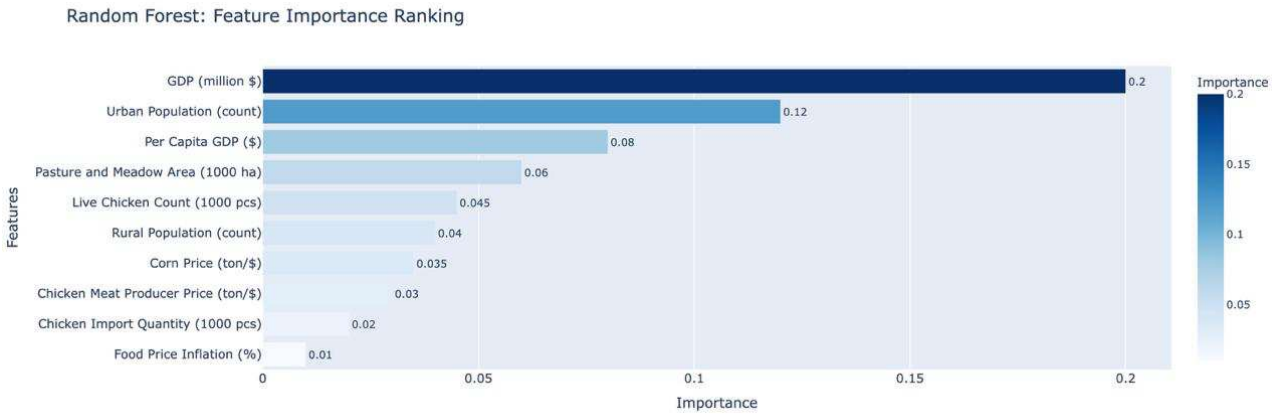

Figure 2. Analysis of feature importance for chicken meat production prediction.

4. Conclusion

In this study, which examined the applicability of machine learning models to predict chicken meat production, different models were developed using LR, RF and k-NN algorithms and comparative performance analysis was performed. The results revealed that the LR algorithm exhibited the best performance with higher accuracy (R^2) and lower error rates (MAE, MSE, RMSE) compared to other methods. These findings show that data-driven prediction models are a powerful tool for optimizing production processes, improving resource utilization and supporting decision-making processes in the poultry sector. The 62-year meteorological, agricultural and economic data used in the study increased the accuracy of machine learning models and demonstrated the feasibility of long-term predictions. However, the sectoral use of such models is directly related to the quality and diversity of available data sources. Inclusion of larger data sets and different attributes in the model can increase the accuracy and generalizability of future prediction models. The findings emphasize the importance of machine learning applications in the poultry sector. In industry, integrating such models into decision support systems can provide significant benefits, both economically and environmentally.

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First record of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) in Türkiye

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ABSTRACT

There are approximately 50000 species of algae. Algae contain economically valuable molecules, such as astaxanthin and polyunsaturated fatty acids (PUFA), and are used in food, cosmetics and animal feed industries. Green microalga *Haematococcus pluvialis* was considered to contain the highest amount of astaxanthin for many years. Recent research suggests that another green microalga, *Dysmorphococcus globosus,* contains more astaxanthin than *H. pluvialis*. *D. globosus* hasn't been studied extensively in the literature. In this study, approximately 1600 bp of the DNA barcoding gene *18S rRNA* was sequenced for identification of a new green alga from the Kaş district. Molecular identification and microscopy analysis show this new isolate belongs to genus *Dysmorphococcus*. This is the first record of genus *Dysmorphococcus* H. Takeda and *Dysmorphococcus globosus* H.C. Bold & Starr in freshwater algal flora in Türkiye.

1. Introduction

Algae are polyphyletic organisms which contain unicellular and multicellular species. They can be found in terrestrial, freshwater and marine environments. They are the primary producers in the marine ecosystem and produce 50-70% of oxygen on the planet. They have a great diversity in life cycles; many of them have a sexual cycle (Raven and Giordano 2014). Algae can be defined broadly as organisms which have oxygenic photosynthetic activity that is not higher plant. Currently, there are four kingdoms, fourteen phyla and sixty-three classes of algae. The fourteen phyla are, Cyanobacteria, Charophyta, Chromeridophyta, Glaucophyta, Rhodophyta, Chlorophyta, Euglenophyta, Dinoflagellata, Heterokontophyta (Ochrophyta), Picophyta, Haptophyta, Cryptophyta, Rhodelphiophyta, Prasinodermatophyta. There are 50589 species of living algae and 10556 fossil species. Chlorophyta (green algae) is the most diverse with eleven classes (Guiry 2024).

There are 5480 recorded algal species in Türkiye and genus *Dysmorhococcus* has not yet been recorded (Maraşlıoğlu and Gönülol 2025). The aim of this study was to isolate and characterize carotenoid containing algae. The *18S rRNA* sequence and cell morphology analysis show that the new isolated green algal strain belongs to the genus *Dysmorphococcus*. According to our database searches, the *Dysmorphococcus* species has not been recorded in Türkiye. We have reported the first isolation of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) from the Kaş district of Antalya.

2. Materials and Methods

2.1. Sample collection

The water sample was taken from a small rock pool that formed after rain water accumulation in the Kaş district of Antalya in February 2023 (Figure 1). The coordinates of the sample collection area are 36°21'24.28" N and 29°19'13.41" E and the elevation is 60 m. The color of the water was red, which suggested carotenoid containing algae could be present. The water sample was brought to the laboratory and examined under a microscope. Microscope observation showed that it contained several different microalgae as well as rotifer, which is known to feed on algae.

2.2. Growth conditions and isolation of the strain

A few milliliters of the water sample were inoculated into liquid tris acetate phosphate (TAP) medium (Gorman and Levine 1965) and kept under white light (approximately 40 µmol photons s^{-1} m^{-2}) at room temperature (20-25°C). The water sample was also spread onto solid TAP medium to allow colony formation. After visible colony formation, colonies were streaked several times to obain axenic cultures. Single cells were separated under stereoscope with a flame drawn Pasteur pipette to obtain monoclonal cultures.

Figure 1. Location of the water sample collected for the isolation of the newly described *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu).

2.3. Morphological identification

The morphological species identification key prepared by Bold and Starr in 1953 was used for the identification of *Dysmorphococcus globosus* (Bold and Starr 1953). A Leica MC 190 HD light microscope was used for observation and to take photographs. Cells were observed using 40X objective lens and oil immersed 100X objective lens.

2.4. 18S rRNA gene sequencing

Primers 16S1N (5'-TCCTGCCAGTAGTCATATGC-3') and 16S2N (5'-TGATCCTTCT/CGCAGGTTCAC-3') were used to amplify *18S rRNA* gene (Grzebyk et al. 1998). 2X GCTempase Mix (Amplicon, Denmark) was used in PCR reaction as recommended by the manufacturer. A total 35 cycles of PCR was used to amplify the gene. Annealing temperature was 55 °C and extension time was 2 minutes in each cycle. Agarose gel was used to visualize the product. The PCR product was purified and the sequence was determined by Sanger sequencing (BM Labosis, Ankara).

2.5. Molecular identification and phylogenetic analyses

The readings obtained from Sanger sequencing were used for molecular identification and phylogenetic relatedness. The forward read and the reverse read were trimmed from the end to remove the low-quality sequences, and then they were used to construct a more extended sequence of 1576 bp. This sequence was used to complete a search in the Basic Local Alignment Search Tool database (BLAST) from the National Center for Biotechnology Information (Zhang et al. 2000; Morgulis et al. 2008). Then phylogenetic analysis was perfomed by constructing a Maximum Likelihood phylogenetic tree, the tree was constructed using MEGA 12 (Kumar et al. 2024) with a bootstrapping value of 10000 replicates, and the tree was built using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) of nucleotide substitutions and the tree with the highest log-likelihood. The tree was rooted in *Chlorella vulgaris* (X13688) as an outgroup.

2.6. Scientific naming of the new taxon

The new taxon was named according to the literature (Takeda 1916; Bold and Starr 1953). "Instructions for Turkish Scientific Names of Plant, Fungi, Algae and Bacteria (Türkçe Bilimsel Bitki, Mantar, Suyosunu ve Bakteri Adları Yönergesi)" has been used for the naming of the new taxon (Menemen et al. 2021).

3. Results and Discussion

3.1. Morphology of the new isolate Dysmorphococcus globosus H.C. Bold & Starr (Kınık su yosunu)

Light microscope images of the isolate are shown in Figure 2. The strain is very similar to morphology reported in the literature (Bold and Starr 1953; Jannel et al. 2023). Cells are round which is about 30 µm in diameter for fully grown cells; younger cells are smaller (Figure 2). As reported in the literature (Zohir et al. 2022) cells that are not stressed are green (Figure 2) and cells under stress have an orange color indicating accumulation of carotenoid astaxanthin (Figure 2c). Although the cells contain two flagella, motile cells were rarely seen in the observations, as also reported in the literature (Jannel et al. 2023). The Turkish name of this species is suggested as "Kınık su yosunu" according to the guidelines of Menemen et al. (2021).

3.2. Amplification of 18S rRNA gene and sequencing

The expected product size for green algal species is about 1700 bp. As expected, a single specific band of about 1700 bp was formed in the PCR reaction (Figure 3). Green alga *Chlamydomonas reinhardtii* was used as a control in lane #3. The purified PCR product was sequenced using the same primers used for PCR in forward and reverse directions.

3.3. Molecular identification and phylogenetic analysis

The BLAST search results summarized in Table 1 shows that the isolate has 100% similarity with *Dysmorphococcus globosus*

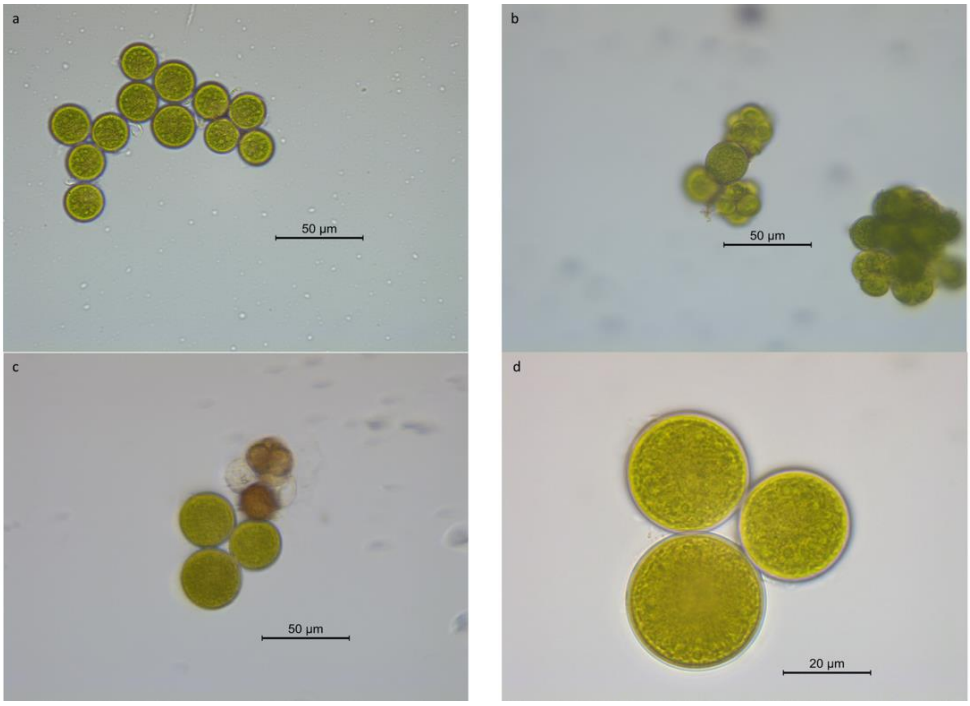

Figure 2. Microscope images of *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu). a) Green cells with 40X objective lens. b) Green cells that are dividing, 40X. c) Cells starting to accumulate carotenoid which have yellowish/orange color, 40X. d) Yellowish cells visualized using oil immersed 100X objective lens.

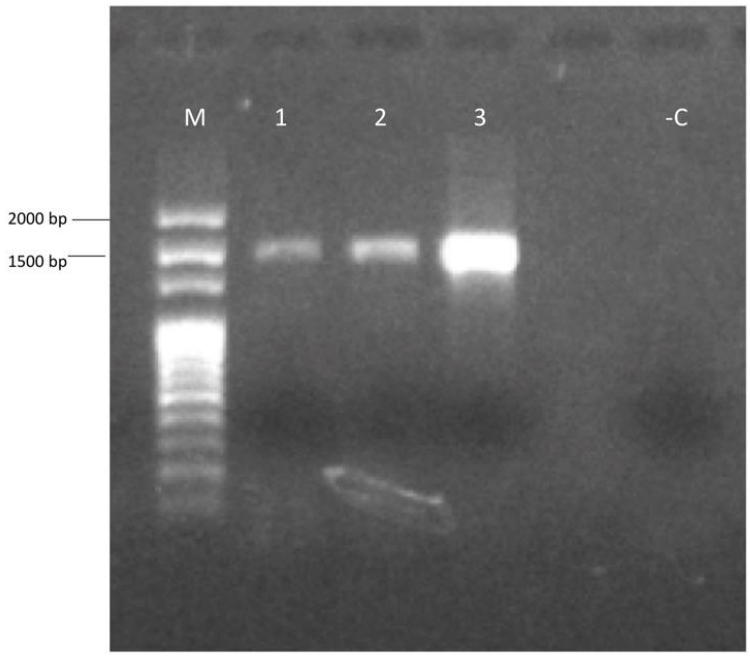

Figure 3. Amplification of 18S rRNA gene by PCR. A specific band of about 1700 bp was obtained. Line 1 and 2 are *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) product, line 3 is *Chlamydomonas reinhardtii* product. M; DNA size marker, -C; negative control.

Table 1. Summary of Blast search results

Sample	Scientific Name (Accession no.)	Max Score	Total score	Query Cover	E value	Percentage of identity
Dysmorphococcus globosus H.C. Bold & Starr (Kınık su yosunu) 1576 bp	*Dysmorphococcus globosus* (PQ632470.1)	2911	2911	100%	0	100
	Dysmorphococcus globosus (PQ002471.1)	2904	2904	100%	0	99.94
	Dysmorphococcus globosus SAG 20-1 (KM020136.1)	2556	2556	100%	0	96.18

(PQ632470.1) strain that was isolated from China, whilst it showed 96.18% similarity with the *Dysmorphococcus globosus* SAG 20-1 strain that is available at the Culture Collection of Algae at Göttingen University. This confirms that this isolate belongs to the *Dysmorphococcus* genus. Unfortunately, there are only a few sequences available for the *18S rRNA* in the GenBank. In order to clarify the phylogenetic relation among these sequences and others, a phylogenetic tree was constructed for these sequences in addition to other sequences from the related species that were obtained from culture collections worldwide (Figure 4). The results showed that the isolate that was obtained from Türkiye clustered with other isolates from the genus *Dysmorphococcus* as shown in Figure 4.

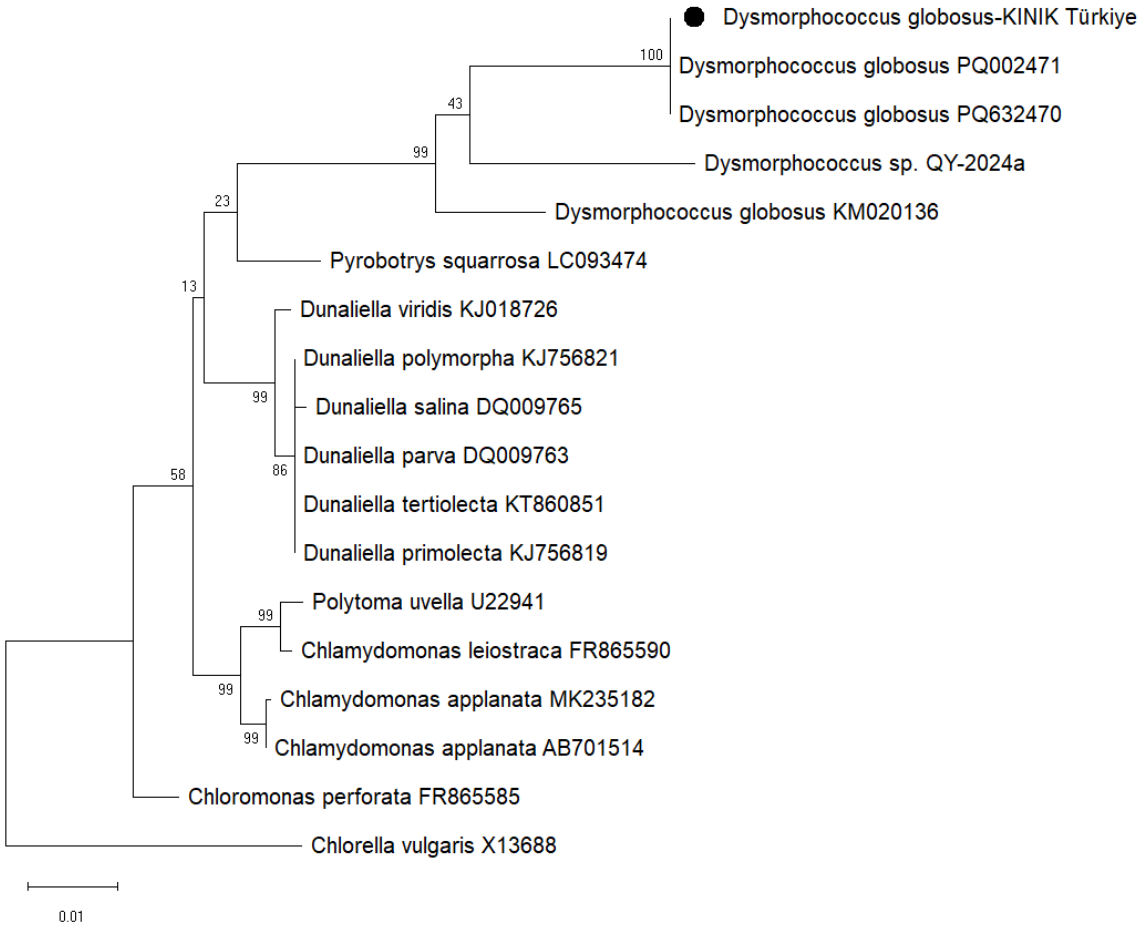

Figure 4. Maximum Likelihood phylogenetic tree constructed using the 18S rRNA. The bootstrapping value is 10000 replicates shown next to the branches, the tree is rooted to the outgroup *Chlorella vulgaris* (X13688).

3.4. Classification of Dysmorphococcus globosus H.C. Bold & Starr (Kınık su yosunu)

Classification according to AlgaeBase database (Guiry and Guiry 2025).

Phylum: Chlorophyta
Subphylum: Chlorophytina
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Phacotaceae
Genus: Dysmorphococcus
Species: *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu)

This taxon occurred in rainwater accumulated in rock cavity formations in the antique city of Xanthos in the Kaş district of Antalya. This is a freshwater species.

4. Conclusion

There are 5480 records of algae in our country (Maraşlıoğlu and Gönülol 2025). Among these, 827 belong to Chlorophyta and with our new addition it reaches to 828 taxons. In this study, *Dysmorphococcus globosus* H.C. Bold & Starr (Kınık su yosunu) which was isolated in Kınık village in the district of Kaş, Antalya has been provided as a new record for Turkish freshwater algal flora in light of molecular and morphological analyses.

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Evaluation of some agro-morphological parameters in commercial sweet corn (*Zea mays* L. saccharata Sturt) hybrids under greenhouse conditions

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ABSTRACT

Sweet corn (*Zea mays* (L.) var. saccharata) is a cereal grown in almost every region of the world and is widely cultivated for human consumption. Moreover, it serves as both a raw and processed ingredient in the global food industry. Its breeding programs optimize yields and many agro-morphological traits through the development of hybrids, which contributes to its worldwide popularity. This study aimed to assess some agro-morphological parameters of 13 commercial sweet corn hybrids during two years under greenhouse conditions. These parameters included plant height (PH), stem diameter (SD), first ear height (FEH), leaf width (LW), number of nodes (NN), number of ears (NE), number of leaves (NL) and tassel initiation day (TI). The two-year average results showed that PH and FEH had a mean of 167.96 and 61.48 cm, ranging from 133.12 to 205.5 and 77 to 31.83 cm, respectively. For SD, the average was 21.28 mm with the highest value recorded by Khan (28.75 mm), followed by Driver (24.62 mm). The earliest TI was observed by Khan (52), succeeded by Challenger, Fragman and SF 2070 with same value of 55 days. In addition, there was a significant variation (at least $P \leq 0.05$) for all traits across the two years. The highest values were observed in SF 2070 for LW, Messenger for NN, 10514 for NE and Febris for NL. Based on the findings of this study, different sweet corn hybrids may be recommended for both breeding programs and cultivation, depending on their specific agro-morphological traits.

1. Introduction

Corn (*Zea mays* L.) is one of the most widely grown cereals in the world (Sanodiya et al. 2023). It belongs to the Poaceae family and was domesticated in Central America (Phiarais and Arendt 2008; Kennett et al. 2020). It is cultivated in almost every region in the world and remains the third most important crop after wheat and rice (Phiarais and Arendt 2008; Kaushal et al. 2023). Its production in 2023 is estimated at 1.24 billion tons on an area covering 208.23 million ha, with the major producers including the USA, China, Brazil, Argentina and India (FAOSTAT 2025). Corn is used for food and feed and, is an ideal source for biofuel production (Courtois et al. 1991; Wallington et al. 2012; Venadan et al. 2024). It is known under different specialized varieties such as sweet corn, which has high sweetness concentration and nutritional values (Hallauer 2001; Dang et al. 2023).

Sweet corn (*Zea mays* (L.) var. saccharata), generally known as a fruit and vegetable product, derives from the spontaneous mutation of the relative gene which regulates the transformation of sugar to starch in the endosperm of kernels (Feng et al. 2020; Yu et al. 2023). It is characterized by light yellow seeds with a high moisture (around 70%) and sugar content 25-30% higher than normal corn varieties (Rah Khosravani et al. 2017; Wang et al. 2023a). It contains precious components such as vitamins, minerals, dietary fiber and phytonutrients while possessing antioxidant, anti-diabetic, anti-inflammatory, and anti-cancer properties which have multiple benefits for human health (Liu et al. 2014; Joshi et al. 2017; Xiao et al. 2022). These properties explain its high preference in the USA, Europe, Asia, and developing countries in the world (Rathinavel et al. 2024). Despite increasing demand for sweet corn, its growth is confronted by rigorous market requirements to satisfy high quality and appearance norms (Alan et al. 2024). It requires important traits such as plant height, ear height and particularly commercial yield for the producers, and it is mostly used in fresh, canned or frozen form (Mahato et al. 2018; Alan et al. 2024).

Nowadays, sweet corn is largely improved through selection and hybridization (Szymanek et al. 2015). These hybrid varieties have higher yields and offer better uniformity and quality (Kumari et al. 2006). Many agro-morphological traits contribute to the overall hybrid productivity of the crop, including plant height, stem diameter, first ear insertion height, etc. Plant height is regarded as a crucial parameter for assessing crop growth conditions and predicting yield potential (Zhao et al. 2024). This trait significantly contributes to enhancing the lodging resistance of corn and grain yields (Liu et al. 2021). In the diverse range of agronomic parameters in corn, stem diameter also plays a crucial role in obtaining high yield. It functions not only as a predictor for yield estimation and evaluation of lodging resistance but also as an important metric for forecasting seasonal biomass accumulation in corn (Kelly et al. 2015: Liu et al. 2022). Enhanced plant development increases the probability of leaf production (Subaedah et al. 2018). The presence of more leaves

and greater leaf areas facilitates the capture of increased solar energy. This could accelerate the photosynthesis process at an increased rate, ultimately resulting in plants that exhibit enhanced growth and productivity (Mansfield and Mumm 2014).

The aim of the present study was to evaluate some agro-morphological parameters, including plant height, stem diameter, first ear height, number of nodes, ears and, leaves of commercial sweet corn hybrids, in two years. Our results will assist the understanding of agro-morphological parameters underlying sweet corn yield-related traits.

2. Materials and Methods

This research was conducted in the greenhouse of Akdeniz University (36°53' N, 38°30' E and altitude of 15 m) from February to June 2023 and 2024. Thirteen sweet corn hybrids were used in the present study which have been released by international companies in Türkiye. The experiments were conducted in a randomized complete block design with three replicates. Prior to the seeding process, the plant residues were removed, and a drip irrigation system was installed in the greenhouse. The recommended standard practices were adhered to throughout the procedure. Seeds were planted in rows measuring 5 m in length, with a distance of 0.7 m maintained between each row and a spacing of 0.2 m between individuals. The weather data recorded for the growth period during 2023 and 2024 revealed that average weekly minimum and maximum temperatures ranged from 12.1 to 41°C and 13.8 to 44.5°C, respectively.

From each plot, three plants were randomly selected at harvest stage for evaluation of agro-morphological parameters. Plant height (PH) was measured as the distance from the soil of the plant to top of the tassel (cm). The measurement of the stem diameter (SD) at the third node from the base of the main stem was conducted utilizing a digital caliper in conjunction with an LCD Stainless Electronic Ruler Micrometer (Clockwise Tools DCLR-0605 Electronic Digital Caliper) (mm). First ear height (FEH) was measured from the ground to first ear (cm). The leaf width (LW) was determined at harvest by selecting the largest part of each leaf selected and recorded in cm. The number of nodes (NN), ears (NE) and leaves (NL) were counted. Tassel initiation day (TI) was determined as the day from planting to tassel emergence.

Analyses of variance (ANOVA, PROC GLM) was performed with SAS 9.0 software.

3. Results and Discussion

Sweet corn is an agricultural crop cultivated for human consumption and an important component of the world's food sector (Swapna et al. 2020). Sweet corn breeding programs, like field corn, prioritize enhancing yield through the development of commercial hybrids. Many agro-morphological traits contribute to the overall hybrid productivity and yield of the crop. In this study, PH, FEH, SD, LW, NN, NE, NL and TI, which are among the important traits in commercial hybrid varieties, were evaluated over two years using 13 hybrid cultivars in a greenhouse. The results indicated that the variations among cultivars were significant (at least $P \leq 0.05$) across all traits. However, the effect of the years was statistically significant only for FEH and NE (Table 1).

PH is a crucial component of sweet corn growth, which influences its yield and provides economic benefits (Thapa et al. 2024). The results indicated that the two-year average PH ranged from 133.12 to 200.50 cm with a mean of 167.96 (Table 2). These results were significantly different from those of Ekiz (2021) in similar conditions, which indicated that PH varied from 152.20 to 337.80 cm. Atakul (2011) and Karacadal (2017) reported that

Table 1. Analysis of variance for some agro-morphological parameters sweet corn hybrids across the two years

Source	PH	SD	FEH	LW	NN	NL	NE
Year (Y)	NS	NS	*	NS	NS	NS	**
Cultivar (C)	**	**	**	**	**	**	**
Y x C	NS	NS	NS	NS	NS	**	NS

*, **: indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; NS indicates not significant; PH: plant height; FEH: first ear height; SD: stem diameter; LW: leaf width; NN: number of nodes; NE: number of ears; NL: number of leaves.

Table 2. Mean values of sweet corn hybrids for PH, FEH, SD, LW, NN, NL and NE across the two years

Cultivars	PH (cm)	FEH (cm)	SD (mm)	LW (cm)	NN	NL	NE
Messenger	169.87	74.50	24.50	7.62	11.87	9.00	1.50
Challenger	154.00	53.50	17.00	6.12	8.87	7.25	1.25
Khan	167.00	56.75	28.75	7.75	9.87	7.25	1.50
10514	166.62	61.25	20.62	6.93	10.87	9.75	2.37
SHY6RH1036	177.25	59.12	21.80	8.00	10.25	8.37	2.00
Sentinel	197.87	77.00	16.62	6.25	9.87	9.00	1.25
Vega	170.06	53.52	17.87	6.00	8.75	9.50	1.25
Caramelo	133.12	56.12	23.50	6.81	9.12	7.00	1.37
Fragman	172.08	56.15	18.75	5.93	8.87	9.50	1.75
SF 2070	142.20	31.83	22.62	8.15	8.25	9.37	1.75
SF 1280	158.50	72.50	22.37	7.00	9.75	7.75	1.12
Driver	200.50	71.75	24.62	5.87	10.87	9.62	1.75
Febris	174.46	75.36	17.62	6.86	10.00	11.62	1.37
Means	167.96	61.48	21.28	6.86	9.61	8.84	1.55
LSD	15.53**	10.53**	3.51**	0.96**	0.82**	0.87**	0.61**

*, **: indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; PH: plant height; FEH: first ear height; SD: stem diameter; LW: leaf width; NN: number of nodes; NL: number of leaves; NE: number of ears.

it varied between 170.00-204.00 cm and 205.00-248.00 cm, respectively. This variation was probably due to humidity, precipitation, and temperature affecting PH (Turhal 2010). It was also influenced by its production environment (Yozgatlı et al. 2019). The results of the average for the two years showed that the highest PH value was recorded by Driver (200.50 cm), followed by Sentinel (197.87 cm) and SHY6RH1036 (177.25 cm). Caramelo had the shortest PH with 133.12 cm. Our result for Caramelo was similar to Tezel et al. (2021) who reported that it (128 cm) had the shortest PH value over two years. SD represents stem thickness and is considered to be an essential agronomic yield component of sweet corn (Iqbal et al. 2015). For the average of two years SD was 21.28 mm and Khan recorded the highest value (28.75 mm), followed by Driver (24.62 mm). Our results were different compared to those obtained by Ağaçkesen and Öktem (2022) which showed SD ranging between 21.90 and 23.90 mm. This difference can be attributed to different genotypic factors, variability between years, and characteristics of the plants (Hallauer et al. 2010; Ağaçkesen and Öktem 2022).

FEH is one of the phenotypic characteristics that provide the information on the vertical structure of the sweet corn and affect its yield (Wang et al. 2020; Wang et al. 2023b). In this study, Sentinel recorded the highest FEH with 77.00 cm, while SF 2070 exhibited the lowest with 31.83 cm, resulting in an average FEH of 61.48 cm over the two-years (Table 2). Similar results were confirmed by Tezel et al. (2021) and Kılınç et al. (2021), who reported that FEH ranged from 35.20 to 79.80 cm and from 45.83 to 70.68 cm, respectively. This trait is influenced by genetic and environmental factors (Sönmez 2000; Yozgatlı et al. 2019). Recent studies reported that there are significant variations between genotypes for the FEH, which varies depending on PH (Anıl and Sezer 2003; Öktem and Öktem 2006; Tezel et al. 2021). Sentinel showed this finding by exhibiting one of the tallest plant heights recorded in this research.

LW, which is considered an important characteristic in plant light competitiveness (Sinoquet and Caldwell 1995; Gao et al. 2021), is an important parameter in plant architecture that considerably impacts photosynthesis and yield (Gao et al. 2021). An average of two-year results indicated that LW ranged between 5.87 and 8.15 cm, with an average of 6.86 cm (Table 2). Our result was lower than the results obtained by Utari et al. (2023), who found a mean value of 9.40 cm. Research by Lu et al. (2024) in two years showed that it had an average value of 8.10 and 8.30 cm in 2015 and 2016, respectively. In our study, the lowest LW was observed in Driver F1 (5.87 cm), followed by Fragman (5.93 cm), while the highest LW was identified in SF 2070 (8.15 cm). This difference shows the variation in LW of sweet corn among cultivars (Utari et al. 2023).

NN plays an important role in maintaining the plant upright and in nitrogen fixation (Zheng et al. 2023). Significant differences were detected at $P \leq 0.01$ level for NN among cultivars, while years and, cultivar x year interaction was not statistically significant across the two years. In this study, NN was ranged between 8.25 and 11.87 with an average of 9.61 (Table 2). A study conducted by Heuer et al. (2001) under greenhouse conditions showed that it could be expected to rise up to 12.00. This explains the variation in NN among sweet corn cultivars (Heuer et al. 2001). It is crucial in regulating lodging and its decrease affects the length of sweet corn (Dong et al. 2023; Heuer et al. 2001). It also improves dry matter accumulation and allocation in the stem, which boosts the yield and yield stability of plants (Liang et al. 2025).

NE is accepted as one of the most important factors that influence sweet corn yields (Ekiz 2021). Statistical analysis showed a significant difference in NE for cultivars ($P \leq 0.01$) and the results showed that across the two years, it had an average of 1.55 and varied from 1.12 to 2.37. 10514 (2.37) recorded the highest value followed by SHY6RH1036 with the value of 2.00 (Table 2). Our results were different from İdikut et al. (2005) and Turgut and Balci (2002), which showed that NE ranged from 1.00 to 1.30 and 1.35 to 1.68, respectively. It generally varied according to the cultivar, sowing dates and environment (Bozokalfa et al. 2004; Eşiyok and Bozokalfa 2005; Kılınç et al. 2021).

NL remains an important factor affecting vegetation cover, photosynthesis and yield of sweet corn (Stansluos et al. 2020). It varied from 7.00 to 11.62, with an average of 8.84 across the two years (Table 2). Our result was similar to those found by Sönmez et al. (2013), which showed that it ranged from 7.90 to 11.10 and lower than that obtained by Alan et al. (2011), which ranged between 9.16 to 12.60. NL differs based on the cultivar, various characteristics, and the production environment (Ağaçkesen and Öktem 2022; Susanti et al. 2023). This affects the yield and the quantity of light received by sweet corn (Stansluos et al. 2020; Susanti et al. 2023).

The TI of sweet corn varies generally according to the genotypes (İdikut et al. 2015). The results indicated that the cultivar Messenger (69 days) had the highest number of days, followed by SF 1280 and Febris, which both had a TI value of 65 days (Figure 1). The lowest IT was observed by Khan (52), followed by Challenger, Fragman and SF 2070 with a value of 55 days. Our results were higher than those obtained by İdikut et al. (2016) and Karacadal (2017), which showed that it ranged between 60-64 and 48-52 days, respectively. However, our results were lower than the results found by Atakul (2011) with a value of 51-77 days and by Alan et al. (2011) with a value of 76-81 days. These variations can be explained by the differences between genetic structures and environmental conditions (Özata 2019; Tezel et al. 2021). It is possible to significantly reduce the TI under greenhouse conditions and obtain an early crop of sweet corn which is suitable for commercialization (Kul 2012; Ekiz 2021).

4. Conclusion

There is great interest in producing sweet corn under greenhouse conditions as the income per unit area is getting close to the income of other vegetables such as lettuce, cucumber, tomato and pepper. The fact that the labor in the greenhouse is significantly less in sweet corn compared to these vegetables reduces the input costs. Therefore, studies on yield characteristics in greenhouse conditions contribute valuable information. This study was to evaluate some agro-morphological parameters such as PH, FEH, SD, LW, NN, NE, NL and TI of 13 commercial sweet corn hybrids under greenhouse conditions. All the traits were significantly varied at least $P \leq 0.05$ across the two years. Driver and 10514 could be recommended for PH and NE, respectively. Khan cultivar was identified as the earliest TI as well as the highest SD. However, more research and additional agro-morphological traits are necessary to evaluate the performance of these commercial sweet corn hybrids under different environments.

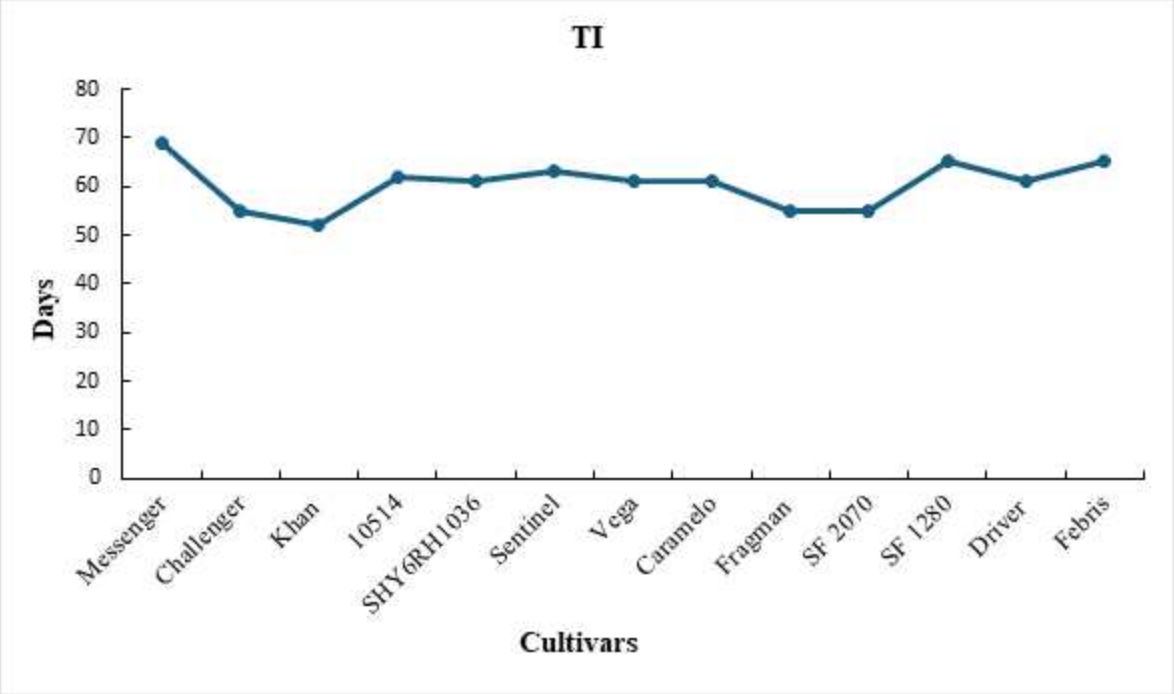

Figure 1. The average tassel initiation (TI) day of different sweet corn hybrids across the two years.

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Determination of *CAST*/*Msp*I gene polymorphism in selected sheep breeds reared in Türkiye*

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Sheep

ABSTRACT

Sheep breeding plays a crucial role in meeting the increasing demand for high-quality meat, milk, and wool. Traditional selection methods based on phenotypic traits have led to genetic improvement; however, progress remains slow for traits with low to moderate heritability. Marker-assisted selection (MAS) has emerged as a promising complementary approach, enabling faster genetic gains. Among candidate genes studied for MAS, the calpastatin (*CAST*) gene is notable for its association with meat yield and quality. This study aimed to identify *CAST/Msp*I gene polymorphisms in four sheep breeds reared in Türkiye (Central Anatolian Merino-CAM, Pırlak-PRL, Romanov-RMV, and Suffolk-SFK) and assess their potential use in MAS. A total of 176 individuals were genotyped using the PCR-RFLP method. All breeds in this study were found to be polymorphic for the *CAST* gene. The frequency of the M allele ranged from 0.67 in CAM to 0.76 in SFK. Genotype frequencies for MM ranged from 0.40 (CAM) to 0.71 (SFK), for MN from 0.10 (RMV and SFK) to 0.54 (CAM), and for NN from 0.06 (CAM) to 0.20 (RMV). Significant deviations from Hardy-Weinberg equilibrium were observed in CAM, RMV, and SFK populations, but not in PRL. Observed heterozygosity ranged from 0.10 to 0.54 and expected heterozygosity from 0.37 to 0.44. The presence of all three genotypes and substantial genetic variation suggests that the *CAST* gene may be a valuable marker in MAS for these breeds. However, further association studies are required to confirm the relationship between *CAST* genotypes and economically significant traits related to meat production.

1. Introduction

Traditional selection methods, which have been employed for many years to increase yields in animal production, are often slow, laborious, labor-intensive, and costly. Most economically important traits in livestock are quantitative in nature, characterized by low heritability and influenced by a multitude of genes as well as environmental factors. Genetic progress tends to be slower for phenotypic traits such as meat or milk yield, which typically have low heritability and can only be measured in the later stages of an animal's life. However, the early identification of candidate genes associated with various performance traits, and their subsequent application in marker-assisted selection (MAS) programs, can significantly accelerate genetic improvement (Karslı et al. 2017; Demir et al. 2022). Identifying candidate genes suitable for MAS is a complex process that requires establishing associations between genotypic and phenotypic data (Javanmard et al. 2010; Kania et al. 2019). Calpastatin (*CAST*) gene is one of the most studied genes reported to be associated with meat yield and quality in different livestock species (Javanmard et al. 2010; Ropka-Molik et al. 2014; Kania et al. 2019).

In sheep, approximately one-quarter of the total body weight consists of skeletal muscle. Skeletal muscle growth in animals is primarily determined by three factors: the number and size of muscle cells, the rate of muscle protein synthesis, and the rate of protein degradation (Ibrahim et al. 2015). Various physiological and genetic factors influencing muscle growth act upon one or more of these fundamental mechanisms. However, in domestic animals, variations in the rate of muscle growth are more commonly attributed to differences in protein degradation rather than changes in protein synthesis (Goll et al. 1998).

Muscle protein degradation, or proteolysis, is mediated by several well-characterized proteolytic enzyme systems, among which the calpain-calpastatin system (CCS) is particularly prominent. This system comprises calpains, a family of calcium-dependent cysteine proteases, with at least fifteen isoforms identified and their endogenous inhibitor, calpastatin. The role of the CCS has been well established in both normal and postnatal skeletal muscle development, as well as in muscle wasting conditions. In general, increased skeletal muscle growth is associated with reduced rates of protein degradation and decreased calpain activity, primarily resulting from elevated calpastatin activity (Goll et al. 1998). Importantly, the calpain-to-calpastatin ratio is considered to be a key determinant of muscle accretion in animals (Kania et al. 2019; Valencia et al. 2022). In contrast, during the post-mortem conversion of muscle to meat, reduced calpastatin activity permits enhanced proteolysis, which significantly contributes to meat tenderization (Kawasaki and

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Kawashima 1996; Goll et al. 1998; Ciobanu et al. 2004; Jawasreh et al. 2019).

The *CAST* gene, which plays a critical role in post-mortem meat tenderization and skeletal muscle development, is located on chromosome 5 in sheep and comprises 29 exons spanning a total of 89576 base pairs (NCBI 2021). Polymorphisms in the *CAST* gene have been reported to be associated with several economically important traits, including birth weight, growth performance, growth rate, muscle development, and carcass yield. In addition to these production traits, variations in *CAST* have also been linked to meat quality characteristics such as pH, meat color, cooking loss, and tenderness (Palmer et al. 1997; Chung and Devis 2012; Knight et al. 2012; Ramadevi et al. 2020). In this context, the aim of the present study was to identify *CAST/Msp*I gene polymorphisms in four sheep breeds in Türkiye (CAM, PRL, RMV, and SFK) and to evaluate their possibilities for use in MAS studies.

2. Materials and Methods

Ethical approval and permission for this study was obtained from Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (Date: 09/10/2020; Decision No: 809).

To carry out CAST gene polymorphism in sheep breeds, blood samples were taken from the jugular vein of animals into sterile vacuum tubes containing EDTA. Information about the blood samples used in the study (breed, number of samples and where collected) is shown in Table 1. Genomic DNA from whole blood was extracted using a commercial DNA isolation kit (BLIRT DNA isolation kit, EM13-250) according to the manufacturer's instructions. DNA quality and quantity were determined using gel electrophoresis (1%) and ND 1000 NanoDrop spectrophotometer (A260/A280 nm).

Table 1. Sampling regions and sample sizes

District	Breed (Abbreviation)	Sample size (n)
Çifteler	Central Anatolian Merino (CAM)	32
	Pırlak (PRL)	8
	Romanov (RMV)	10
	Suffolk (SFK)	21
Sivrihisar	Central Anatolian Merino (CAM)	64
Mahmudiye	Central Anatolian Merino (CAM)	41
Total		176

Polymorphisms in the *CAST* gene were determined in the studied sheep breeds using the PCR-RFLP method. After the DNA isolation step, the primers reported by Palmer et al. 1998 were utilized for the amplification of the *CAST* gene by PCR. To amplify the 622 bp region of the cast gene, 25 µl of reaction mixture was prepared by adding 100 ng DNA, 10 X PCR buffer, 0.6 mM of each dNTP, 1.5 mM $MgCl_2$, 1 pM of each primer and 1 U of Taq polymerase. The PCR amplification was performed using 35 cycles of 95˚C for 1min, 62˚C for 1min and 72˚C for 2 min, followed by 72˚C for 10 min. The 622 bp products were digested with *Msp*I restriction enzyme. The bands were visualized using ultraviolet transillumination and the size of the amplified fragments was compared to a Solis Biodyne 100 bp DNA ladder (Cat 07-11-00050).

Based on the results of electrophoresis, the presence of the relevant genotypes was identified. The Popgene software package was used to calculate allele and genotype frequencies, observed (Ho) and expected heterozygosity (He), and Hardy-Weinberg equilibrium for the CAST gene (Yeh et al. 1997). The chi-squared test ($\chi 2$) was also used to test whether or not the populations were in Hardy-Weinberg equilibrium.

3. Results and Discussion

PCR amplification successfully produced 622 bp *CAST* gene fragments, and a 100 bp ladder was used for comparison of the amplification length. The PCR products were then digested with the restriction enzyme *Msp*I and separated on 2% agarose gel electrophoresis. As a result of PCR-RFLP, the presence of polymorphism in *CAST* gene presented two alleles M and N. Enzyme *Msp*I produced two fragments of 336 bp and 286 bp for allele M, whereas the PCR product remained uncut for allele N. The PCR-RFLP profile of M allele homozygous animals (MM) showed two bands of 336 and 286 bp. All three genotypes were observed, and the *CAST* gene locus was found to be polymorphic in all populations studied. The heterozygous genotype (MN) showed three bands of 622, 336 and 286 bp and homozygous for the N allele (NN) showed only one band of 622 bp, as shown in Figure 1.

The gene frequencies of the *CAST* locus were estimated using both the Popgene V.1.31 (Yeh et al.1997) software package and counting the number of genes. The chi-squared test (χ^2) was used to test whether or not the populations were in Hardy-Weinberg

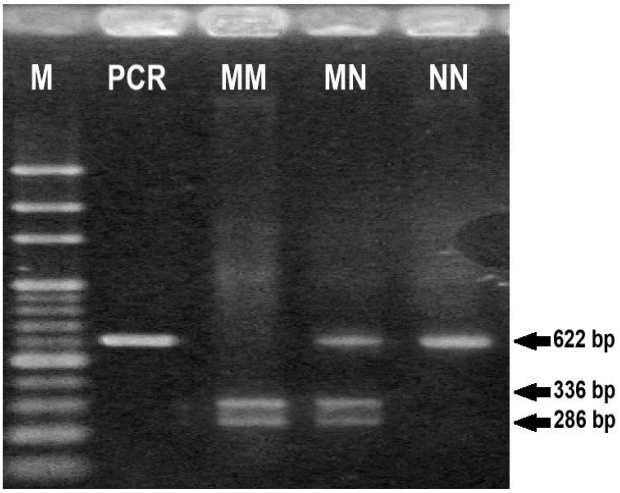

Figure 1. Agarose gel image of *CAST* genotypes detected using the PCR-RFLP.

equilibrium. The frequencies of genotypes and alleles of the *CAST* locus are shown in Table 2.

The estimated genetic diversity parameters for the *CAST* gene in the studied sheep breeds are presented in Table 3.

In all breeds, except the Central Anatolian Merino, the homozygous MM genotype had the highest frequency, and M was the most common allele. The allele frequencies reported from previous studies on different sheep breeds are summarized in Table 4.

The calculated allele frequencies (M and N) in the Pırlak, Romanov and Suffolk sheep breeds were consistent with studies by Shahroudi et al. (2006) in Karakul breed, Szkudlarek-Kowalczyk (2011) in Polish merino, Gharahveysi et al. (2012) in Zel breed, Tohidi (2013) in Sanjabi sheep, Sunilkumar et al. (2014) in Bandur sheep, Avanus et al. (2015) in Karakul sheep, Bozhilova-Sakova et al. (2020) in Northeast Bulgarian Merino breed and Bayraktar and Shoshin (2022) in Awassi sheep. The

Table 2. Genotype and allele frequencies of CAST gene

Breed	N	Genotype Frequencies			Allele Frequencies	
		MM	MN	NN	M	N
Central Anatolian Merino	137	0.40 (55)	0.54 (74)	0.06 (8)	0.67	0.33
Pırlak	8	0.63 (5)	0.25 (2)	0.12 (1)	0.75	0.25
Romanov	10	0.70 (7)	0.10 (1)	0.20 (2)	0.75	0.25
Suffolk	21	0.71 (15)	0.10 (2)	0.19 (4)	0.76	0.24

Table 3. Genetic diversity parameters in the studied breeds

Breed	n	Ho	He	Ne	χ^2
Central Anatolian Merino	137	0.54	0.44	1.78	6.86
Pırlak	8	0.25	0.40	1.60	0.88
Romanov	10	0.10	0.39	1.60	5.37
Suffolk	21	0.09	0.37	1.56	12.64

n: sample size, Ho: Observed heterozygosity; He: Expected heterozygosity, Ne: Number of effective alleles. $\chi^2_{1,0.05}$: 3.841 Hardy-Weinberg equilibrium (P<0.05).

Table 4. The allele frequencies in different sheep breeds

Breed	Allele Frequencies		Reference
	M	N	
Karagül	0.79	0.21	Shahroudi et al. (2006)
Tsigai x Lacaune	0.90	0.10	Gabor et al. (2009)
Tsigai	0.91	0.09	
Polonya Merinos	0.76	0.24	Szkudlarek-Kowalczyk et al. (2011)
Berrichon du Cher	0.92	0.08	
Ile de France	0.95	0.05	
Atabi	0.64	0.36	Nanekarani et al. (2011)
Zel	0.75	0.25	Gharahveysi et al. (2012)
Balkhi	0.88	0.12	Khan et al. (2012)
Kajli	0.86	0.14	
Lohi	0.87	0.13	Suleman et al. (2012)
Kajli	0.81	0.19	
Thalli	0.90	0.10	
Sanjabi	0.72	0.28	Tohidi (2013)
Ghezel	0.69	0.31	
Afshari	0.63	0.37	
Makui	0.88	0.12	
Bandur	0.72	0.28	Sunilkumar et al. (2014)
Lori	0.63	0.37	Asadi et al. (2014)
Güney Karaman	0.67	0.33	Balcıoğlu et al. (2014)
Akkaraman	0.69	0.31	
Awassi	0.59	0.41	
Kangal	0.92	0.08	
Karayaka	0.89	0.11	
Morkaraman	0.87	0.13	
Gökçeada	0.99	0.01	Yılmaz et al. (2014a)
Kıvırcık	0.85	0.15	
Karacabey Merino	0.80	0.20	
Kıvırcık	0.84	0.16	Yılmaz et al. (2014b)
Karagül	0.73	0.27	Avanus et al. (2015)
Shumen	0.92	0.08	Georgieva et al. (2015)
Northeast Bulgarian Merino	0.73	0.27	Bozhilova-Sakova et al. (2020)
Awassi	0.78	0.22	Bayraktar and Shoshin (2022)

allele frequencies calculated in Central Anatolian Merino sheep breed were similar to the results of Nanekarani et al. (2011) in Atabi sheep, Tohidi (2013) in Ghezel and Afshari sheep, Asadi et al. (2014) in Lori sheep and Balcıoğlu et al. (2014) in Güney Karaman, Akkaraman and Awassi sheep breeds.

However, this study is not in agreement with the following studies:- Gabor et al. (2009) in Tsigai x Lacaune and Tsigai sheep, Szkudlarek-Kowalczyk et al. (2011) in Berrichon du Cher and Ile de France sheep, Khan et al. (2012) in Balkhi and Kajli sheep, Suleman et al. (2012) in Lohi, Kajli and Thalli sheep, Tohidi (2013) in Makui sheep, Balcıoğlu et al. (2014) in Kangal, Karayaka and Morkaraman sheep, Yılmaz et al. (2014a, 2014b) in Gökçeada, Kıvırcık, Karacabey merino sheep and Georgieva et al. (2015) in Shumen sheep. The frequency of the M allele in these studies is considerably higher than in the results of this study.

There was no statistical significance between the observed and expected frequency differences for the *CAST* gene in the Pırlak population. In terms of the *CAST* polymorphism, the Pırlak population was found to be in Hardy-Weinberg genetic equilibrium (Table 3, $P<0.05$). However, the higher $\chi 2$ values calculated for the Central Anatolian Merino, Suffolk, and Romanov populations indicated that the $\chi 2$ values for these three breeds were significant (Table 3, $P<0.05$). The chi-square test showed that these sheep populations were not in Hardy-Weinberg equilibrium for the *CAST* locus. This unexpected result could be due either to a sampling error or to the fact that a few rams were used for breeding.

Polymorphisms in the *CAST* gene in sheep influence various growth traits and carcass traits. Most of the studies reported a positive effect of the MN genotype on the weight of the animals measured at different ages. In the same way, in different breeds, the MN genotype was reported to have a 15.4% higher birth weight than the NN genotype (Ramadevi et al. 2020; Valencia et al. 2022) and for weaning weight, MN was 1.04 kg higher than MM (Gorlov et al. 2016). It has also been reported that daily weight gain from birth to weaning is greater in MM than in MN and NN (Nassiry et al. 2006; Yilmaz et al. 2014b, Gorlov et al. 2016 and Jawasreh et al. 2017). However, Afanasyeva et al. (2019) reported that higher growth of MM is limited up to weaning in the West Siberian mutton sheep breeds. In most studies, the M allele was superior for all growth-related traits, with few exceptions, for example, in the Prydniprovska meat sheep and Altai Mountain breed, animal weight was in favour of the N allele rather than the M allele (Pomitun et al. 2019; Selionova et al. 2020). In addition, Jawasreh and Ismail (2019) reported that MN lambs of the Awassi breed may be healthier due to a higher neutrophils and neutrophil to lymphocyte (N/L) ratio, high triiodothyronine (T3) and cortisol in their blood hematology and serum studies.

In addition to growth traits, *CAST* variants influence carcass and meat quality in sheep. The pre-slaughter live weight of MN genotyped sheep from Volgograd was 3.7 kg more than that of MM genotyped sheep (Kolosov et al. 2021). Palmer et al. (1999) found that the ac genotype had a 15-18% higher age-corrected carcass weight and MN had a higher chilled carcass weight. Furthermore, the aa genotype had the highest percentage of muscle and lowest percentage of fat in the hindquarters, while the ac genotype had the highest intramuscular fat in the ram loin of the two synthetic breeds (Kania et al. 2019). Ibrahim et al. (2015) also reported that the lean and fat percentages of sheep meat were also influenced by the *CAST* gene. MM and MN genotypes tended to have the highest longissimus muscle width, and MN had heavier longissimus muscle than MM (Jawasreh et al. 2017; 2019). MM and MN had higher backfat thickness and skin plus backfat thickness values of the loin eye muscle in Kıvırcık sheep than in NN genotypes (Yilmaz et al. 2014b). In a study by Selionova et al. (2020), N was found to be superior (NN genotype) in carcass mass, carcass performance and meat percentage in the Altai Mountain breed.

Kumar et al. (2018) found that the Warner-Bratzler shear force score of the NN genotype was significantly ($P<0.001$) lower than that of the MM genotype in Bandur ram lambs, and the NN genotype indicated higher meat tenderness. In the Dorset Down breed, ac genotypes had higher shear force (SF) in fillets from ewes (Palmer et al. 1997). Meat samples from ab showed a higher initial mean pH in the Dorset Down breed and aa reported a higher final pH (after ten days) in the Santa Inês breed (Palmer et al. 1997; Esteves et al. 2020). Jawasreh et al. (2017) reported that MN had lighter meat and MN showed lower cooking loss than MM in Awassi sheep.

In contrast, various studies found no association between the *CAST* gene and different traits (Sutikno et al. 2011; Dehnavi et al. 2012; Nikmard et al. 2012; Peirvisi et al. 2020; Bayram et al. 2019). The available research has shown a significant correlation between *CAST* SNPs and various traits in sheep. It is important to note that the same allele may have different effects in different breeds and therefore breed specific studies are required before using the *CAST* gene as a marker in selection.

4. Conclusions

The *CAST/Msp*I polymorphism was investigated in the Central Anatolian Merino, Pırlak, Romanov and Suffolk sheep breeds by PCR-RFLP method. In this gene region, variations were observed at different frequencies across all three genotypes (MM, MN, and NN) of the CAM sheep breed. Additionally, a high level of genetic diversity was found within the CAM population. Based on these findings, it is proposed that the relevant gene region could be utilized in Marker-Assisted Selection (MAS) studies aimed at enhancing meat yield and quality in the CAM breed. However, prior to initiating MAS studies, it would be prudent to conduct correlation analyses between the identified genotypes and the phenotypic data associated with meat yield and quality.

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*Ethical approval and permission for this study was obtained from Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (Date: 09/10/2020; Decision No: 809).

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