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Table of Contents

Research Articles

- 1. THE USAGE OF MARINE PLANT-BASED BIO-FERTILIZER FOR TOMATO GROWING IN MOGADISHU, SOMALIA**
Mohamed Mohamud SALAH, Mohamed Hassan ABDI, Mohamed AHMED, Mohamed Ali KAHIE, Arunachalam SIVAKUMAR.....197-202
- 2. FORECASTING SEASONAL MILK PRODUCTION USING MARS ALGORITHM FOR MULTIPLE CONTINUOUS RESPONSES IN HOLSTEIN DAIRY CATTLES**
Demet ÇANGA BOĞA, Mustafa BOĞA, Mutlu BULUT.....203-214
- 3. A DETAILED EXAMINATION OF TÜRKİYE'S PROJECTED PRECIPITATION AND GROWTH SEASON TRENDS UNDER CLIMATE CHANGE CONDITION**
Eser ÇELİKTOPUZ.....215-223
- 4. THE EFFECTS OF FERTILIZER AND DIFFERENT SOIL CONDITIONER TO YIELD AND YIELD COMPONENTS IN CHICKPEA CULTIVARS**
Zekeriya KARA, Cengiz YÜRÜRDURMAZ, Alihan ÇOKKIZGIN.....224-232
- 5. EFFECTS OF DIFFERENT MIXTURE RATIOS OF COMMON VETCH AND TRITICALE ON FORAGE YIELD AND SILAGE QUALITY**
Mehmet ÖTEN, Mertcan KOÇ, Alperen GÜMÜŞ.....233-239
- 6. FRUIT QUALITY AND BIOCHEMICAL CHARACTERISTICS OF DIFFERENT STRAWBERRY TREE (*Arbutus unedo L.*) GENOTYPES GROWN IN GİRESUN**
Serap ÇAKMAK, Muharrem YILMAZ.....240-248
- 7. THE EFFECT OF TREATED WASTEWATER LEVELS ON THE ROOTING OF BLACKBERRY (*Rubus fruticosus L.*) GREEN CUTTINGS**
Ayşegül ÇAKMAKCI, Merve KARAKOYUN, Sinem ÖZTÜRK ERDEM.....249-256
- 8. EVALUATION OF AGRICULTURAL MECHANIZATION AS AN INDICATOR OF AGRICULTURAL DEVELOPMENT: A COMPARATIVE STUDY FOR TÜRKİYE AND EGYPT**
Hüseyin DURAN, Nashwa A. GHALY, Gürkan Alp Kağan GÜRDİL.....257-265
- 9. EFFECTS OF BREEDING PRACTICES ON REPRODUCTIVE EFFICIENCY OF EWES AND FATTENING PERFORMANCE OF LAMBS**
Hilal TOZLU ÇELİK, Mustafa OLFAZ, Tefvik DAĞ.....266-273
- 10. DETERMINATION OF ERGONOMIC FACTORS OF THE BACKPACK BLOWERS USED IN THE WINDROW OF HAZELNUTS**
Hüseyin SAUK, Kübra Meriç UĞURLUTEPE.....274-279
- 11. EFFECTS OF SALICYLIC ACID SEED PRIMING ON GERMINATION OF LENTIL (*Lens culinaris Medik.*) EXPOSED TO SALT STRESS**
Gizem KAMÇI TEKİN, Behiye Tuba BİÇER, Fatma BAŞDEMİR.....280-286
- 12. BOSCALID + PYRACLOTROBIN RESISTANCE AND EXPRESSION OF SUCCINATE DEHYDROGENASE GENES (*sdhA* and *sdhB*) IN CHESTNUT BLIGHT FUNGUS *Cryphonectria parasitica***
Ebru DERELLİ TÜFEKÇİ, Deniz ÇAKAR, Seçil AKILLI ŞİMŞEK.....287-297

13. ASSESSING ALUMINUM STRESS RESILIENCE IN COMMON BEAN ROOTS: PHENOTYPIC, HISTOCHEMICAL, AND PVGST/PVPOD GENE EXPRESSION ANALYSIS	
<i>Mehmet Zahit YEKEN</i>	298-304
14. COMPARISON OF NONLINEAR MODELS TO DESCRIBE THE GROWTH OF TUJ AND ROMANOV X TUJ (F1) LAMBS	
<i>Ülkü DAĞDELEN, Nurinisa ESENBUĞA</i>	305-309
15. EFFECTS OF DIFFERENT SALT STRESS AND TEMPERATURE APPLICATIONS ON GERMINATION IN MUNG BEAN (<i>Vigna Radiata</i> (L.) R. WILCZEK) GENOTYPES	
<i>Onur OKUMUS, Akife DALDA SEKERCI</i>	310-316
16. GLOBAL RESEARCH ON BLUETONGUE: A BIBLIOMETRIC ANALYSIS	
<i>Hakan SERİN, Muslu Kazım KÖREZ, Sinan SİREN</i>	317-326
17. THE ENVIRONMENTAL IMPACT ON ECONOMICALLY SIGNIFICANT TRAITS IN CENTRAL ANATOLIAN MERINO SHEEP	
<i>Yunus ARZIK, Sedat BEHREM, Mehmet KIZILASLAN, Simge TÜTENK DOĞDAŞ, Çağatay YILDIZ</i>	327-334



THE USAGE OF MARINE PLANT-BASED BIO-FERTILIZER FOR TOMATO GROWING IN MOGADISHU, SOMALIA

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Abstract: Seaweed and seagrass contain plant growth hormones and mineral nutrients such as proteins, lipids, amino acids, phytohormones, carbohydrates, antimicrobial compounds and osmoprotectants. Hence, the present work was undertaken to assess the potential of marine plant-based bio-fertilizer, prepared as a mixture of six seaweed species and three seagrass species, in comparison with goat manure fertilizer, mixed fertilizer, conventional and no-fertilizer (control) applications on the growth and yield of tomato (*Solanum lycopersicum*) under greenhouse conditions. The experiment was designed in a split plot with a randomized complete block design. Fifteen biological replications were used from each treatment for measuring plant height, number of fruits and fruit weight. In this study, treatment of conventional, marine plant bio-fertilizer, goat manure, mixed fertilizer and control was applied. This study revealed that tomato plants supplied with a mix of traditional fertilizers had the highest plant height (178 cm) and the highest number of fruits (150 fruits/plant), while the plants treated with marine plant bio-fertilizer produced fruits of the highest weight (3132 grams/15 fruits). This is the first study on the utility of marine plant as bio-stimulants for agricultural production in Somalia. The application of eco-friendly and user-friendly marine bio-fertilizers can be suggested to farmers in getting higher yields and better growth of tomato plants.


Keywords: Traditional fertilizers, Seaweed, Seagrass, Tomato, Marine, Bio-fertilizer


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1. Introduction

Marine plant bio-fertilizers have been traditionally utilized in stimulating agricultural production since ancient times (Zafar et al., 2022). Seaweeds have been traditionally used as bio-fertilizers in farming practices in the Roman Empire, China, France, Japan, Spain, Britain, and others. Seaweeds are reported to be a potential source of plant-growth-promoting hormones and nutrients necessary for crop plant growth. Additionally, the seaweeds comprise various chemical constituents such as carbohydrates, proteins, amino acids, lipids, and antimicrobial compounds (Jayasinghe et al., 2016; Nabti et al., 2017; Vinoth et al., 2017; Pramanick et al., 2017; Raghunandan et al., 2019). Today, there is an increasing need for eco-friendly agricultural output of healthy and quality food to feed the rising population of the globe. In this context, marine plants possess many benefits for crop production, including enhancement of seed germination, improvement of growth and health, enhanced absorption of water and nutrients, stress tolerance to saline and frost, bioremediation of pollutants, and resistance to phytopathogens (Nabti et al., 2017; Raghunandan et al., 2019).

Seaweed and seagrass extracts are available as fertilizers in the forms of powder, Seaweed Liquid Fertilizers (SLF), and Seagrass liquid fertilizer (SGLF) (Patel et al., 2017; Dineshkumar et al., 2018; Sheeja, 2019; Dineshkumar et al., 2019a). Seaweed extracts are efficient for improving soil fertility and the quality of crop yield (El-Din, 2015; Patel et al., 2017). The effectiveness of the SLF is experimentally evaluated at various concentrations as 1%, 0.8%, 0.6%, 0.4% and 0.2%, applied to tomato plants by foliar spray, seed and soil treatments and recorded the highest yield and growth of the tomato at 0.6% concentration (Sasikala et al., 2016). The combined SLF and SGLF are reported to be highly effective on seed germination at 10% concentration compared to SGLF and SLF used individually (Sheeja, 2019). Recently, several studies showed that seaweed extracts enhance seed germination, plant nutrient uptake, and shelf life of horticultural products (El-Din, 2015; Ghaderiardakani et al., 2019; Ahmed et al., 2021). Nevertheless, a higher concentration of SLF may inhibit growth and yield, as reported in tomato, wheat and eggplants (El-Din, 2015; Ramya et al., 2015; Sasikala et al., 2016).



The application of 0.5 g of seaweed biomass is reported to increase plant growth rates, rooting ability, 100% survival rates, higher number of leaves, longer shoots, higher stem thickness and total chlorophyll content. Additionally, the seaweed increases the levels of total phosphorus, nitrogen and potassium in the treated soil as compared to chemical fertilizer (Saadaoui et al., 2019). The combination of seaweed extract and chemical fertilizer was also examined. *Kappaphycus* extract at 7.5% along with 100% of the recommended dose of chemical fertilizers, are reported to improve the growth, yield and quality of potatoes (Pramanick et al., 2017). Moreover, the efficacy of the organic palm leaf, seaweed extract, rice residuals and conventional was evaluated on the growth of Broccoli (*Brassica oleracea var. italica L.*) and recorded higher growth rates, enhanced head produce and good quality at six ml/l of seaweed extract and rice residual (Manea and Abbas, 2017).

Seaweeds are reported to be a potential source of polysaccharides with a good effect on plant biomass, chlorophyll content and seed germination (Chbani et al., 2015; Dineshkumar et al., 2017; Mzibra et al., 2018). *Turbinaria murayana* (TM) was proven to be a bio-stimulant in tomato plants. The TM extract at 4% considerably increased vegetative growth, plant height, shoot branches, and leaf number (Sunarpi et al., 2020).

The lady's finger (*Abelmoschus esculentus*) seeds were treated with seagrass extract in the form of foliar spray (FS) and soil drench (SD), recorded increased pods and number of flowers by FS, whereas enhanced weight and length of pods by SD, and suggested the marine plants as an alternate source of bio-stimulants for organic farming (Muniswami et al., 2021). Foliar sprays of seaweed mixtures are known to substantially increase growth and biochemical parameters in *Ocimum sanctum* when treated with a combination of *Turbinaria ornata*, *Sargassum wightii* and *Caulerpa racemosa* (Uthirapandi et al., 2018). The treatment of seaweed mixture was also proven to reduce heavy metal pollutants. The application of a combination of *Ulva fasciata* and *Sargassum lacerifolium* appeared to decrease the levels of Cu, Pb, Ni and Zn to their normal rates in the soil samples and to reduce the levels of Cr, Cd, Mn and Fe to tolerable limits (Ahmed et al., 2021). To sum up, there has never been any previous research done on the application of marine plant bio-fertilizer in agricultural production in Somalia. Hence, the present work was undertaken to investigate the potential of marine plant-based bio-fertilizer, in comparison with organic and inorganic fertilizers for the growth and yield of tomato.

2. Material and Methods

This experiment was performed at the greenhouse of the Department of Agriculture, City University of Mogadishu, Somalia. Before the beginning of the trial, soil samples were randomly collected and analyzed at the Filson Somali Labs to identify soil chemical and physical properties (Table 1).

Table 1. Physical and chemical features of the soil of the experimental site

Parameter	Unit	Value
pH	-	6.87
Electrical conductivity	ds.m ⁻¹	0.25
Total N	mg.kg ⁻¹	120
Exchangeable K	mg.kg ⁻¹	80
Available P	mg.kg ⁻¹	0.08
Available S	mg.kg ⁻¹	0.05
Organic matter	mg.kg ⁻¹	0.96
Soil texture:		
Clay	%	57.1
Sand		14.3
Silt		28.6
Calcium as Ca(OH) ₂	g/kg of soil	15.35

Note that pH refers potential of hydrogen, N refers to nitrogen, K refers to kalium, the Mediaeval Latin word for potassium, P refers to phosphorus, and S refers to sulfur.

The crop plant selected for this experiment was tomato (*Solanum lycopersicum*). Seeds in uniform size, weight and colour were used for raising seedlings in trays with dimensions of 28 × 53 cm and 200 cells containing coco peat. The seedlings were applied NPK fertilizers and watered daily with 1 litre during their growing period. The trays were kept in the greenhouse, maintained with a temperature of 29-38 °C, relative humidity of 60 to 40 %, and a photoperiod of 12 hours. The greenhouse had a total area of 320 square meters with 40 m length and 8 m width, and there were six rows of 39 m in length and 30 cm in width each. Each row had two irrigation lines with 60 cm between plants and spaced 30 cm between irrigation lines. When seedlings were 8–12 cm in height, they were transplanted at a distance of 60 cm between plants, and there were 120 plants in each row. The seaweeds used in this study were *Sargassum sp.*, *Ulva lactuca*, *Codium fragile*, *Asparagopsis sp.*, *Corallina sp.* and *Chondrus sp.* (Table 2).

Table 2. The different types of seaweeds/seagrasses available in Lido area, Mogadishu, Somalia

Scientific Name	Common Name	Local Name
Seaweeds		
<i>Sargassum sp.</i>	Japanes Wireweed	Teli
<i>Ulva lactuca</i>	Sea Lettuce	Cowsbaded
<i>Codium fragile</i>	Dead man's Fingers	Cowska Badda
<i>Asparagopsis sp.</i>	Red seaweed	Cowsbadeed
<i>Corallina sp.</i>	Red alga	Cowsbadeed
<i>Chondrus sp.</i>	Irish Man	Cowsbadeed
Seagrasses		
<i>Enhalus sp.</i>	Eelgrass	Teli
<i>Zostera Marina</i>	Common eelgrass	Teli
<i>Syringodium sp.</i>	Noodle seagrass	Teli

They were freshly collected from the coastal area of the Indian Ocean, Lido (2° 2' 48.9624" N-45° 19' 5.3796" E) in Mogadishu, Somalia. The seagrasses used in this study were *Enhalus*, *Zostera Marina* and *Syringodium*, collected

from the same coastal area. The plant samples were collected during low tide, washed thoroughly to remove sand particles and epiphytes, and transported in bags to the laboratory.

The marine plant samples were dried at room temperature. After drying, they were mixed and allowed to decompose in a pit with a length of 2.3 m, width of 1.7 meters and depth of 1 m. After three months, the decomposed marine plants were removed from the pit. Five of the six rows of soil beds in the greenhouse were used for fertilizer treatment in the amount of 14 kg per 0.08 decares as a soil drench, and the sixth row was maintained as control without any treatment. Each row was applied with one of these five fertilizer treatments: decomposed marine plant bio-fertilizer, goat manure, DAP fertilizers (conventional), and a mixture of fertilizers. The last row was used as a control. 15 plants were selected from each row for analysis. The trial was designed in a split plot with a randomized complete block design. Fifteen biological replications were used from each row for measuring plant height, number of fruits and fruit weight. The data collection continued for 17 weeks.

3. Results

Growth performance was assessed for the number of fruits, fruit weight and plant height of tomatoes under the treatment of marine plant bio-fertilizer, goat manure, chemical fertilizer, mixed fertilizer, and control (Tables 3-5). The growth performance was analyzed under five developmental stages (31, 62, 92, 123, 153) for five months.

3.1. Plant Height

The plant height steadily increased under the five growth stages in four fertilizer treatments (Table 3). The plant height was recorded to be the highest for conventional, followed by marine plant bio-fertilizer, mixed fertilizer and goat manure, with an increase of 19.9%, 13.4, 7.5% and 1% over control, respectively.

3.2. Number of Fruits Harvested

The fruit yield was analyzed every 30 days on 92 days, 123 days and 153 days of plant growth (Table 4). A total of 571 fruits were collected from the experimental and control plants.

Table 3. Impact of basal fertilizer application of marine plant bio-fertilizer, goat manure, mixed fertilizer, traditional fertilizer (DAP) and control on growth parameters (plant height (cm)) of tomato plant (*Solanum lycopersicum*)

Fertilizer Treatment	Plant Height (cm) under different days of growth				
	31	62	92	123	153
Marine plant	26.09	86.7	132.0	173.0	178.0
Goat manure	21.4	71.0	115.3	151.0	158.5
Mixed fertilizer	22.0	94.1	115.5	163.6	168.7
Conventional	31.6	114.7	139.3	174.6	188.3
Control	20.5	74	115.5	151	157

Table 4. Impact of basal fertilizer applications of marine plant bio-fertilizer, goat manure, mixed fertilizer and traditional fertilizer (DAP) on growth parameters (fruit number) of tomato plant (*Solanum lycopersicum*)

Fertilizer treatment	Number of fruits harvested			
	92	123	153	Total
Marine plant	81	39	14	134
Goat manure	44	16	17	77
Mixed fertilizer	77	44	14	135
Conventional	99	39	12	150
Control	36	28	11	75

Table 5. Impact of basal fertilizer applications of marine plant bio-fertilizer, goat manure, mixed fertilizer, Traditional fertilizer (DAP) and control on growth parameters (fruit weight) of tomato plant (*Solanum lycopersicum*)

Fertilizer treatment	Fruit weight (gram)		
	92 DAT	123 DAT	153 DAT
Marine plant	3132	1450	579.1
Goat manure	1825	525.5	717.9
Mixed fertilizer	2482	1086.5	471.9
Conventional	3210	1337.4	421.3
Control	1255	836	258.6

The total number of fruits harvested was recorded to be the highest for conventional, followed by mixed fertilizer, marine plant bio-fertilizer and goat manure, with an increase of 100%, 80%, 79% and 3% over control, respectively. The total harvest of fruits was 337, 166 and 68 on 93, 123, 153 days of growth, respectively. Thus, the yield reduced with the increment of plant growth.

3.3. Fruit Weight

The fruit weight was analyzed every 30 days from 92, 123 and 153 days of plant growth (Table 5). The average fruit weight was the highest for marine plant bio-fertilizer, followed by conventional, mixed fertilizer and goat manure, with increases of 120%, 112%, 72% and 31% over control, respectively. The total harvest of fruit weight was 2381, 1047, and 490 g on 93, 123 and 153 days of growth, respectively. Therefore, the fruit weight is reduced with the increase of plant growth.

4. Discussion

In general, agricultural soil is affected by indiscriminate application of chemicals such as pesticides and synthetic fertilizers, and intensive cropping practice reduces soil nutrients. However, there is an upsurge in practicing organic farming without using inorganic fertilizers. Hence, seaweeds are cut into pieces and boiled with 1 Liter of distilled water for 1 h. After cooling, the extract is filtered through Whatman No: 1 Paper and stored at 4 °C for future analysis (Vijayakumar et al. 2018). The filtrate is considered a 100% concentration of the seaweed liquid fertilizer (SLF) to boost the growth of crops, seed germination, number of leaves, shoot length, root length and number of lateral roots (Zodape et al., 2010; Haider et al., 2012; Singh et al., 2016). The seaweed extracts are reported for better seed germination and growth performance in green gram (Ashok-Kumar et al., 2012), tomato (Sasikala et al., 2016) and black gram (Kalaivanan and Venkatesalu, 2012). A recent study has successfully attempted to use the extract of *Gracilaria sap* and *Kappaphycus alvarezii* with or without a conventional (Pramanick et al., 2014). The present study observed a similar effect in tomato plants. The marine plant-based bio-fertilizer increased tomato fruit by 120%, the number of fruits by 79% and plant height by 13% over plants without fertilizer treatment (Tables 3-5). This finding is similar to an earlier report that seaweed extract induces tomato plants to produce fruits of larger size (Hussain et al., 2021).

The seaweeds are rich in macro-nutrients (Ca, K, P), particularly *Caulerpa sertifoloides*; *Caulerpa cf. brachypus*; *Undaria pinnatifida* and *Ulva lactuca* (van Ginneken and de Vries, 2018). Liquid seaweed fertilizers contain potassium, an essential element for meristematic growth, translocation of photosynthesis, and disease resistance. Seaweed extracts have a role in activating enzymes, elongating cells, and stabilizing cell structures. Plant hormones known to induce physiological responses, are also found in seaweed extracts (Pramanick et al., 2013). Moreover, brown and green

seaweed extracts are known to contain several betaines and betaine-like elements (MacKinnon et al., 2010). Betaines contain an osmolyte at higher concentrations, which induces stress tolerance to drought and salinity. They also serve as a nitrogen source at lower concentrations (Zecher et al., 2020).

Liquid seaweed fertilizers play a role to control the water status of plants by stomatal regulation and photosynthesis-related processes. The seaweed-extract-treated plants were reported to have more flowers than control plants (Dookie et al., 2021). The number of flowers and their maturity determine the crop yield. The flowering is influenced by phytohormones such as cytokinins (Bartrina et al., 2017), auxins (Ke et al., 2018), gibberellins (Moeller et al., 2013) and ethylene (Iqbal et al., 2017) and also the physiological age of plants determines initiation and development of flowers. The phytohormones found in seaweed extracts are reported to promote flowering by stimulating more rapid plant growth (Pramanick et al., 2013). Early flowering produces early fruiting and ripening, which usually bring better market prices for the farmers.

The micro-algal fertilizers play a role in releasing crucial chemicals needed for producing high-quality fruits and preventing leaching and excessive nutrient losses. The plants provided with micro-algal bio-fertilizers lead to enhanced fruit quality attributes due to the abundance of nutrients at the top of the plant when compared with the use of conventional (Dineshkumar et al., 2018; Dineshkumar et al., 2019b; Dineshkumar et al., 2020a; Dineshkumar et al., 2020b). The micro-algal extracts were used as a foliar spray (Supraja et al., 2020) and dry biomass as a soil drench (Dineshkumar et al., 2018) for energizing plant growth. Application of *Chlorella vulgaris* as foliar spray, soil drench and mixture of *C. vulgaris* bio-fertilizer + cow dung treatments have shown to improve soil quality, plant growth and greater yield of maize, paddy, black gram and onion (Dineshkumar et al., 2017; Dineshkumar et al., 2018). Furthermore, application of a mixture of *C. vulgaris* and Cow dung has increased the release of plant nutrients and their availability for improving yield attributes. The positive effect of *C. vulgaris* bio-fertilizer on the chemical composition and quality of tomato fruits was also reported (Suchithra et al., 2021). Similarly, tomato plants treated with dried *Acutodesmus dimorphus* biomass showed to improve the number of flowers and shoot branches of tomatoes (Garcia-Gonzalez and Sommerfeld, 2016). In addition to growth, the microalgal and organic fertilizer treatments influence the chemical constituents of tomatoes (Pangaribuan and Monica, 2016). Thus, the beneficial impacts of algal extracts are agronomically substantial for incrementing tomato fruit quality and output. This is in agreement with the present results and other published papers.

5. Conclusion

Seagrass and seaweed contain phytohormones and macro- and micronutrients required for plant growth. In this study, conventional treatment showed the highest increment in the number of fruits and plant height, whereas marine plant-based bio-fertilizers exhibited the highest fruit weight. The existence of inorganic elements in seaweed and seagrass fertilizers makes them a potential choice for organic fertilizers. The areas where conventional are not abundantly available, seaweed/seagrass bio-fertilizers might give an eco-friendly alternative to plant nutrient management. The use of marine plant-based bio-fertilizer is cheaper and cost-effective for agricultural applications due to the abundant availability of seaweeds and seagrasses along the coastal areas of Somalia. The application of eco-friendly and user-friendly marine bio-fertilizers can be suggested to farmers in getting higher yields and better growth of tomato plants. Future studies are required on using seaweed/seagrass liquid extracts as soil drenches and foliar spray.

Author Contributions

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

	M.M.S.	M.H.A.	M.A.	M.A.K.	A.S.
C	20	20	20	20	20
D	20	20	20	20	20
S	20	20	20	20	20
DCP	20	20	20	20	20
DAI	20	20	20	20	20
L	20	20	20	20	20
W	20	20	20	20	20
CR	20	20	20	20	20
SR	20	20	20	20	20
PM	20	20	20	20	20

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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FORECASTING SEASONAL MILK PRODUCTION USING MARS ALGORITHM FOR MULTIPLE CONTINUOUS RESPONSES IN HOLSTEIN DAIRY CATTLES

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Abstract: In this study, seasonal milk yield estimation will be made using multivariate adaptive regression spline (MARS) algorithm for multiple continuous responses in dairy cattle (Holstein hybrid). For the research, milking records for the years 2020-2021 were collected from 157 dairy animals using Holstein hybrid dairy cattle from a research farm in Konya, Türkiye. The amount of feed given in this experiment was not changed and the effect of the season on the estimation of milk yield was investigated in the study. The analyzed independent variables used in the study were pregnancy status (PS), number of days milked (MDN), Lactation Number (LN), age of cows (months), average seven-day milk yield (7-Day Average Milk-SDMY), last lactation milk yield (last_MY), number of inseminations (IN), peak yield (Pik_Yield) and target variables were calculated as $(Yield_{Autumn/winter/spring/summer} \text{ (kg)}) = \text{Mean milk mean of season}$. In this context, the ehaGoF package was used to measure the prediction performance of the simultaneous MARS model established with the earth package for MARS analysis. MARS estimation equations obtained simultaneously for four dependent variables (multiple responses) are given. By looking at the MARS equation, the MARS model estimation equation was determined for the optimum milk yield, the threshold values, the three threshold values determined in the model were determined as MDN, Age, Peak_Yield, and the corresponding values were respectively; 159 days, 39.6 (months) and 37.1 kg/day. Considering the estimation equation, it is seen that the independent variables MDN, SDMY and LN are the most important variables in determining the estimation equation. It is seen that the best fitting value for the estimation equation of the dependent variables is the $Yield_{Winter}$ variable.


Keywords: MARS, Multiple response, Dairy cattle, Milk yield

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1. Introduction

As a hybrid race adopted for the conditions of Türkiye, Holstein has a milk yield of 6000-9000 kg (Torshizi, 2016). Many different factors such as the physiological status of this breed (age, number of lactations, number of days in lactation) can affect milk yield (Boğa et al., 2020; Omar, 2022). However, important environmental factors that do not have a genetic effect on milk yield are lactation period, calving age, calving season and calving stage (Torshizi, 2016). In addition, the effects of environmental factors such as calving year, calving interval, calving season, number of births, herd and milking frequency on milk yield should be investigated (Javed et al., 2007; Eydurun et al., 2013).

Therefore, to study these relationships, machine learning methodologies from traditional statistical methods have been increasingly adopted. In light of this, researchers commonly use machine learning approaches to improve predictive efficiency (Nayana et al., 2022). These

algorithms develop by distinguishing and defining the consistency of educational knowledge patterns that may apply to complex nonlinear datasets between yield and parameters. In recent years, many machine learning approaches have been used in the literature on predictive modeling in agriculture and animal data (Küçükönder et al., 2014; Küçükönder et al., 2015). Investigated the effect of the number and duration of lactation of Holstein breed of cows on milk yield with the artificial neural network (ANN) method. In their study, it was determined that this model created with the artificial neural network converged well with the real values and that the performance in milk yield estimation could give more successful results by increasing the number of parameters. Similarly, in the study of Boğa et al. (2020), the effect of the number of lactations, lactation days, first calving and reproductive age, and the number of inseminations (ratio) on cattle milk yield (mean last seven days) was determined. They evaluated the data on the use of a deep neural network in dairy cattle farms



and suggested that additional controlled management was needed in livestock and that the errors of the farm should be corrected. Akin et al. (2020a), applied MARS algorithm in agricultural applications. Altay et al. (2022) found that it would benefit herd management by closing the gaps in mastitis diagnosis with the development of data mining methods. Therefore, they stated that the application of CART and MARS algorithms could be a good choice for cattle breeders to find the threshold values of effective milk characteristics that accurately distinguish healthy and unhealthy cows. Nayana et al. (2022) compared wheat yield estimation for India and the most wheat-producing countries using MARS after extracting the main characteristics with Principal Component Analysis (PCA), considering parameters such as cultivated area and production for 1962-2018. Çanga (2022) developed MARS prediction models with first-order interaction effects using the MARS algorithm to predict carcass yield. The carcass weight of cattle of various breeds was determined using a MARS Data Mining Algorithm based on training and test sets. Akin et al. (2020b) used MARS statistical approach to predicting macronutrient-related growth responses of three strawberry species. Çelik and Yilmaz (2021) investigated the effects of silage type, silage consumption, and birth type and birth weight on body weight after fattening in curly lambs using MARS and Bagging MARS algorithms. Tyasi et al. (2021) conducted the body weight estimation of the Hy-Line Silver Brown Commercial Layer chicken breed using MARS. In this research, estimation equations were created to examine seasonal milk yield estimation, performance was evaluated with error analyzes such as RMSE, ME, Rsq, and the most suitable MARS model was selected using cross-validation and user-defined parameter optimization. Therefore, this study aims to develop prediction models that best predict milk production using the MARS algorithm.

2. Material and Methods

2.1. Data Identification

The experimental data set was taken from a private farm in Türkiye with Holstein hybrid dairy cattle used with an automatic milking system in a private farm in Konya province. For a period of twelve months, milking records obtained from 157 milking animals for 2020-2021 were collected. It was performed with daily milking number and collective (by placing corn silage, grass silage, wheat straw, soybean meal) or individual (pellet feed distributed through automatic feeder) consumption. Each cow is milked twice a day by the automatic milking system. In this experiment, the amount of feed given was not changed and the effect of the season on milk production estimation was investigated in the study. The milking dairy cattle used in the experiment were Holstein, and group fattening was done. Only all milking animals were given 3 kg/day extra milking milk feed during milking. During each animal group feeding, wheat straw: 0.5 kg/day, alfalfa hay: 6.5 kg/day, corn silage: 15 kg/day, dairy feed: 5.5 kg/day, corn flake: 2 kg/day, cottonseed meal per animal: 1.5 kg/day, soybean meal: 1kg/day, barley paste: 2.5 kg/day, premix: 0.05 kg/day, calcid: 0.05 kg/day. On average, 58% roughage and 42% concentrate feed are mixed and given to the animals daily as a total mixed ration.

The independent variables used in the study (Table 1), pregnancy status (PS), number of days milked (MDN), LN Lactation Number (LN), age of cows (months), daily mean milk yield (7 Day Mean Milk-SDMY), last lactation milk yield (last_MY), the number of insemination (IN), peak yield (Pik_Yield), insemination number (IN); dependent variables were formed as $Yield_{Autumn/winter/spring/summer}$ (Kg) = Mean milk mean of season. The study was conducted by MARS to build and train the most suitable model for the 4 dependent variables.

Table 1. Descriptive statistics of variables to be used in modeling

	N	Minimum	Maximum	Mean	Std. dev
MDN	157	9	998	170.40	151.74
LN	157	1	6	2.38	1.34
Age(month)	157	26	111	50.55	19.04
SDMY (kg)	157	10	46	26.51	8.29
Pik_Yield (kg)	157	16	48	32.17	6.15
last_MY(kg)	157	3155.00	9450.00	6444.76	1223.99
IN	157	0	9	1.80	2.06
YieldAutumn (kg)	157	45	983	422.07	284.56
Yieldwinter (kg)	157	58	1116	606.61	240.50
Yield spring (kg)	157	510	1184	504.73	354.26
Yieldsummer (kg)	157	42	879	321.32	267.38
				Frequency	
PS		0		120	
		1		37	

MDN= number of days milked, LN= lactation Number, Age= cow's age (month), PS= pregnancy status (1:Ppregnant; 0:Nonpregnant), SDMY= daily mean milk yield (7 day mean milk), Pik_Yield= peak yield of daily mean milk yield, last_MY= last lactation milk yield, IN= insemination number.

2.2. Multivariate Adaptive Regression Spline (MARS)

MARS algorithm used by Friedman (1999) to capture nonlinear relationships between predictors and response variable(s) is a powerful approach that does not require assumptions about functional relationships between dependent and input variables. The model that emerges as the weighted total basic function including the BFi (x) function is given by Equation 1 below (Akin et al., 2020a; Eydurán et al., 2020; Çanga and Boğa 2020; Çelik et al., 2021; Çanga 2022).

$$y = \sum_{i=1}^k a_i BFi(x) \tag{1}$$

Mars algorithm is formed by the linear breakdown of the basic function of BFi (x) with the following Equation 2a and 2b.

$$BF_1 = \max(0, x - t) \begin{cases} x - t, & x > t \\ 0, & x \leq t \end{cases} \tag{2a}$$

$$BF_2 = \max(0, t - x) \begin{cases} t - x, & x > t \\ 0, & x \leq t \end{cases} \tag{2b}$$

here, x is the variable range; t is the node. The linear combination of the basic functions obtained accordingly as in Equation 3:

$$Y_i = a_0 + a_1 BF_1 + a_2 BF_2 + \dots + a_k BF_k \tag{3}$$

and the estimation equation is obtained. Here Y_i is dependent variable, a_0 intercept, and a_1, \dots, a_k are coefficients of the related basic functions (Emamgolizadeh et al., 2015; Everingham and Sexton 2011, Çanga and Boga 2019; Akin et al., 2020a).

2.2.1. A MARS model application

“earth” and “chaGoF” packages were used for the MARS model in the study (Eydurán et al., 2019; Eydurán et al., 2020). With the earth package, the same basic functions are generated for MARS prediction models created simultaneously for more than one dependent variable. So MARS models produced with the “earth” package have different coefficients. To make this estimate, the Generalized cross-validation (GCV) method, a computational solution for linear models that provide an estimated exclusion cross-validation error metric, is used. According to the GCV criterion, MARS generalizes the model by eliminating the terms. GCV is given by Equation 4, a form of regulation that balances model complexity with the goodness of fit (Eydurán et al., 2019; Akin et al., 2020a; Akin et al., 2020b).

$$GCV = \sum_{i=1}^N \frac{(y_i - \hat{y})^2}{\left(1 + \frac{C}{N}\right)^2} \tag{4}$$

Here, $C = 1 + cd$, is the number of items in the N dataset; d is a degree of freedom; c is the basic function addition penalty. Y_i is an independent variable and \hat{Y} is an estimated value (Eydurán, 2020; Akin et al., 2020a).

2.2.2. Parameter tuning

The maximum degree of interaction and the number of

terms in the final state are two important setting parameters for the MARS model. These two values are set by the “prune” and “degree” caret application, respectively. The maximum number of terms of the pruned model is “prune”. The actual degree of interaction is calculated by “degree”. The prune can be calculated automatically by the user or by using an external resampling technique and the default pruning protocol uses GCV. In addition, the earth package helps to evaluate possible interactions between the functions of various functions by reducing the number of nodes. To find the best hyperparameter combination, we use cross-validation (for k = 10 times) using the “caret” function. In the last case, the closest CVRSq to RSq is obtained using the FOR loop created in the R package program. In other words, with the FOR loop, the best CVRSq value, that is, the optimum CVRSq value was created out of 100 cycles. MARS algorithm has the advantage of using input variables that only increase the accuracy of the model and obtain an automated type of feature selection. This will be installed with the necessary parameters and run in each dataset using all features and the Spline model as a classifier. Each dataset gives the most appropriate feature subset rated based on its relative importance. Based on the highest overall accuracy, the smallest number of attributes collected, and the lowest false alarm error, the best optimal feature subset was selected (Eydurán et al., 2019; Akin et al., 2020a).

2.2.3. Model validity

The most common model fit criteria to be used in measuring the predictive accuracy of the MARS algorithm (Goodness of Fit Criteria) are the goodness of fit criteria such as R-square, RMSE and MAE mentioned below (Eydurán and Zaborski, 2017; Eydurán et al., 2019; Akin et al., 2020a; Çelik et al., 2021; Nayana et al., 2022). The model was evaluated according to these values.

1) Determination coefficient (R^2):

It is the percentage of the total variation in the response variable explained by the regression line. The Equation 5 is expressed by X.

$$R^2 = 1 - \frac{SSE}{SST} \tag{5}$$

where $SSE = (y_i - \hat{y})^2$ is the sum of the squares of the differences between the predicted and the observed value, and $SST = (y_i - \bar{y})^2$ is the sum of the squares of the differences between the observed and the overall average value?

2) Average square error (RMSE), average estimation error (is the square root of the average square error). The formula is stated as given in Equation 6:

$$RMSE = \sqrt{\sum_{i=1}^n (y_i - \hat{y})^2} \tag{6}$$

3) Average error (ME) is the average estimation error. It is less sensitive to outliers. It is given by the formula as in Equation 7:

$$ME = \frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}) \quad (7)$$

4) Mean absolute deviation (MAD) is the mean absolute estimate error. It is less sensitive to outliers. The formula is given as in Equation 8:

$$MAD = \frac{1}{n} \sum_{i=1}^n |y_i - \hat{y}| \quad (8)$$

5) Pearson correlation coefficient between actual values and estimated values in terms of a dependent variable (r) (Equation 9):

$$PC = r_{y_i \hat{y}} = \frac{Cov(y_i, \hat{y})}{S_{y_i} S_{\hat{y}}} \quad (9)$$

6) Akaike information criterion (AIC) (Equation 10a and 10b):

$$AIC = n \ln \left[\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y})^2 \right] + 2k; \text{ If } \frac{n}{k} > 40 \quad (10a)$$

$$AIC_C = n \ln \left[\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y})^2 \right] + 2k + \frac{2k(k+1)}{n-k-1}; \text{ otherwise;} \quad (10b)$$

Standard deviation ratio (SD_{ratio}):

S_m: Standard deviation of model error terms,

S_d: The standard deviation of the dependent variable,

Cov(y_i, ŷ): The covariance between actual and predicted values in terms of a dependent variable,

S_{y_i}: The standard deviation of the actual values of the dependent variable and

S_ŷ: It refers to the standard deviation of the mined values of the dependent variable.

3. Results and Discussion

The basic functions (BF_i) and coefficients of MARS prediction equations obtained simultaneously for four dependent variables (multiple responses) are presented in Table 2.

Table 2. Basic functions and corresponding coefficients of MARS model for the estimation of seasonal dependent variables

Terms	Coefficients (a _i)				Basis functions
	Yield _{Autumn}	Yield _{winter}	Yield _{Spring}	Yield _{summer}	
1	-284.15	1682.55	635.20	579.83	Intercept
2	16.16	-9.36	1.78	-4.02	max(0, MDN - 89)
3	5.74	-10.58	3.64	1.78	max(0, 159 - MDN)
4	-15.21	9.63	-0.11	11.18	max(0, MDN - 167)
5	-2.88	-1.23	7.72	-6.69	max(0, MDN - 276)
6	3.09	1.11	-7.90	-0.10	max(0, MDN - 360)
7	50.20	15.60	245.78	99.27	max(0, LN - 2)
8	-7.67	-3.11	-25.60	-19.12	max(0, Age - 39.6)
9	5.56	1.87	12.17	13.70	max(0, Age - 51.7)
10	-1.56	18.89	4.39	1.42	max(0, 35.8 - SDMY)
11	-14.53	-7.24	-13.52	-5.16	max(0, 37.11 - Pik_Yield)
12	15.02	9.43	22.59	25.94	max(0, Pik_Yield - 37.11)
13	-0.02	0.00	-0.10	-0.04	max(0, 7797 - last_MY)
14	-0.02	0.01	-0.05	-0.02	max(0, last_MY - 7797)
15	0.43	-0.04	4.03	1.57	max(0, Age - 39.6) * PS
16	0.16	0.24	-0.12	-0.05	max(0, 120 - MDN) × max(0, 35.8 - SDMY)
17	-0.05	-0.01	-0.10	-0.04	max(0, MDN - 120) × max(0, 35.8 - SDMY)
18	-0.15	0.03	-0.22	-0.20	max(0, 159 - MDN) × max(0, Pik_Yield - 32.71)
19	0.00	0.00	-1.3896e-0.5	0.00	max(0, 89 - MDN) × max(0, 7797 - last_MY)
20	0.00	-8.54	0.00	0.00	max(0, 159 - MDN) × max(0, last_MY - 7063)
21	0.00	0.00	0.00	0.00	max(0, 159 - MDN) × max(0, 7063 - last_MY)
22	2.21	-0.63	6.17	0.17	max(0, 3 - LN) × max(0, 35.8 - SDMY)
23	0.00	0.00	0.00	0.00	max(0, Age - 39.6) × max(0, 4580 - last_MY)

The model consists of 23 terms left between each node and 31 basic functions with four-way interaction. The three threshold values determined in the model for MDN, Age, Peak_Yield are 159day, 39.6 (month), and 37.11kg, respectively, and these values are the most common in the multi-response MARS model equation that constitutes the basic functions. These values are selected to simplify the multi-response MARS model by deleting the corresponding basis functions. As a result, this simplified MARS model is made up of marked coefficients and basic functions (Akin et al., 2020b).

For the MARS model estimation equation for optimum milk yield, the corresponding values for the three threshold values MDN, Age, Peak_Yield are 159d, 39.6 (month), and 37.1kg, respectively. Threshold values are the most common ones in the multi-response MARS model equation, which constitutes the basic functions, and the equations based on these values are deleted. Thus, it is selected to simplify the multi-response MARS model by deleting the relevant basic functions (Akin et al., 2020a; Akin et al., 2020b). As a result, the simplest form of the model is composed of coefficients and basic functions related to the MARS model simplified in this way. To further optimize the target responses and to ensure optimum milk yield for the tested dependent variables, the $Yield_{Autumn/winter/spring/summer}$ estimation equations was first defined by looking at the MARS equation in Table 3.

First of all, when the four prediction models are examined by looking at both Table 3 and the prediction equations, the sign differences regarding some coefficients of the same basic functions are remarkable (Akin et al., 2020b; Çelik and Yılmaz 2018; Çanga 2022; Çanga and Boğa, 2019). Since the threshold value for the lactation day, which is one of the variables discussed in Table 2, is LD=159, in the case of $\max(0, MDN - 89)$, there is an increase of 16.16 units and 1.78 units in $Yield_{Autumn}$ and $Yield_{Spring}$ milk yield, respectively; $Yield_{winter}$ and $Yield_{summer}$ milk yields decreased by 9.36 and 4.02 units, respectively. When the third term in the prediction model is examined, when $MDN > 159$, the effect of $Yield_{winter}$, $Yield_{Autumn}$, $Yield_{Spring}$, and $Yield_{Summer}$ on milk yield is masked. $MDN < 167$ the effect of the fourth, fifth and sixth term is masked. When $LN > 2$, a positive effect is seen in all dependent variables, while the biggest effect is $Yield_{Spring}$ with 245.78 units. When $AGE > 39.6$, the effect of all dependent variables is masked. If $MDN > 120$ and $SDMY < 35.8$, the interaction effect will be negative in all dependent variables, while if $MDN < 120$ or $SDMY \geq 35.8$, the interaction effect will be masked. Similar comments can be made about other situations (Emamgolizadeh,

2011; Çelik et al., 2021; Fatih et al., 2021; Faraz et al., 2021). Within the scope of the Response Surface Method, it can be suggested that there is the same directional relationship between the dependent variables discussed with the optimization logic in terms of the ease of interpretations to be made. Therefore, similar to this study, when the study on tissue culture conducted by Akin et al. (2020b) was examined, the results obtained from the optimal design response surface method for the optimization of three dependent variables with the same directional relationship between them were analyzed with MARS algorithm. While it is observed that the basic functions of MARS equations produced for the three dependent variables have the same marked coefficients, when the 3 estimation equations are examined, the sign differences regarding some coefficients of the same basic functions draw attention. This is because there is an inverse relationship between the two variables (due to the negative correlation).

From here, the estimation equation for the milk yield for the autumn is obtained as follows:

$$Yield_{Autumn} = 284.15 + 16.16 \times \max(0, MDN - 89) - 15.21 \times \max(0, MDN - 167) - 2.88 \times \max(0, MDN - 276) + 3.09 \times \max(0, MDN - 360) + 50.20 \times \max(0, LN - 2) + 5.56 \times \max(0, Age - 51.7) - 1.56 \times \max(0, 35.8 - SDMY) - 0.02 \times \max(0, 7797 - last_MMY) - 0.02 \times \max(0, last_MMY - 7797) + 0.16 \times \max(0, 120 - MDN) - 0.05 \times \max(0, MDN - 120) \times \max(0, 35.8 - SDMY) + 0.00 \times \max(0, 89 - MDN) \times \max(0, 7797 - last_MMY) + 2.21 \times \max(0, 3 - LN) \times \max(0, 35.8 - SDMY)$$

Finally, when we substituted the threshold values for maximizing responses, i.e. $MDN = 159$, $Age = 39.6$, $Peak_Yield = 37.11$, the corresponding basic functions were deleted according to the rules in equation 2. For example, according to equation 2a and equation 2b ($\max(0, MDN - 167)$), since $MDN = 159$, the basic functions of those terms are masked as equal to 0, and in this case, the model of the relevant dependent variable is deleted and simplified (Akin et al., 2020; Faraz et al., 2021).

Table 3. MARS model created after the elimination process

Terms	coefficients (a_i)				Basis functions
	$Yield_{Autumn}$	$Yield_{winter}$	$Yield_{Spring}$	$Yield_{summer}$	
1	-284.15	1682.55	635.20	579.83	Intercept
2	16.16	-9.36	1.78	-4.02	$\max(0, MDN - 89)$
4	-15.21	9.63	-0.11	11.18	$\max(0, MDN - 167)$
5	-2.88	-1.23	7.72	-6.69	$\max(0, MDN - 276)$
6	3.09	1.11	-7.90	-0.10	$\max(0, MDN - 360)$
7	50.20	15.60	245.78	99.27	$\max(0, MDN - 2)$
9	5.56	1.87	12.17	13.70	$\max(0, Age - 51.7)$
10	-1.56	18.89	4.39	1.42	$\max(0, 35.8 - SDMY)$
13	-0.02	0.00	-0.10	-0.04	$\max(0, 7797 - last_MY)$
14	-0.02	0.01	-0.05	-0.02	$\max(0, last_MY - 7797)$
16	0.16	0.24	-0.12	-0.05	$\max(0, 120 - MDN) \times \max(0, 35.8 - SDMY)$
17	-0.05	-0.01	-0.10	-0.04	$\max(0, MDN - 120) \times \max(0, 35.8 - SDMY)$
22	2.21	-0.63	6.17	0.17	$\max(0, 3 - MDN) \times \max(0, 35.8 - SDMY)$

The final models for Yield_{Autumn/winter/spring/summer} are obtained as given in Equation 11:

$$\begin{aligned}
 \text{Yield}_{\text{Autumn}} &= \begin{cases} 284.15 + 16.16 \times \max(0, \text{MDN} - 89) + 50.20 \times \max(0, \text{LN} - 2) - \\ 0.02 \times \max(0, 7797 - \text{last}_{\text{MY}}) - 0.02 \times \max(0, \text{last}_{\text{MY}} - 7797) - \\ 0.05 \times \max(0, \text{LD} - 120) \times \max(0, 35.8 - \text{SDMY}) + 2.21 \\ \max(0, 3 - \text{LN}) \times \max(0, 35.8 - \text{SDMY}) \end{cases} \\
 \text{Yield}_{\text{winter}} &= \begin{cases} 1682.55 + 9.36 \times \max(0, \text{MDN} - 89) - 15.60 \times \max(0, \text{LN} - 2) \\ + 18.89 \times \max(0, 35.8 - \text{SDMY}) - 0.01 \times \max(0, \text{last}_{\text{MY}} - 7797) \\ - 0.01 \times \max(0, \text{MDN} - 120) \times \max(0, 35.8 - \text{SDMY}) - 0.63 \\ \max(0, 3 - \text{LN}) \times \max(0, 35.8 - \text{SDMY}) \end{cases} \\
 \text{Yield}_{\text{spring}} &= \begin{cases} 635.20 + 1.78 \times \max(0, \text{MDN} - 89) + 245.78 \times \max(0, \text{LN} - 2) \\ + 4.39 \times \max(0, 35.8 - \text{SDMY}) - 0.10 \times \max(0, 7797 - \text{last}_{\text{MY}}) - \\ - 0.01 \times \max(0, \text{MDN} - 120) \times \max(0, 35.8 - \text{SDMY}) - 6.17 \\ \max(0, 3 - \text{LN}) \times \max(0, 35.8 - \text{SDMY}) \end{cases} \\
 \text{Yield}_{\text{summer}} &= \begin{cases} 579.83 - 4.02 \times \max(0, \text{MDN} - 89) + 99.27 \times \max(0, \text{LN} - 2) \\ + 1.42 \times \max(0, 35.8 - \text{SDMY}) - 0.04 \times \max(0, 7797 - \text{last}_{\text{MY}}) \\ - 0.02 \times \max(0, \text{last}_{\text{MY}} - 7797) - 0.05 \times \max(0, 120 - \text{MDN}) \\ \times \max(0, 35.8 - \text{SDMY}) - 0.04 \times \max(0, \text{MDN} - 120) \times \max(0, 35.8 \\ - \text{SDMY}) + 0.17 \times \max(0, 3 - \text{LN}) \times \max(0, 35.8 - \text{SDMY}) \end{cases}
 \end{aligned} \tag{11}$$

MARS optimizes all stages of model design and implementation, including variable selection, transforming predictive variables with a nonlinear relationship, determining interactions of predictive variables, and creating new nested variable strategies to deal with missing values and avoid overfitting with comprehensive self-tests (Akin et al., 2020a). After MARS analysis, the overall GRsq, CVRSq, and RSq values for all dependent variables were found to be quite high and very close to each other for optimum simultaneous MARS modeling (Table 3). When all dependent variables are examined, it is seen that the highest value among GCV, GRsq, Rsq, sd and CVRSq values belongs to Yield_{Winter} dependent variable. Some metrics for MARS prediction models for four dependent variables are summarized in Table 4.

Table 4. Summary performance of MARS prediction models of dependent variables

	GCV	GRsq	CVRSq
Yield _{Autumn}	0.0213	0.906	0.838
Yield _{Winter}	0.0055	0.944	0.954
Yield _{Spring}	0.0225	0.853	0.625
Yield _{Summer}	0.0171	0.878	0.644
All	0.0664	0.887	0.765

When the earth package has more than one (k) continuous dependent variable (multiple responses), k simultaneous prediction models are created. This package tries to minimize the sum of GCV values of k-dependent variables ($GCV_1 + GCV_2 + GCV_3 + \dots + GCV_k$) (Milborrow, 2019). $GCV(\text{Yield}_{\text{Spring}}) + GCV(\text{Yield}_{\text{Summer}})$ is based on the principle of being minimum. When the following outputs are examined, GCV total value is equal to $GCV_{\text{ALL}} = 0.0213 + 0.0055 + 0.0225 + 0.0171 = 0.0664$. Therefore, GRsq, Rsq, CVRSq values were found to be quite high and close to each other. Therefore, it can be said that the generalization ability of simultaneous MARS

modeling is very good, that is, there is no excessive adaptation problem (Akin et al., 2020a).

Since the R package in question tries to optimize all models simultaneously, the results of MARS analysis to be obtained for more than one dependent variable (the GRsq value calculated simultaneously for all dependent variables) will not be as good as MARS analyses to be obtained separately for each dependent variable (i.e. the GRsq values calculated for each of the dependent variables) (Milborrow, 2019).

The ehaGoF package was used to evaluate the predictive performance of the MARS model established for all dependent variables, and the results obtained are shown in Table 5 below.

It can be argued that MARS models established due to the very low values of RMSE, RRMSE, CV, RAE, MAD, MAPE and MRAE goodness of fit criteria have a very good fit. When the literature is examined, the fact that the standard deviation rate of a model is lower than 0.10 means that the predictive accuracy of that model is quite good (Grzesiak and Zaborski, 2012; Eyduran and Zaborski, 2017; Eyduran et al., 2019; Faraz et al., 2021). It has been reported that the standard deviation rate should be lower than 0.40 to say that an established regression model can have a good fit (Grzesiak and Zaborski, 2012). In this study, Yield_{Winter} MARS result with the best (0.17) value of Standard deviation rate (SDR) was observed. In other words, it can be said that the MARS model established for this value has a much better fit than other values. As here, it is seen that the mean of ME, that is, error terms, is theoretically zero, and this is the desired value.

The fact that the determination coefficient (Coefficient of Determination, Rsq) and the Adjusted Coefficient of Determination (AdjRsqu = 0.998) of the established MARS regression model are close to 1 means that the said model explains almost all of the total difference (variation) of the dependent variable. In the study, when

Table 5. The goodness of fit criteria MARS algorithms

Criteria	MARS Results			
	Yield _{Autum}	Yield _{Winter}	Yield _{Spring}	Yield _{summer}
1 Rootmeansquareerror (RMSE)	0.35	0.04	0.43	0.07
2 Relative root mean square error (RRMSE)	14.81	6.73	19.27	20.70
3 Standard deviation ratio (SDR)	0.22	0.17	0.28	0.25
4 Coefficient of variation(CV)	14.85	6.75	19.33	20.76
5 Pearson's correlation coefficients (PC)	0.98	0.99	0.96	0.97
6 Performance index (PI)	7.50	3.39	9.82	10.51
7 Mean error (ME)	0.00	0.00	0.00	0.00
8 Relative approximation error (RAE)	0.02	0.00	0.03	0.03
9 Mean relative approximation error (MRAE)	0.01	0.01	0.01	0.01
10 Mean absolute percentage error (MAPE)	0.21	0.61	0.31	0.32
11 Mean absolute deviation (MAD)	43.89	29.02	72.03	49.60
12 Coefficient of determination (Rsqr)	0.0-95	0.97	0.92	0.94
13 The adjusted coefficient of determination (ARsqr)	0.94	0.97	0.91	0.93
14 Akaike's information criterion (AIC)	1344.42	1210.78	1483.22	1363.93
15 Corrected Akaike's information criterion (CAIC)	1352.72	1219.08	1491.52	1372.73

Model Selection (all responses)

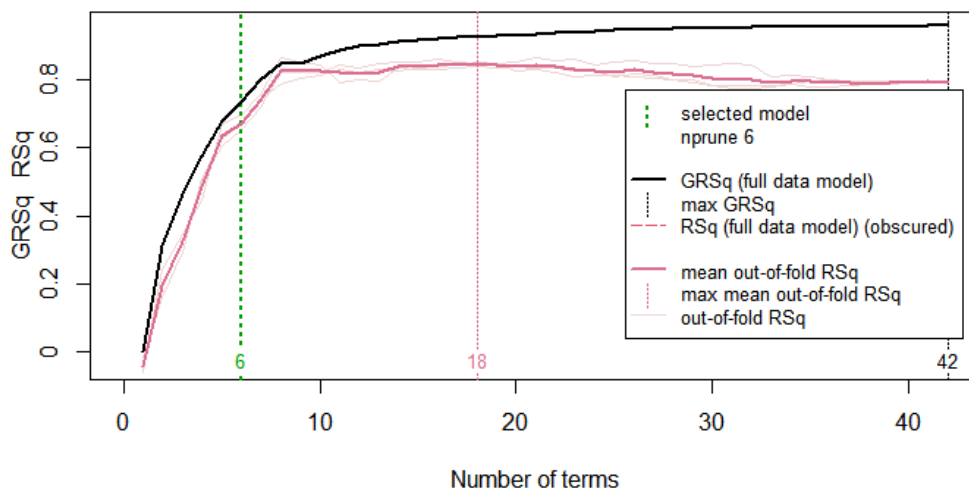


Figure 1. Selection graph of MARS model for all dependent variables.

Table 5 is examined, it is seen that the Rsq and AdjRs values of all dependent variables are very close to 1 and the highest value belongs to the dependent variable Yield_{Winter} (Rsq=0.971, AdjRsqr=0.966). Similar comments It is desirable that the adjusted coefficient of determination (Adjusted Coefficient of Determination, AdjRsqr =0.998) is close to the coefficient of determination (Rsq =0.999) (Akin et al., 2020a; Çelik et al., 2021).

The Pearson correlation coefficient (PC) between the observed and estimated values in terms of milk yield is desired to be very close to 1 for all dependent variables. When Table 5 was examined, it was determined that the Pearson correlation between the actual and predicted values was quite high and statistically significant in terms of the dependent variables examined. It is seen that the Yield_{Winter} value has the highest correlation (r=0.9869) (Çelik and Yılmaz, 2018; Akin et al., 2020a).

3.1. Graphical Representation of MARS Model

In the MARS model with four simultaneous continuous dependent variables, the black horizontal bold line represents the common GRSq value, the red horizontal dashed line represents the common Rsq value, the pale pink fine lines represent the corresponding Rsq value in each folk, and the horizontal thick pink line represents the common CVRsqr value, the dashed red vertical line represents the optimum number of terms corresponding to the common CVRsqr value in the peak, and the dashed fine dotted black vertical line (common for the four dependent variables) represents the point where the GRSq value is the maximum (Akin et al., 2020a). However, it can be stated that the dashed vertical dotted red line showing the point where the horizontal thick red line is maximum indicates the ideal number of terms determined by cross-validation. The earth pack accepts as the appropriate number of terms the number of terms

corresponding to the dashed vertical dotted black line, indicating the point where the GRSq value is maximum. In general, dashed vertical dotted red and black lines overlap. However, when Figure 1 is examined, it is seen that the MARS prediction model is established based on the GRSqvalue, that is, the GCV criterion has 6 terms for the MARS prediction model established based on cross validity for four dependent variables (Akin et al., 2020a). The Yield_{Spring} dependent variable is given as a graph showing the error value corresponding to each prediction value (Figure 2). In linear models, the constant variance assumption of errors is ideal. Therefore, it is desirable that the error values should spread evenly against the increase of the relevant estimation values and that the red straight horizontal line should be on the horizontal gray zero line. However, the fact that the

distribution of errors against the increasing Yield_{Spring} estimation values is in the form of a pipe around the zero point is proof of this assumption. However, it has been stated that the constant variance assumption, which is one of the most important assumptions of the linear model, is not important for MARS models (Milborrow, 2019; Akin et al., 2020a). Therefore, in the graph in Figure 2, it can be said that the constant variance is provided because the red horizontal straight line is almost above the zero horizontal line. This was confirmed to be in line with the results of the study by Akin et al (2020b). It is seen that observations 43, 67, and 135 for the Yield_{Spring} feature are outlier values that increase the error variance. Similar interpretations are made for other dependent variables.

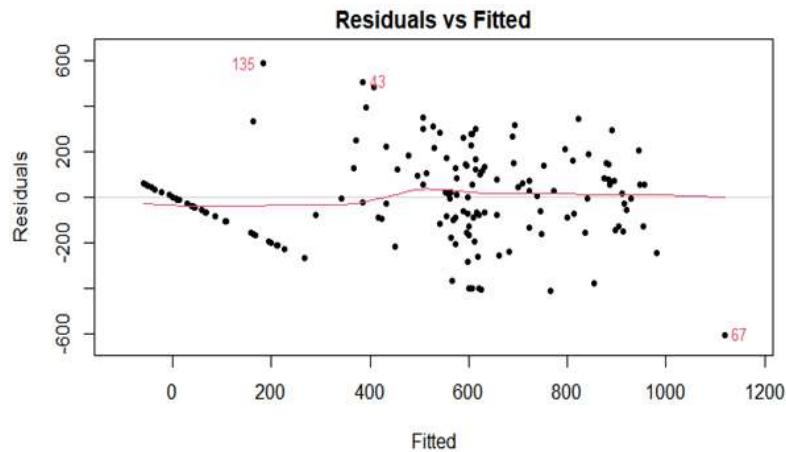


Figure 2. Distribution graph of errors according to the estimated Yield_{Spring} dependent variable.

Milborrow (2019) reported that the normal distribution of errors is generally not important for MARS models. The fact that the error values are almost above and around the cross line means that the errors show normal distribution. As can be seen from the graph in Figure 3, it can be said that observation values 43, 67 and 135 produce large error values (Figure 3). Similar to this research, Çanga (2022) compared the goodness-of-fit criteria of the training and test set model, reducing the bias by cross-validation. It represents the test data against the estimated graph obtained using the MARS model (Figure 4, 5, 6 and 7).

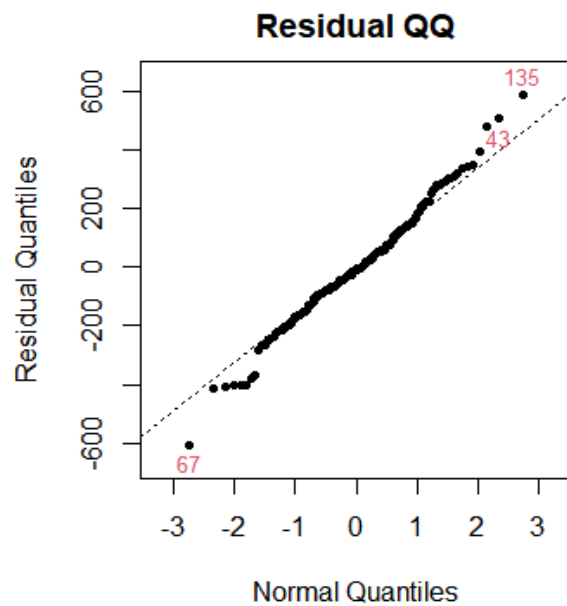


Figure 3. QQ graph of errors for Yield_{Spring} dependent variable.

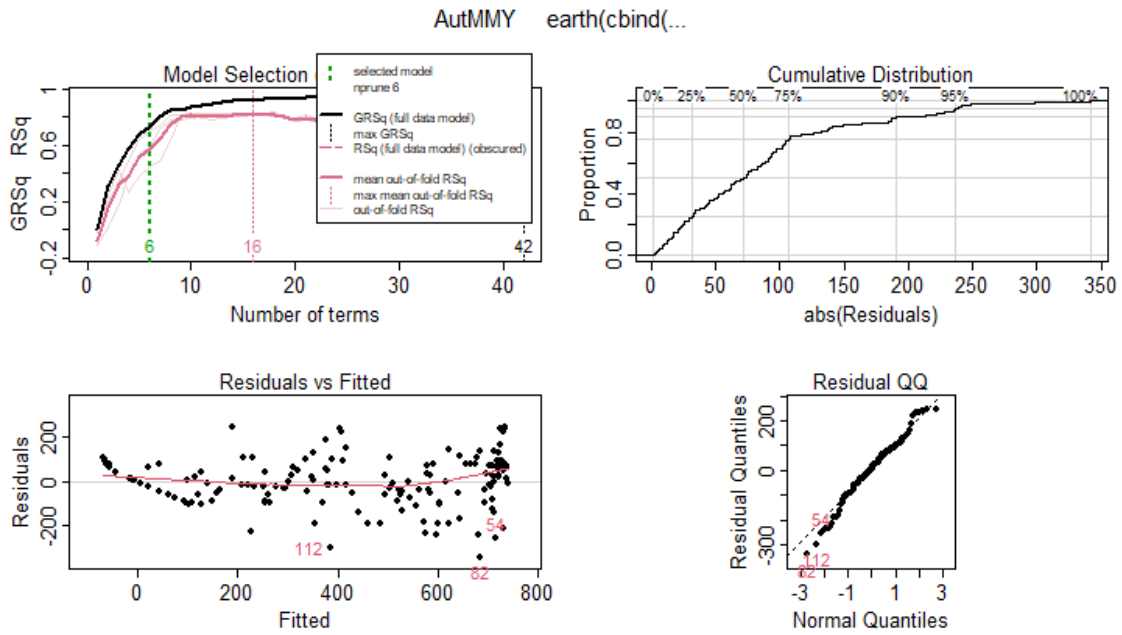


Figure 4. A graphical representation of the terms that make up the MARS model for Yield_{Autumn} estimation.

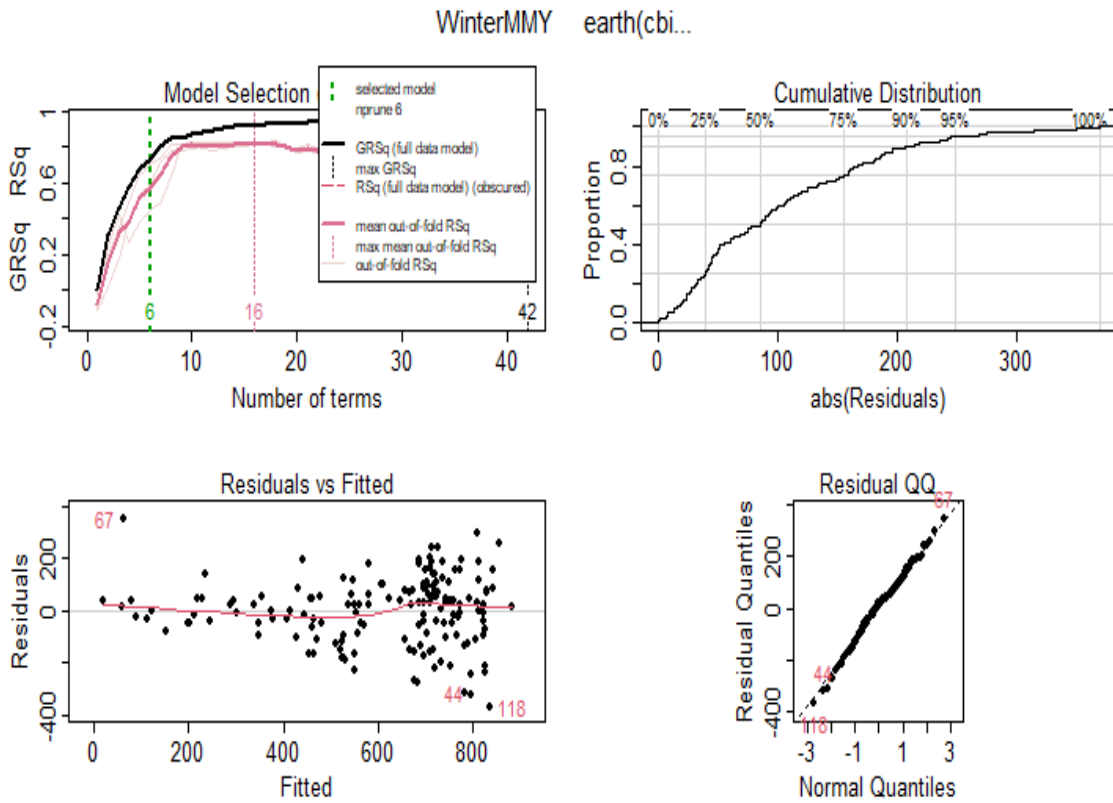


Figure 5. A graphical representation of the terms that make up the MARS model for Yield_{Winter} estimation.

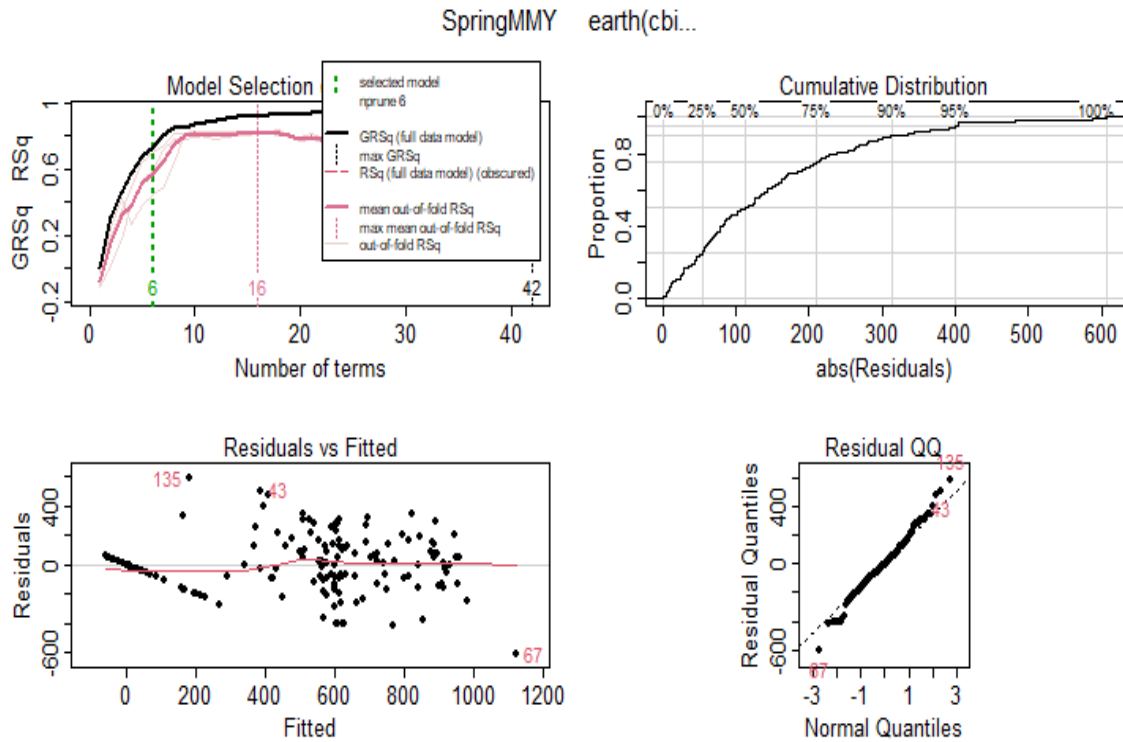


Figure 6. A graphical representation of the terms that make up the MARS model for Yield_{Spring} estimation.

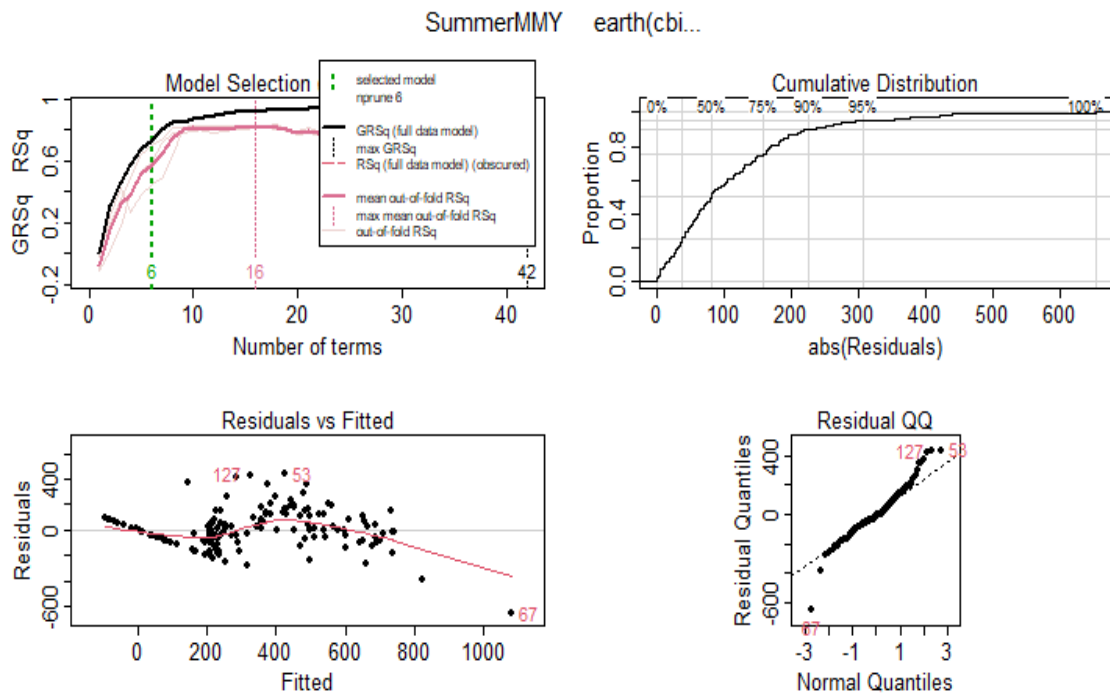


Figure 7. A graphical representation of the terms that make up the MARS model for Yield_{Autumn} estimation.

A more detailed interpretation can be made by giving a complete graphical holistic representation. In their study, Bayril and Yilmaz (2017) reported that the milk yields of animals differ in spring, summer, autumn, and winter and that milk yields are decreasing in summer and autumn. This situation is consistent with the results of the study. In parallel with this study, Novak et al. (2009) investigated the effects of hot months, lactation period,

and many lactations on milk yield. According to their findings, milk yields gradually increased from May to June and decreased in December. On the other hand, Bouallegue et al. (2013) reported that the milk yields of cattle were less in the summer months. Consistent with this study Vijayakumar et al. (2017) found that there was a relationship between the number of lactations, the number of days of lactation, the lactation phase-time, and

milk yield. Here, it is clear that the graphs that best fit among the four dependent variables are the Yield_{Winter} variable. In addition, when Table 5 was examined, it was observed that the Yield_{Winter} value had the highest correlation ($r=0.9869$) in terms of the dependent variables examined.

5. Conclusion

MARS algorithm is a method to advance a more understandable and easy prediction of agricultural and livestock. In the study, the direct effect of the parameters and complex interactions of the MARS model was clearly shown. It is seen that the best fit value for the estimation equation of four dependent variables is the Yield_{Winter} variable. The effect of the season on milk yield can be further investigated in this study by taking into account different parameters. Models with more and fewer features can be examined to determine the best-performing model. However, the results clearly show that machine learning models such as MARS perform more detailed and better than traditional statistical models when used in animal milk yield estimation.

It shows that the MARS model is suitable for Türkiye as a whole and for farmers producing milk to have information about the best yield periodically on the farm. Milk production estimation based on accepted statistics should be used to create short- and long-term plans to deal with future milk production, especially.

Author Contributions

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

	D.Ç.B.	M.Bo.	M.Bu.
C	90	10	
D	100		
S	100		
DCP		100	
DAI	90	10	
L	70	20	10
W	80	10	10
CR	70	30	
SR	75	5	20
PM	80	10	20

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. Livestock enterprises' own data was taken and there was no experiment conducted at the farm. Also, a signed consent form was also obtained from the owners

of the farms.

Acknowledgments

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A DETAILED EXAMINATION OF TÜRKİYE'S PROJECTED PRECIPITATION AND GROWTH SEASON TRENDS UNDER CLIMATE CHANGE CONDITION

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Abstract: This study presents a comprehensive analysis of Türkiye's changing precipitation patterns and growing season dynamics in the context of global warming, utilizing the Coupled Model Intercomparison Project Phase 5 (CMIP5) and various Representative Concentration Pathway (RCP) scenarios. In light of global warming, this study provides a thorough analysis of Türkiye's evolving precipitation patterns and growing season dynamics using multiple RCP scenarios and CMIP5. The research aims to fill a crucial gap in climate research by combining historical data and future projections to assess Türkiye's precipitation path under different greenhouse gas emission scenarios. The study employs linear regression for trend analysis and uses data from the Climate Change Knowledge Portal (CCKP), with a focus on precipitation data from 1986 to 2100. According to various RCP scenarios, this study's findings show a considerable variation in precipitation trends over the 21st century. The RCP 8.5 scenario predicts a significant decrease in precipitation, which would present difficulties for the management of water resources and agricultural productivity. In contrast, the least severe RCP 2.6 pathway shows a fairly stable pattern of precipitation. Complex seasonal hydrological responses to climate change are revealed by monthly precipitation analysis; RCP8.5 predicts an increase in the frequency of periods of drought and heavy precipitation events. The impact of these changes in precipitation on Türkiye's agricultural growing seasons was further investigated in this study. In high-emission scenarios, there was an initial tendency towards longer growing seasons, which were subsequently followed by shorter ones. This suggests that although global warming might initially result in an extended growing season, it might ultimately cause a reduction in it, particularly in situations where mitigation efforts are minimal. The need for adaptable strategies that can respond to long-term climate trends as well as seasonal variability was highlighted by this research. It draws attention to the fact that to mitigate the effects of climate variability, informed policy decisions and integrated resource management are essential. The results highlight the need for quick action to lower the risks associated with water and highlight the potential advantages of intensive mitigation efforts in stabilizing and extending growing seasons.

Keywords: Climate change projections, Hydrological variability, RCP scenarios analysis

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1. Introduction

Türkiye, which occupies a special geographical location, is experiencing notable climatic shifts attributed to global warming. Sen et al. (2012) and Turkes et al. (2020) have demonstrated through recent studies that there are increasing temperatures and altered precipitation patterns across the country. These changes have implications for various sectors, particularly agriculture and water resources. While these changes align with global trends, they manifest uniquely in Türkiye due to its diverse topography and climatic conditions.

The utilization of the Coupled Model Intercomparison Project Phase 5 (CMIP5) in this research is consistent with its significant contribution to the Fifth Assessment Report of "The Intergovernmental Panel on Climate Change" (IPCC). Türkiye's future climate scenarios can be understood and projected using the robust framework provided by CMIP5, renowned for its comprehensive

climate modeling capabilities (Taylor et al., 2012; Dimri et al., 2023). This model was chosen because of its widespread adoption and thorough verification within the climate science community.

For estimating future climatic conditions under various levels of greenhouse gas emissions, the use of Representative Concentration Pathway (RCP) scenarios is essential in this study (Jiang and O'Neill, 2017; O'Neill et al., 2020; Gurney et al., 2022). Each RCP scenario, from high-emission to optimistic trajectories, provides insights into potential future climates and their implications for Türkiye. Successful planning and implementation of climate adaptation and mitigation plans require this approach.

Conducting a comprehensive analysis of Türkiye's precipitation and growing season length trends is crucial in light of the anticipated changes in both regional and global climates. The complicated relationship among



climatic variables, including temperature, greenhouse gas emissions, and regional topography, demands a thorough examination of the likely evolution of precipitation patterns. Planning for agriculture, water management, and overall environmental sustainability in Türkiye depends on a deep understanding of these dynamics.

This study aims to address a significant research gap in climate change by conducting a comprehensive analysis of Türkiye's precipitation patterns and growing season length trends under different scenarios of climate change. The study provides important insights into the temporal and spatial changes in precipitation and growing season length trends, addressing both short- and long-term challenges posed by climate change. This is achieved by integrating historical data with future projections.

2. Materials and Methods

2.1. Data Acquisition and Selection for Climate Projection

2.1.1. Data source

The data utilized in this study were obtained from The World Bank Group's Climate Change Knowledge Portal (CCKP) (Taylor et al., 2012; Dimri et al., 2023). This comprehensive database includes various climate-related data, encompassing historical records and future projections based on different Representative Concentration Pathway (RCP) scenarios (Dimri et al., 2023). Our analysis focused specifically on precipitation data spanning from 1986 to 2100.

2.1.2. Projection model

Indicator CMIP5 was used for the projections. The Fifth Assessment Report of the IPCC is greatly appreciated for the solid framework that CMIP5 provided for climate modeling.

2.1.3. Spatial resolution

The data was displayed at a spatial resolution of $1.0^\circ \times 1.0^\circ$, or roughly 100 km x 100 km. This resolution strikes a balance between computational feasibility and detail, rendering it suitable for capturing significant climatic trends over large geographic areas.

2.1.4. Selection justification

The use of CMIP5 indicators has been widely accepted and validated within the climate science community, therefore, the credibility of these indicators justifies their inclusion in our study. The model's accurate projections are essential for comprehending potential future climate scenarios (Taylor et al., 2012; Dimri et al., 2023)

2.2. Scenarios Selection and Setup

2.2.1. Climate scenarios used

Detail the specific climate models included in the Multi-Model Ensemble (MME) used for projections. The MME approach provides a more reliable projection of climate variables by integrating the outputs from multiple climate models. This technique minimizes the uncertainties and biases associated with individual models, improving the accuracy of climate projections.

The MME provides an extensive overview of potential climate futures by combining data from multiple models, which makes it an ideal tool for evaluating long-term climate trends and variability.

2.2.2. RCP scenarios

RCPs are scenarios that outline different greenhouse gas concentration trajectories (O'Neill et al., 2020). Each RCP represents a specific radiative forcing pathway:

RCP 2.6: Assumes significant mitigation efforts, leading to a peak in radiative forcing at approximately 3.0 W/m^2 before declining to 2.6 W/m^2 by 2100.

RCP 4.5 and 6.0: Intermediate scenarios, with radiative forcing stabilizing at around 4.5 W/m^2 and 6.0 W/m^2 , respectively, without significantly decreasing emissions.

RCP 8.5: Represents a high greenhouse gas emissions trajectory, with radiative forcing reaching over 8.5 W/m^2 by 2100, reflecting minimal mitigation efforts.

Each RCP encompasses assumptions about greenhouse gas emissions, land use, and technological changes, influencing the extent of future climate change (Jiang and O'Neill, 2017; Gurney et al., 2022).

2.2.3. Projection periods

Near-Term (2020–2039): Emphasizing policy interventions and the direct consequences of climate change.

Mid-Century (2040–2059): Examining the transitional phase of climate impacts and current mitigation measures.

Late-Century (2060–2079): Evaluating the effects of present emission trends over the long run.

End-of-Century (2080–2099): Providing information on how the climate will change under various greenhouse gas scenarios at the end of the 21st century.

2.2.4. Regression analysis

To analyze the trends in precipitation data, a linear regression model was employed. The purpose of the regression analysis was to calculate the annual rate of change in precipitation for each RCP scenario. The process involved calculating the slope of the linear trend line indicating the annual change in precipitation.

The regression model was constructed as follows:

Data cleaning: Firstly, the dataset was carefully examined to detect any errors or missing data. Subsequently, the data were preprocessed to ensure compliance with the specifications for the regression analysis.

Model fitting: Linear regression was applied to each set of data corresponding to the historical and RCP scenario-specific series. The 'scipy.stats' Python library facilitated this process, with the linregress function simplifying the calculation of the slope and intercept.

Projection and analysis: By projecting future precipitation scenarios, this model offers insights into how Türkiye's precipitation may vary under different conditions.

Visualization tool: The seaborn and matplotlib libraries for Python, which are well-known for their adaptability and effectiveness in data visualization, were used to create the heatmaps.

3. Results

3.1. Precipitation Projections under Different RCP Scenarios for Türkiye

Precipitation projections under different RCP scenarios indicate a significant variance in trends over the 21st century (Figure 1). The historical data indicates a decreasing precipitation trend, with a slope of -0.94 mm/year. This trend can be attributed to ongoing climate shifts, possibly influenced by human activity. Under the least severe pathway of the RCP 2.6 scenario, a slope of -0.08 mm/year suggest a relatively stable precipitation pattern. This implies the potential effectiveness of mitigation strategies aimed at reducing greenhouse gas concentrations. The slopes for the intermediate emissions and radiative forcing RCP 4.5 and RCP 6.0 scenarios are -0.51 mm/year and -0.59 mm/year, respectively. These intermediate trends, possibly influenced by delayed effects of emissions controls, exhibit a more noticeable decrease in precipitation, however at a slower rate compared to historical data. The RCP 8.5 scenario is predicted to bring about the most significant changes, with an accelerated decrease in precipitation indicated by a slope of -1.40 mm/year. This scenario implies that future precipitation patterns could be significantly changed, posing potential challenges to water resources management and agricultural productivity. It is characterized by high greenhouse gas emissions and a lack of effective mitigation efforts.

3.2. Monthly Precipitation Analysis under Different RCP Scenarios for Türkiye

Monthly precipitation anomalies by decade under various RCP scenarios are analyzed, and the results show a complex relationship between seasonal hydrological responses and climate change (Figure 2). The heatmaps show variations in precipitation in a striking way, with color gradients indicating the size of anomalies over time. Assuming a strict mitigation pathway, the range of variations in precipitation anomaly is relatively limited under the RCP 2.6 scenario. The first few decades exhibit minimal variations, but as the twenty-first century goes

on, a slight trend toward several months of wetter weather becomes apparent. The decreased intensity of anomalies in this scenario highlights the possible advantages of aggressive climate mitigation measures. In contrast, the anomalies clarify an entirely different tale under the RCP 8.5 scenario, characterized by high greenhouse gas emissions and little mitigation efforts. Negative anomalies become more prevalent, especially during typically rainier months, which indicates a trend towards drier weather. The last few decades, especially those after 1970, have shown a noticeable increase in the frequency of dry spells. This indicates a potential disruption in the water cycle, which could have a significant impact on ecosystem services, agriculture, and water resources.

These findings highlight the critical role of temporal and seasonal scales in understanding the impacts of climate change on precipitation. The decadal analysis provides insight into the progressive nature of climate change, revealing how the manifestation of anomalies can shift from one decade to the next. This highlights the challenges in predicting and preparing for future hydrological conditions.

The results emphasize the urgency for adaptive strategies that address both seasonal variability and long-term climatic trends. The clear trend of increasing precipitation anomalies, especially under high-emission scenarios, calls for an integrated approach to water management that considers the full spectrum of potential future climates.

The projected monthly precipitation by decade patterns (Figure 3), stratified across different RCPs, provide a compelling visual narrative of the potential hydrological shifts induced by climate change. The temporal range includes the near-term (2020–2039), mid-century (2040–2059), late-century (2060–2079), and end-of-century (2080–2099) periods. The temporal scope extends from historical baselines to the end of the twenty-first century.

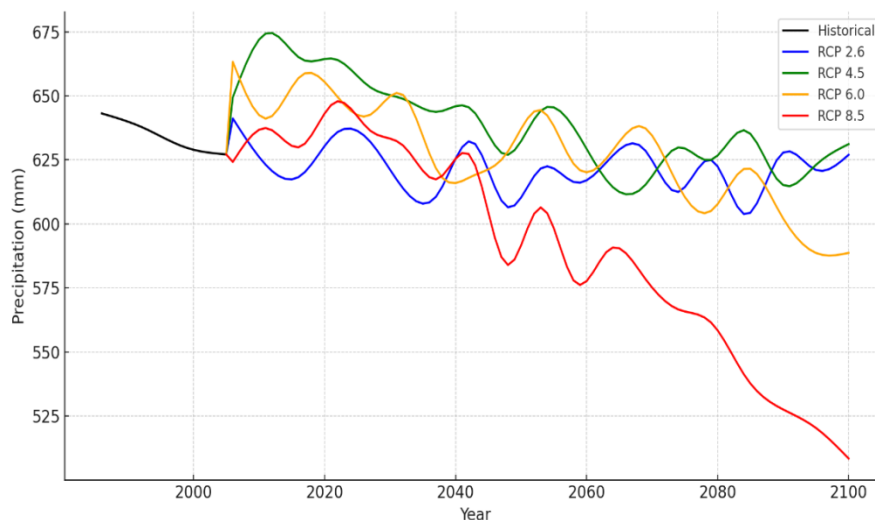


Figure 1. Precipitation projections under different RCP scenarios.

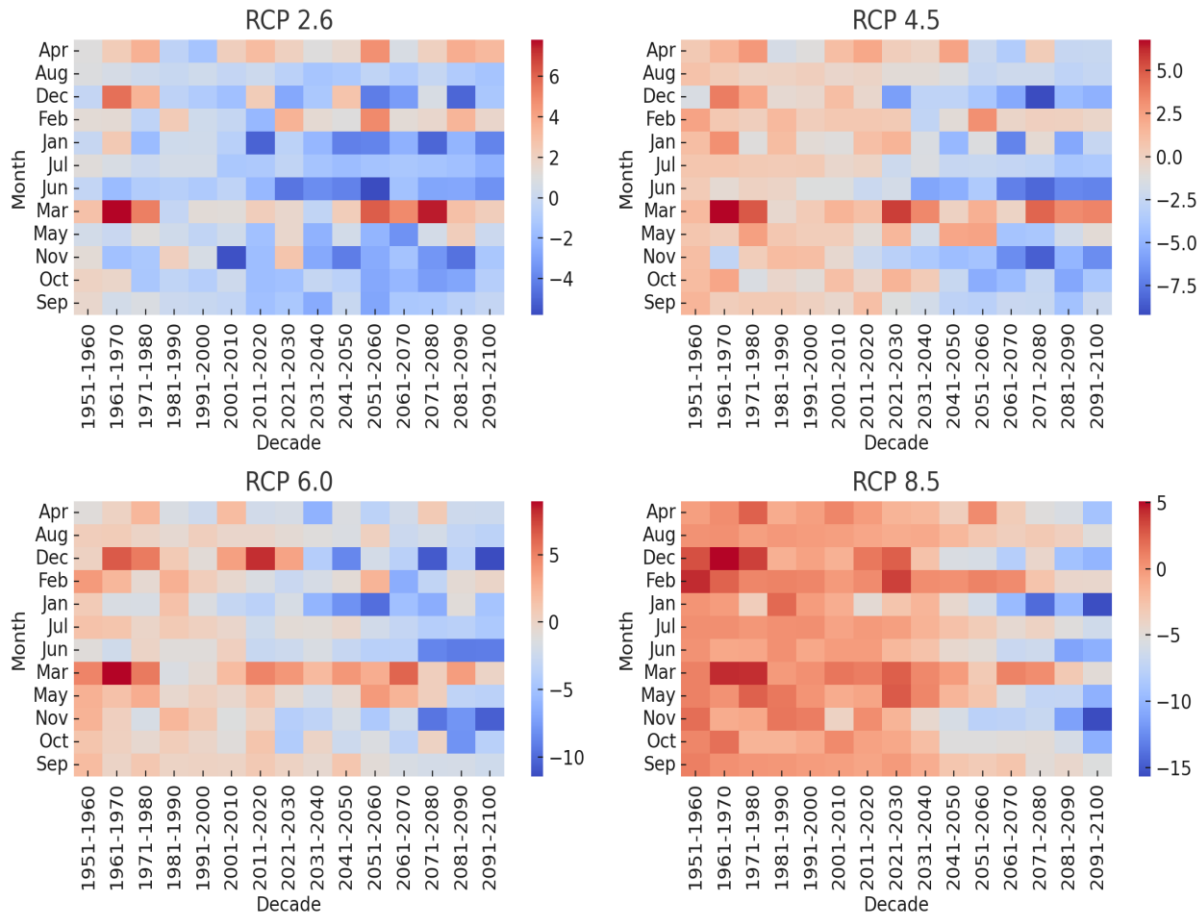


Figure 2. Monthly precipitation anomalies by decade under different RCP scenarios.

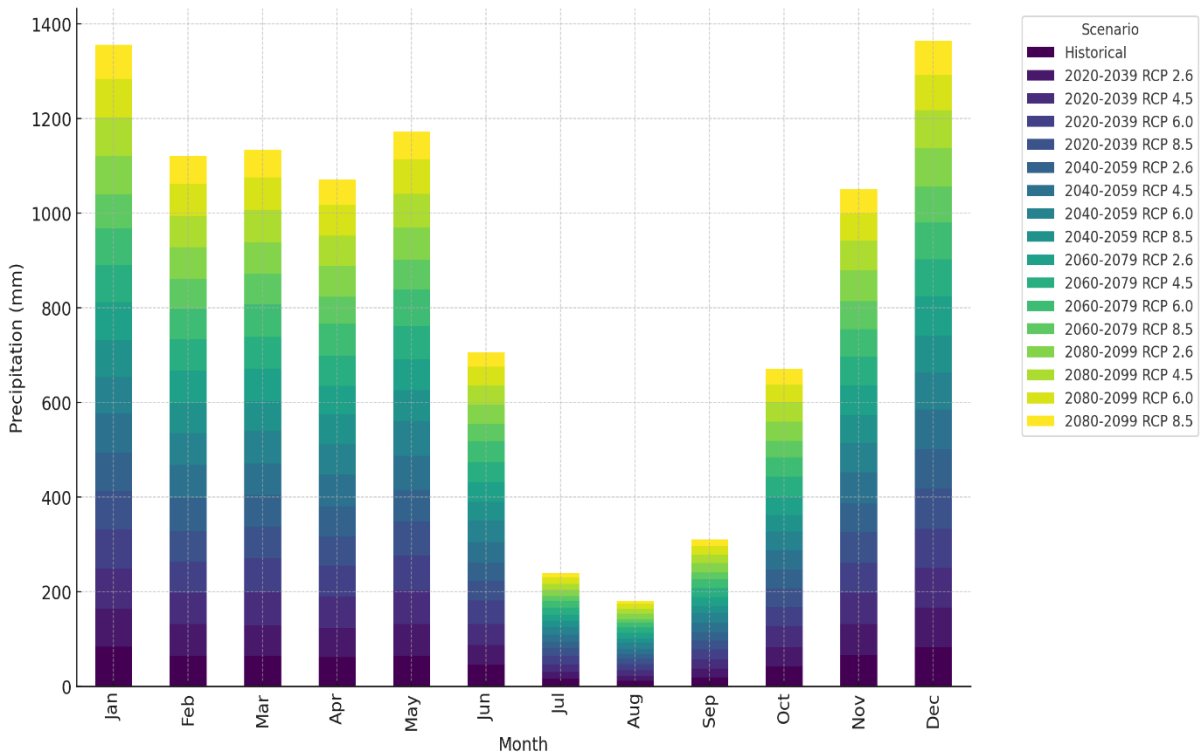


Figure 3. Monthly precipitation by decade across RCP scenarios.

The baseline comprises historical precipitation data, where the natural climatic rhythm is defined by the seasonal peaks and troughs of precipitation. It is

predicted that under different scenarios of greenhouse gas concentrations, this rhythmic pattern will change significantly. All RCPs' near-term projections (2020-

2039) demonstrate a continuation of the historical pattern, with a few minor variations that point to the beginning of change.

The deviations from historical norms become more noticeable as the century goes on. All RCPs, including the most optimistic RCP 2.6, start to show changes in the mid-century projections (2040–2059), with certain months displaying significant deviations from the historical precipitation levels. The projections for the late century (2060–2079) show a clear divergence, especially with the RCP 8.5 scenario, which shows the greatest reductions in precipitation. This trend becomes more pronounced by the end of the century (2080–2099), when the RCP 8.5 scenario predicts a significant decrease in precipitation during the traditionally rainy months, while RCP 2.6 maintains more in line with historical patterns, however with some modifications. The scenarios presented in RCP 4.5 and 6.0 illustrate intermediate flows and a complex trajectory. The moderate precipitation decline in these scenarios suggests that mid-range mitigation measures might not be enough to prevent substantial changes in the monthly precipitation distribution. It is noteworthy to observe the variation in the anticipated alterations among the RCPs, underscoring the intricate interplay between emissions scenarios and precipitation dynamics interact. Despite the general decline in precipitation, the distribution and degree of change are closely related to the emissions pathways that are being followed.

This analysis highlights the importance of considering both temporal and spatial scales in precipitation projections. The significance of taking into account both temporal and spatial scales in precipitation projections is highlighted by this analysis. Monthly precision enables a better understanding of potential seasonal resource challenges. The information highlights the urgency of developing focused adaptation plans that can handle the extremes and unpredictability of future climates.

3.3. The Analysis of 20mm+ Precipitation Events under Different RCP Scenarios for Türkiye

When significant amounts of precipitation (more than 20 mm) are analyzed historically and projected for the future, a pattern that is consistent with the hydrological cycle increasing under elevated greenhouse gas concentrations is revealed (Figure 4). The historical data provides a baseline by demonstrating variability in the frequency of these events but not an obvious long-term trend. The frequency of heavy precipitation events first declines under the RCP 2.6 scenario, which reflects a pathway of significant mitigation, and then gradually increases towards the end of the century. This implies that vigorous emissions reductions may lead to the hydrological cycle's eventual stabilization and recovery. On the other hand, heavy precipitation events are projected to occur more frequently under RCP 8.5, which assumes high greenhouse gas emissions in the lack of major mitigation efforts. This pattern suggests that the hydrological cycle is becoming more intense, which will probably lead to more frequent flooding and related socioeconomic effects. The frequency of heavy precipitation events varies in scenarios RCP 4.5 and RCP 6.0, which correspond to intermediate emission levels, but there is an overall increasing trend. In contrast to RCP 6.0, which exhibits more fluctuation, the RCP 4.5 scenario predicts a more stable increase. This suggests that variations in emissions paths could lead to variability in precipitation patterns.

This analysis highlights the increased likelihood of extreme precipitation events in all future scenarios, with the highest emissions scenario showing the greatest increase in risk. The observed trends emphasize the urgent need for adaptation plans to mitigate the risk of flooding and other water-related disasters. The data also suggests taking proactive steps to mitigate these changes by limiting greenhouse gas emissions to slow down the rate at which they progress.

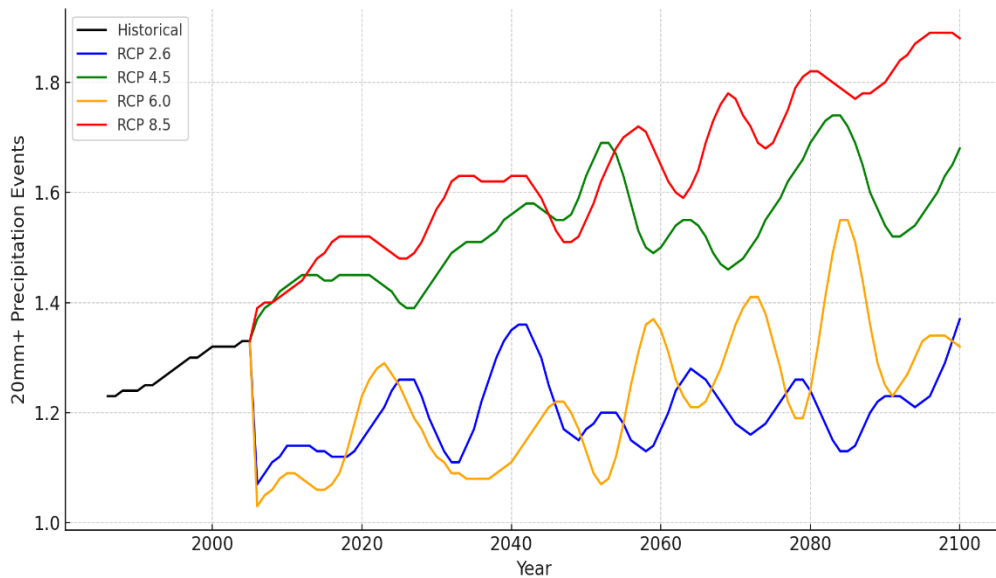


Figure 4. Change in annual 20mm+ precipitation events from 1986 to 2100 based on historical data and RCP scenarios.

The heat maps of monthly anomalies in heavy precipitation events (exceeding 20 mm) that are displayed provide light on the subtle changes that are predicted to occur under different RCP scenarios in the 20th and 21st centuries (Figure 5). Understanding the distinct effects of climate change on monthly precipitation extremes depends on this temporal-spatial analysis.

The anomalies exhibit fluctuations within a narrow band in the RCP 2.6 scenario, which assumes strict efforts to mitigate climate change. This indicates a relatively stable pattern of heavy precipitation events. Despite slight increases or decreases in certain decades, overall stability indicates that precipitation extremes could be decreased with effective mitigation.

The moderate emission reduction pathway known as RCP 4.5 exhibits a mixed pattern of anomalies, with some periods most notably the mid-21st century pointing to possible increases in heavy precipitation events. This hypothetical scenario highlights the complexity of intermediate climate responses, in which some areas might see increased variability in extreme weather occurrences. The heat map for RCP 6.0 shows that as the century goes on, the climate will become drier with more

notable negative anomalies. This raises the possibility of fewer heavy precipitation events, which could have a significant impact on disaster mitigation and the management of water resources.

The most notable negative anomalies are found in RCP 8.5, the pathway with the least mitigation and the highest greenhouse gas emissions, especially in the second half of the twenty-first century. A drier climate with fewer heavy precipitation events may cause more droughts, affecting ecosystem services, agriculture, and water security. The differences between various emission scenarios and their possible effects on heavy precipitation events are highlighted by the heat map analysis. It emphasizes the importance of understanding seasonal and localized variations in precipitation to develop effective risk management and adaptation plans. The presented data highlights the necessity for emissions reductions to mitigate the negative effects of climatic changes on precipitation extremes. Therefore, it recommends a forward-looking approach to climate policy, emphasizing the urgency of addressing emissions to safeguard against the potential consequences of altered precipitation patterns.

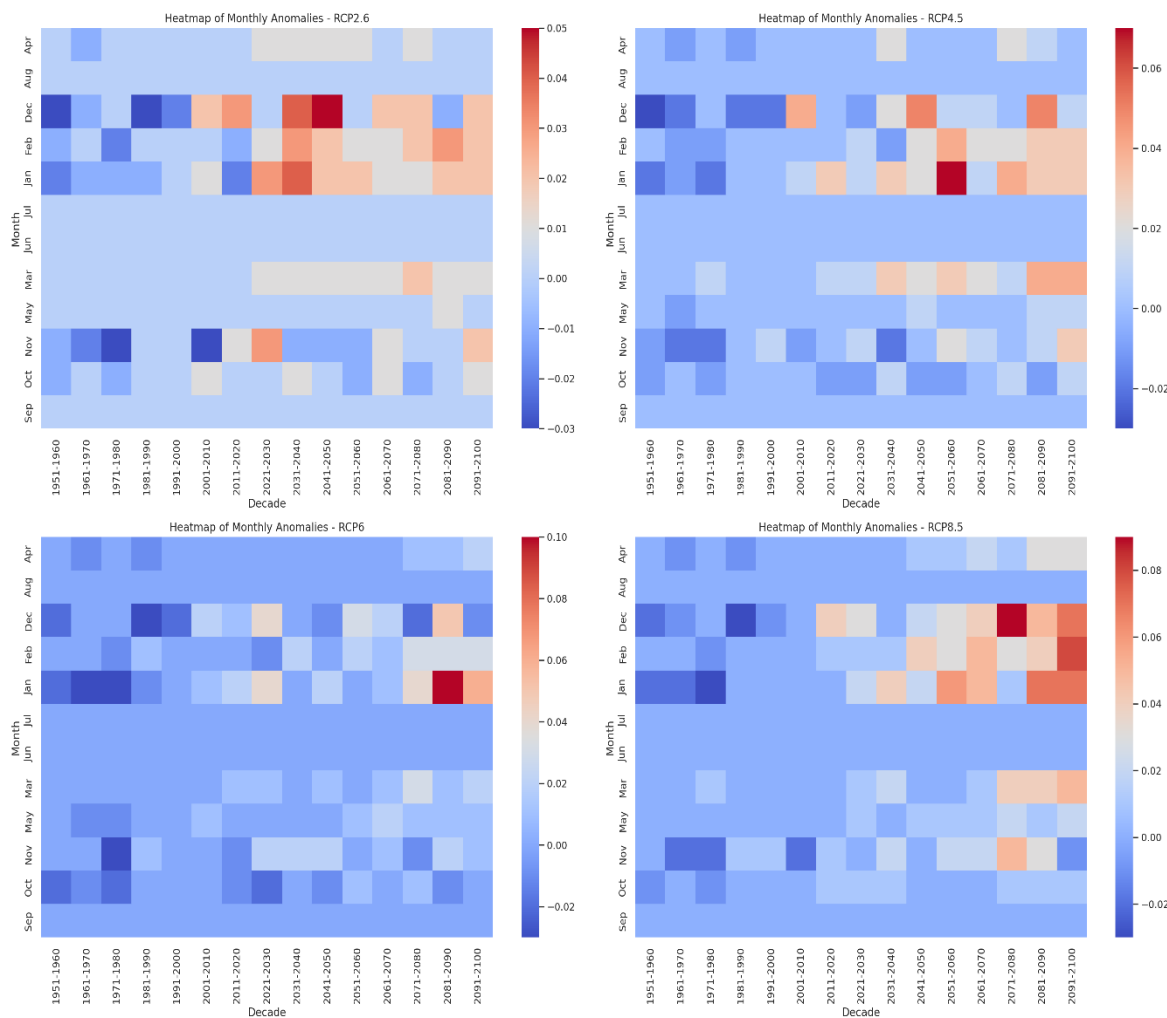


Figure 5. Monthly 20mm+ precipitation events anomalies under different RCP scenarios.

3.4. Growing Season Length under Different RCP Scenarios for Türkiye

The growing season length's temporal evolution, as shown by data from observations and projections under various RCP scenarios (Figure 6), offers a deep understanding of climate change impacts on agriculture. A baseline is set by the historical reference period (1986–2005) against which notable expansions and contractions of the growing season are evaluated.

Under the RCP 2.6 scenario, characterized by low greenhouse gas emissions and significant mitigation efforts, the length of the growing season exhibits moderate fluctuations with an overall trend of slight increase. This implies that strict climate regulations may help to stabilize and potentially improve the growing season for agriculture, protecting against the more extreme climate change effects. It is possible to observe a more identified variability in the growing season length when we proceed to RCP 4.5, an intermediate scenario. There is an initial increase, which is followed by a period of stability and then a decline towards the end of the century. This illustrates the mitigation strategies related

to this pathway's limited and partial efficiency. A tendency toward a shorter growing season in the later half of the century is indicated by the RCP 6.0 scenario, which shows a similar pattern to RCP 4.5. This highlights the necessity for more effective mitigation strategies to maintain agricultural productivity.

In RCP 8.5, which assumes high emissions without significant mitigation efforts, the growing season starts longer, peaks in the mid-century, and then experiences a drastic decrease. According to this scenario, agricultural viability may decline as the century goes on, which could have a significant impact on both food security and the stability of the agricultural industry's economy.

The variations in growing season length under various emissions scenarios highlight the critical dependence of agricultural systems on climate conditions. These findings imply that, despite the warming climate may temporarily lengthen the growing season in some places, the risks of increased variability and possible decline in the absence of substantial emissions reductions are likely to outweigh these benefits.

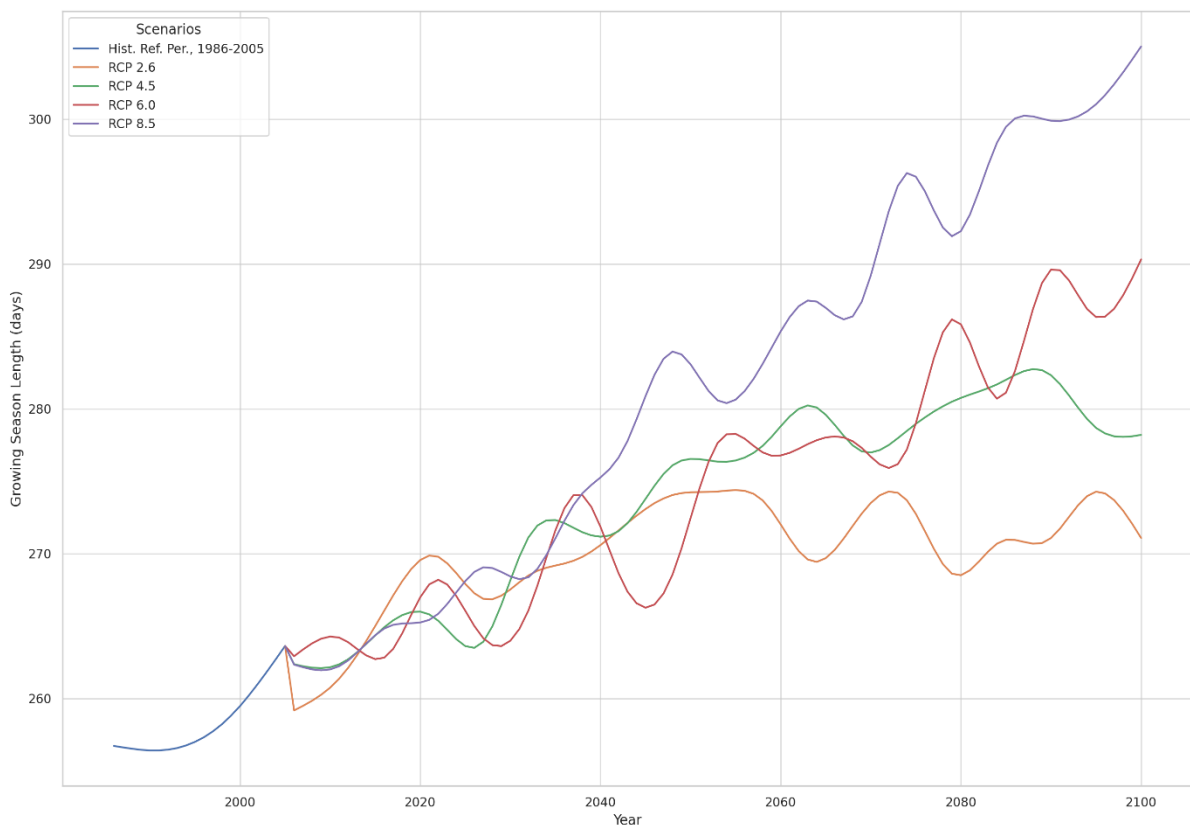


Figure 6. Growing season length under different RCP scenarios.

4. Discussion

The current study reveals that, under various RCP scenarios, Türkiye's growing season length varies noticeably. In the early decades, there was a tendency toward longer growing seasons, which were then followed by shorter ones, particularly in high-emission scenarios. A global perspective on the effects of climate change is given by Ruosteenoja et al. (2011), who

highlight changes in temperature and precipitation. Their results are consistent with the current study's observation of a longer growing season initially and a shorter one later on in Türkiye, indicating that global warming may initially extend the growing season but may also eventually cause it to decrease in Türkiye. Their findings about the lengthening growing season as a result of rising temperatures directly relate to the work being

done now, especially when considering the implications for Turkish agriculture. Vızıy et al. (2015) highlight how local climate systems can react differently to global climate drivers by focusing on regional climate variability. This study provides evidence in favor of the hypothesis that regional traits, like those present in Türkiye, can have a major impact on the local display of global climate trends. In their study, Arslantaş and Yeşilirmak (2020) investigate the climatic growing season in Türkiye's western Anatolia. Their results regarding the extended growing season as a result of rising temperatures are directly correspond to the present investigation, especially when considering the implications for Turkish agriculture.

The current study, also, provides a thorough examination of Türkiye's precipitation trends under various RCP scenarios. An important reduction in precipitation should be noted, especially in high-emission scenarios (RCP 8.5), which could be a direct consequence of global warming. As noted in studies like Demircan et al. (2014), increased temperatures can disrupt atmospheric circulation patterns, affecting precipitation distribution. Land use changes in Türkiye, including urbanization and deforestation, as discussed by Ciftci and Sahin (2023), might be contributing to local climatic alterations, influencing rainfall patterns. This is consistent with the findings of Bağçacı et al. (2021), who highlighted the impact of anthropogenic changes on local climates. Thus, this indicates possible difficulties in managing Türkiye's water resources and agricultural output.

The Mediterranean climate, which dominates much of Türkiye, is characterized by its own unique set of climatic interactions. According to studies by Aziz and Yücel (2021), regional climatic phenomena like the Mediterranean Oscillation are important factors in determining patterns of precipitation. Regional climate variations were also studied by Demircan et al. (2014) and Demircan et al. (2017), with a particular emphasis on the Mediterranean basin. Their results demonstrate the more extensive regional effects of climate change and confirm the trends found in the current study. These studies' consistency emphasizes how urgent it is to address Türkiye's hydrological changes caused by the climate.

The observation of a reasonably stable precipitation pattern under the RCP 2.6 scenario in the current study is consistent with the findings of Okkan (2014) and Ciftci and Sahin (2023). The combined results of these studies highlight the potential effectiveness of strict climate mitigation measures in stabilizing precipitation patterns, which is especially pertinent for Türkiye's policy-making process.

Bağçacı et al. (2021) and Aziz and Yücel (2021) support the importance of the seasonal and decadal variability in precipitation patterns found in the current study for Türkiye's agricultural sector. In light of changing seasonal rainfall patterns, these studies emphasize the importance of adaptive agricultural practices as a means

of guaranteeing Türkiye's food security.

Seker and Gumus (2022) offer valuable perspectives on beneficial approaches to water resource management. When the results of this study are taken into account, it is evident that Türkiye needs to manage its water resources in a proactive and integrated manner to mitigate the effects of changing patterns of precipitation.

According to the current study and Yeşilköy and Şaylan (2022), changes in the hydrological cycle brought on by warming temperatures may increase in extreme weather events. This covers variations in precipitation intensity and frequency in addition to changes in precipitation quantity. Because of these shifting precipitation patterns, Türkiye's agriculture industry, which is crucial to the country's economy, may encounter serious challenges. Studies like Okkan (2014) and Seker and Gumus (2022) highlight the necessity of adaptive agricultural practices and water management strategies.

5. Conclusion

There is a complicated relationship between growing season dynamics, the frequency of extreme weather events, and precipitation trends. Some individuals may view a longer growing season as a positive outcome of global warming, but these benefits can be overshadowed by the inherent threat of more frequent and severe weather events.

This current comprehensive analysis delineates a future where precipitation patterns, extreme weather events, and agricultural growing seasons are increasingly influenced by climate change. The anticipated changes in hydrological dynamics under all RCPs highlight the need for effective mitigation and flexible approaches. The data highlights the potential benefits of rigorous mitigation efforts in stabilizing and potentially extending the growing seasons also calls for immediate action to reduce water-related risks, particularly under higher emission scenarios. This emphasizes how important it is to make well-informed policy decisions and practice integrated resource management to protect against the various effects of climate variability in Türkiye.

Author Contributions

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

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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THE EFFECTS OF FERTILIZER AND DIFFERENT SOIL CONDITIONER TO YIELD AND YIELD COMPONENTS IN CHICKPEA CULTIVARS

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Abstract: This study was carried out in Kahramanmaraş Sütçü İmam University Faculty of Agriculture Research treatment area in 2022. In the study, 14 different fertilizer applications (no fertilizer, chemical fertilizer, olive bagasse 100 kg/da, olive bagasse 200 kg/da, olive bagasse 400 kg/da, olive bagasse 600 kg/da, worm manure 100 kg/da, worm manure 200 kg/da, worm manure 400 kg/da, worm manure 600 kg/da, leonardite manure 100 kg/da, leonardite manure 200 kg/da, leonardite manure 400 kg/da, leonardite manure 600 kg/da) were made in 2 different chickpea cultivars (Aras, Katran). According to the results obtained from the study, Katran variety had higher grain yield than Aras variety, but 1000 grain weight was found to be lower. On the other hand, the highest grain yield was obtained from olive bagasse 200 kg/da application.


Keywords: Fertilizer, Chickpea cultivars, Yield, Components

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1. Introduction

Agriculture has been the foundation of national economies worldwide. It has achieved significant success, thanks to advancements in science, technology, and the intensive use of land. However, it has also brought about certain agro-ecological and environmental challenges. The widespread use of chemical fertilizers and pesticides in agriculture has resulted in land degradation and environmental pollution in various agricultural ecosystems. The continuous application of chemical fertilizers to agricultural lands has deteriorated soil fertility, leading to a reduction in biodiversity, soil organic matter content, and causing irreversible soil erosion (Yasir et al., 2022). In contrast, organic fertilizers enhance soil organic matter content, soil fertility, microbial activity, and moisture retention capacity (Demirkıran et al., 2012; Meagy et al., 2016). They improve soil quality and crop yield by serving multiple functions in agricultural ecosystems (Jones et al., 2007; Ekici et al., 2023). Organic matter enhances soil quality by improving input use efficiency and ensuring optimum sustainability and environmental safety (Kushwah et al., 2016). The application of organic fertilizer increases soil fertility and, consequently, crop yield (Reddy et al. 2005). Many studies state that organic wastes improve soil

structure, increase soil organic matter content, reduce evaporation, and are effective against erosion (Laamrani et al., 2017; Liang et al., 2012). The application of organic fertilizer enhances soil quality and health by increasing soil carbon and microorganisms beneficial to plant crops (Gand and Singh, 2016). The protein content of wheat grown with organic fertilizers is higher than that of wheat grown with chemical fertilizers (Bahrman et al., 2004; Shivay et al., 2010). Foods produced with organic fertilizers are known to be tastier and have a more balanced vitamin and mineral content than conventionally grown foods (Fuertes-Mendizabal et al., 2010). Organic production is an economical, renewable, and environmentally friendly approach that allows the preservation of natural resources for current and future generations, ensuring the sustainability of production. Leonardite is a low-calorie coal rich in humic acid and organic matter and formed as a result of the oxidation of lignite coal without coalification (Olivella et al., 2002). Leonardite, an organic regulator, improves the structure of the soil, increases water retention capacity and water permeability, maintains soil moisture content and maintains soil pH balance (Kara et al., 2022a). Vermicompost has positive effects on the physical (aggregate stability, water retention), chemical (plant



nutrition elements, soil pH), biological properties (living diversity and biomass) and plant productivity of soils (Dominguez et al., 2003; Ferreras et al., 2006; Azarmi et al., 2008; Saha et al., 2008). Olive bagasse is the solid sub-product remaining after the mechanical processing of olives into oil. When applied to soils as an organic regulator, it improves the physical properties of soils (hydraulic conductivity, aggregate stability, dispersion ratio, field capacity and bulk density) (Kara et al., 2022b). In this study, we investigated the effects of different organic fertilizers (leonardite, worm manure, and olive bagasse) on the yield and quality of chickpea varieties (Aras and Katran).

2. Materials and Methods

This research; in order to determine the yield and yield characteristics of local chickpea genotypes and registered chickpea varieties were conducted in the Kahramanmaraş in 2022 year with Aras and Katran chickpea varieties. It was conducted in the Research and Application area of Kahramanmaraş Sutçu Imam

University, Faculty of Agriculture (it's coordinate 37S X: 307001, Y: 41630333). Wheat plants have been planted before in the area where the study is located. The area of each parcel was determined as 10m² (5m × 2m). The study was conducted as a randomized completely block design with three replications. In the study, no fertilizer, chemical fertilizer, olive bagasse 100 kg/da, olive bagasse 200 kg/da, olive bagasse 400 kg/da, olive bagasse 600 kg/da, worm manure 100 kg/da, worm manure 200 kg/da, worm manure 400 kg/da, worm manure 600 kg/da, leonardite 100 kg/da, leonardite 200 kg/da, leonardite 400 kg/da, leonardite 600 kg/da fertilizer applications were made.

Harvesting operations were done manually. Weeds were physically combated throughout the growing period. On the other hand, chemical control was carried out against diseases and pests. Some physical and chemical properties of the soil used as a trial area are given in Table 1. In addition, some chemical properties of the organic fertilizers used as materials (Olive Bagasse, Worm manure and Leonardite) are given in Table 2.

Table 1. Some physical and chemical properties of the soil where the experiment was established

Sand	Clay	Silt	pH	OM	Lime	P	K
%	%	%		%	%	µg/g	µg/g
41	30	29	7.62	1.68	3.2	13.8	230

Table 2. Some chemical properties of organic regulators

Variables	Leonardite (Kara and Yakupoğlu, 2023)	Olive Bagasse (Kara et al., 2022b)	Worm Manure
OM (%)	55	84.1	52.4
C (%)	31.9	48.14	30.39
N (%)	0.54	1.02	2.06
P (µg/g)	1781	900	4600
K (µg/g)	4170	12000
Ca (µg/g)	92100	2800
Mg (µg/g)	2087	1100
Fe (µg/g)	2780	305.6
Zn (µg/g)	28.26	30.1
Mn (µg/g)	25.88	25.2
Cu (µg/g)	0.9

2.1. The climate characteristic of the study area

The summers in Kahramanmaraş city center are hot and dry, and the winters are warm and rainy. The average temperature and total precipitation values for the year the experiment was conducted are given in Table 3. As reported by other researchers in the study; plant height (cm), first pod height (cm), number of pods per plant (number/plant), 1000 seed weight (g) and seed yield (kg/da) parameters were examined (Colkesen *et al.*, 2014; Girgel *et al.* 2018; Girgel and Cokkizgin, 2019).

2.2. Statistical Analysis

The data obtained from study were analyzed according to one factor randomized complete block design using the SAS package program (SAS, 2004). LSD multiple range test was used to compare the means.

Table 3. Climate data obtained from Kahramanmaraş meteorological station (Anonymous, 2022)

Months	Monthly Total	Monthly Total	Monthly Average	Monthly Average
	Precipitation (mm)	Precipitation (mm)	Temperature (°C)	Temperature (°C)
	2022	1930-2018	2022	1930-2018
January	311.2	4.9	4.47	4.9
February	170.4	6.5	8.7	6.5
March	157.8	10.7	7.1	10.7
April	12.7	15.5	18.2	15.5
May	40.4	20.3	20.4	20.3
June	3.7	25.2	26.1	25.2
July	0.5	28.4	29.6	28.4
August	0	28.5	29.4	28.5
September	10.7	25.2	26.1	25.2
October	12.3	19.1	20.6	19.1
November	72.4	11.7	13.4	11.7
December	32.5	6.7	9.6	6.7
Total	824.6	725.8
Mean	17.8	16.9

3. Results and Discussion

3.1. Plant Height (cm)

Variance analysis table for plant height are given in Table 4. According to the results obtained from the research, different fertilizer applications and different fertilizer X chickpea interaction were found to be insignificant. The statistical difference between chickpea varieties was found to be significant.

In terms of plant height, Aras variety produced taller plants than Katran variety (Table 6). On the other hand, when the situation is evaluated in terms of chemical applications, the highest plant height value was obtained from leonardite fertilizer 600 kg/da application (Table 6). Generally similar results were obtained when looking

at other organic fertilizer application doses (CF, OB 100kg/da, OB 200kg/da, OB 400kg/da, OB 600kg/da, WM 100kg/da, WM 200kg/da, WM 400kg/da; WM 600kg/da, L 100kg/da, L 200kg/da, L 400kg/da). The results obtained from the study are in agreement with other studies (Bakoğlu, 2005; Ekici et al., 2023). Ekici et al. (2023) determined plant height of chickpea between 48 cm and 54 cm with using leonardite and stated that increases in plant height were insignificant.

When the varieties (Aras and Katran) are evaluated within themselves; differences between fertilizer applications were statistically insignificant. Similar opinions reported by Colkesen et al. (2014), Girdel et al. (2018), Girdel and Cokkizgin (2019).

Table 4. Variance analysis table for plant height

Variation Source	DF	SS	MS	F	Prob.
Different Fertilizer Application	13	121.6234155	9.3556473	1.09	0.3833
Chickpea Cultivars	1	62.7644298	62.7644298	7.34	0.0089
Different Fertilizer X Chickpea Interaction	13	42.5274536	3.2713426	0.38	0.9701
Error	56	479.0024000	8.5536143		
Total	83	705.9176988			

DF= degrees of freedom , SS= sum of squares , MS= mean sum of squares , F= F-statistic, Prob= probability

Table 5. Plant height values of cultivars and statistical groups

Cultivars	Means
Aras	48.4660 ^a
Katran	46.7371 ^b

^{a,b} Means with different letters in the same column are significantly different at P<0.05.

Table 6. Plant height (cm) values of different fertilizer applications and statistical groups

DFA	Aras	Katran	Mean
Control	50.043 ND	46.587 ND	48.315 ND
CF	48.587 ND	45.127 ND	46.857 ND
OB 100 kg/da	49.000 ND	45.083 ND	47.042 ND
OB 200 kg/da	47.460 ND	45.377 ND	46.418 ND
OB 400 kg/da	46.670 ND	45.210 ND	45.940 ND
OB 600 kg/da	45.917 ND	47.293 ND	46.605 ND
WM 100 kg/da	47.127 ND	46.170 ND	46.648 ND
WM 200 kg/da	48.503 ND	48.917 ND	48.710 ND
WM 400 kg/da	47.583 ND	46.253 ND	46.918 ND
WM 600 kg/da	49.127 ND	46.627 ND	47.877 ND
L 100 kg/da	48.417 ND	47.627 ND	48.022 ND
L 200 kg/da	49.337 ND	46.833 ND	48.085 ND
L 400 kg/da	49.253 ND	47.087 ND	48.170 ND
L 600 kg/da	51.500 ND	50.130 ND	50.815 ND

ND= non differences, DFA= different fertilizer application, CF= chemical fertilizer, OB= olive bagasse, WM= worm manure, L= leonardite

3.2. First Pod Height (cm)

Variance analyses for first pod height are given in Table 7. In terms of first pod height, different fertilizer applications and different fertilizer X chickpea interaction were found to be insignificant. But chickpea cultivars variation source was found to be significant. Worm manure (200 kg/da) application had highest the first pod height value in chickpea plant. Additionally, in all other applications, the initial pod height was similar (Table 8). When the varieties (Aras and Katran) are

evaluated within themselves; differences between fertilizer applications were statistically insignificant. The results obtained from the study are in agreement with other studies (Bakoğlu, 2005). The results obtained from the study are in agreement with other studies (Kahraman, 2017). The average first pod height of Aras and Katran varieties is given in Table 9. Accordingly, the average first pod height of the varieties (Aras and Katran) was statistically significant.

Table 7. Variance analysis table for first pod height

Variation Source	DF	SS	MS	F	Prob.
Different Fertilizer Application	13	138.7596905	10.6738223	0.89	0.5667
Chickpea Cultivars	1	60.2149333	60.2149333	5.03	0.0289
Different Fertilizer X Chickpea Interaction	13	174.0771000	13.3905462	1.12	0.3640
Error	56	670.695800	11.976711		
Total	83	1043.747524			

DF= degrees of freedom , SS= sum of squares , MS= mean sum of squares , F= F-statistic, Prob= probability

Table 8. First pod height (cm) values of chemical applications and statistical groups

DFA	Aras	Katran	Mean
Control	21.710 ND	25.170 ND	23.440 ND
CF	21.710 ND	25.003 ND	23.357 ND
OB 100 kg/da	22.043 ND	23.920 ND	22.982 ND
OB 200 kg/da	21.420 ND	24.960 ND	23.190 ND
OB 400 kg/da	21.003 ND	23.167 ND	22.085 ND
OB 600 kg/da	21.380 ND	24.587 ND	22.983 ND
WM 100 kg/da	21.380 ND	24.297 ND	22.838 ND
WM 200 kg/da	31.710 ND	23.543 ND	27.627 ND
WM 400 kg/da	21.960 ND	23.17 ND	22.565 ND
WM 600 kg/da	21.460 ND	24.587 ND	23.023 ND
L 100 kg/da	23.127 ND	24.500 ND	23.813 ND
L 200 kg/da	22.003 ND	22.960 ND	22.482 ND
L 400 kg/da	22.503 ND	23.920 ND	23.212 ND
L 600 kg/da	20.670 ND	24.003 ND	22.337 ND

ND= non differences, DFA= different fertilizer application, CF= chemical fertilizer, OB= olive bagasse, WM= worm manure, L= leonardite.

Table 9. First pod height values of cultivars and statistical groups

Cultivars	Means
Aras	22.4343 ^b
Katran	24.1276 ^a

^{ab} Means with different letters in the same column are significantly different at P<0.05.

3.3. Pod Number Per Plant (number/plant)

Variance analysis table for pod number per plant are given in Table 10. According to the results obtained from the research, different fertilizer applications and different fertilizer X chickpea interaction were found to be insignificant. The statistical difference between chickpea varieties was found to be significant in terms of pod number per plant.

Worm manure (400 kg/da) application is the fertilizer application that creates the most pods in the research (Table 11). On the other hand, the Katran variety has higher pods than the Aras variety, and this is statistically

significant (Table 12). The results obtained from the study are in agreement with other studies (Soysal et al., 2020). On the other hand when the varieties (Aras and Katran) are evaluated within themselves; differences between fertilizer applications were statistically insignificant in terms of pod number. Similar opinions reported by Colkesen et al. (2014), Girdel and Cokkizgin (2019). Similarly, Ekici et al. (2023) determined pod number of chickpea with using leonardite as 37-54 number/plant and stated that increases in pod number were not significant.

Table 10. Variance analysis table for pod number per plant

Variation Source	DF	SS	MS	F	Prob.
Different Fertilizer Application	13	2258.40735	173.72364	1.17	0.3252
Chickpea Cultivars	1	11945.98801	11945.98801	80.43	<.0001
Different Fertilizer X Chickpea Interaction	13	2471.55434	190.11956	1.28	0.2524
Error	56	8317.46660	148.52619		
Total	83	24993.41630			

DF= degrees of freedom , SS= sum of squares , MS= mean sum of squares , F= F-statistic, Prob= probability

Table 11. Pod number per plant values (number/plant) of different fertilizer applications and statistical groups

DFA	Aras	Katran	Mean
Control	44.377 ND	72.670 ND	58.522 ND
CF	40.583 ND	67.960 ND	54.272 ND
OB 100 kg/da	39.920 ND	69.170 ND	54.545 ND
OB 200 kg/da	37.797 ND	57.630 ND	47.712 ND
OB 400 kg/da	37.127 ND	62.710 ND	49.918 ND
OB 600 kg/da	38.920 ND	55.000 ND	46.962 ND
WM 100 kg/da	41.213 ND	63.000 ND	52.108 ND
WM 200 kg/da	38.837 ND	67.750 ND	53.295 ND
WM 400 kg/da	41.417 ND	95.290 ND	68.355 ND
WM 600 kg/da	44.293 ND	54.340 ND	49.315 ND
L 100 kg/da	38.543 ND	68.960 ND	53.752 ND
L 200 kg/da	42.463 ND	55.920 ND	49.192 ND
L 400 kg/da	41.793 ND	61.710 ND	51.752 ND
L 600 kg/da	49.630 ND	58.710 ND	54.170 ND

ND= non differences, DFA= different fertilizer application, CF= chemical fertilizer, OB= olive bagasse, WM= worm manure, L= leonardite.

Table 12. Pod number per plant values of cultivars and statistical groups

Cultivars	Means
Aras	41.208 ^b
Katran	65.059 ^a

^{ab} Means with different letters in the same column are significantly different at P<0.05.

3.4. 1000 seed weight (g)

Variance analysis table for 1000 seed weight are given in Table 13. In terms of 1000 seed weight, different fertilizer applications and cultivars were found to be significant but different fertilizer X chickpea interaction variation source was found to be insignificant.

Decare 200 kg olive bagasse application had a maximum weight of 1000 grains, among fertilizers. This was followed by the application of 200 kg of leonardite fertilizer per decare (Table 14). The Aras variety has higher 1000 seed weight than the Katran variety and this is statistically significant (Table 15). This situation is

associated with the fact that the grain sizes of the Katran chickpea variety are smaller than the Aras variety. The results obtained from the study are in agreement with other studies (Kahraman, 2017). When the varieties are evaluated within themselves; Differences between fertilizer applications in Aras cultivar were statistically insignificant. However, the response of the Katran cultivar to fertilizer applications was found to be statistically significant. Olive Bagasse 200 kg/da application had the highest 1000 grain weight value for the Katran variety (282.83 g).

Table 13. Variance analysis table for 1000 seed weight

Variation Source	DF	SS	MS	F	Prob.
Different Fertilizer Application	13	12414.6770	954.9752	2.53	0.0083
Chickpea Cultivars	1	839292.1492	839292.1492	2223.30	<.0001
Different Fertilizer X Chickpea Interaction	13	3871.9638	297.8434	0.79	0.6683
Error	56	21139.8905	377.4980		
Total	83	876718.6805			

DF= degrees of freedom , SS= sum of squares , MS= mean sum of squares , F= F-statistic, Prob= probability

Table 14. 1000 seed weight values (g) of chemical applications and statistical groups

DFA	Aras	Katran	Mean
Control	435.29 ND	225.12 ^B	330.20 ^{BC}
CF	416.55 ND	226.68 ^B	321.61 ^{BC}
OB 100 kg/da	428.01 ND	227.34 ^B	327.67 ^{BC}
OB 200 kg/da	454.26 ND	282.83 ^A	368.55 ^A
OB 400 kg/da	425.33 ND	234.07 ^B	329.70 ^{BC}
OB 600 kg/da	436.88 ND	219.96 ^B	328.42 ^{BC}
WM 100 kg/da	438.19 ND	224.29 ^B	331.24 ^{BC}
WM 200 kg/da	430.92 ND	225.64 ^B	328.28 ^{BC}
WM 400 kg/da	441.89 ND	222.75 ^B	332.32 ^{BC}
WM 600 kg/da	429.14 ND	222.19 ^B	325.67 ^{BC}
L 100 kg/da	409.7 ND	227.37 ^B	318.54 ^{BC}
L 200 kg/da	434.63 ND	246.99 ^B	340.81 ^B
L 400 kg/da	412.53 ND	217.65 ^B	315.09 ^C
L 600 kg/da	431.44 ND	223.06 ^B	327.25 ^{BC}

^{A,BC} Means with different letters in the same column are significantly different at P<0.05. ND= non differences, DFA= different fertilizer application, CF= chemical fertilizer, OB= olive bagasse, WM= worm manure, L= leonardite.

Table 15. 1000 seed weight values of cultivars and statistical groups

Cultivars	Means
Aras	430.340 ^a
Katran	230.424 ^b

^{ab} Means with different letters in the same column are significantly different at P<0.05.

3.5. Seed Yield (kg/da)

Variance analysis table for seed yield are given in Table 16. According to the results in terms of seed yield, different fertilizer applications and different fertilizer X chickpea interaction were found to be insignificant. The statistical difference between chickpea varieties was found to be significant.

Decare 200kg olive bagasse application had the highest BSJ Agri / Zekeriya KARA et al.

value in terms of grain yield. The second highest yield result (230.22 kg/da) was obtained in the parcels where 100 kg of leonardite fertilizer was applied per decare (Table 17). The positive effect of fertilizers on productivity is known (Uçar, 2019).

On the other hand, the Katran variety has higher seed yield (235.48 kg/da) than the Aras variety (198.63 kg/da), and this is statistically significant (Table 18).

When the varieties (Aras and Katran) are evaluated within themselves; differences between fertilizer applications were statistically insignificant. The results obtained from the study are in agreement with other

studies (Bakoğlu, 2005). Our findings are in agreement with similar studies (Arora et al., 2003; Lopez-Bellido et al., 2004; Bakoğlu, 2005; Kamithi et al., 2009; Kahraman, 2017; Soysal et al., 2020; Ekici et al., 2023) (Table 18).

Table 16. Variance analysis table for seed yield

Variation Source	DF	SS	MS	F	Prob.
Different Fertilizer Application	13	8012.55668	616.35051	0.25	0.9958
Chickpea Cultivars	1	28524.11152	28524.11152	11.53	0.0013
Different Fertilizer X Chickpea Interaction	13	16586.51296	1275.88561	0.52	0.9055
Error	56	138572.1791	2474.5032		
Total	83	191695.3603			

DF= degrees of freedom , SS= sum of squares , MS= mean sum of squares , F= F-statistic, Prob= probability

Table 17. Seed yield values (kg/da) of different fertilizer applications and statistical groups

DFA	Aras	Katran	Mean
Control	220.22 ND	237.54 ND	228.88 ND
CF	190.15 ND	231.16 ND	210.65 ND
OB100 kg/da	212.56 ND	216.82 ND	214.69 ND
OB 200 kg/da	202.97 ND	265.72 ND	234.34 ND
OB 400 kg/da	180.97 ND	225.5 ND	203.24 ND
OB 600 kg/da	207.57 ND	214.92 ND	211.24 ND
WM 100 kg/da	210.7 ND	218.26 ND	214.48 ND
WM 200 kg/da	177.51 ND	250.85 ND	214.18 ND
WM 400 kg/da	222.31 ND	208.34 ND	215.33 ND
WM 600 kg/da	199.79 ND	220.12 ND	209.96 ND
L 100 kg/da	200.37 ND	260.06 ND	230.22 ND
L 200 kg/da	202.37 ND	257.57 ND	229.97 ND
L 400 kg/da	164.51 ND	240.42 ND	202.47 ND
L 600 kg/da	188.76 ND	249.45 ND	219.11 ND

ND= non differences, DFA= different fertilizer application, CF= chemical fertilizer, OB= olive bagasse, WM= worm manure, L= leonardite.

Table 18. Seed yield values of cultivars and statistical groups

Cultivars	Means
Aras	198.63 ^B
Katran	235.48 ^A

4. Conclusion

Considering the findings, organic regulators (worm manure, olive bagasse, leonardite) and application doses (100 kg/da, 200 kg/da, 400 kg/da and 600 kg/da) didn't show positive changes on plant yield quality. This situation may be due to the heterogeneous characteristics of the study area. On the other hand, when we compared chickpea varieties among themselves, the katran chickpea variety gave higher yield than the Aras

variety. According to this result, Katran variety can be recommended to Kahramanmaraş region farmers.

In addition, the highest grain yield was obtained from 200 kg/da olive bagasse application. However, it is important to repeat the study in a homogeneous area in order to be able to talk more clearly about organic fertilizers (olive bagasse, leonardite and worm manure) and application doses.

Author Contributions

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

	Z.K	C.Y.	A.C
C	40	30	30
D	40	30	30
S	40	30	30
DCP	30	40	30
DAI	40	30	30
L	50	20	30
W	80		20
CR	30	30	40
SR	80	10	10
PM	40	30	30
FA	40	30	30

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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EFFECTS OF DIFFERENT MIXTURE RATIOS OF COMMON VETCH AND TRITICALE ON FORAGE YIELD AND SILAGE QUALITY

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Abstract: In order to obtain high amounts and quality forage in legume grain mixtures, the mixture ratios must be adjusted well. Since grains are tillered due to their physiology, their ratios in roughage are different from the mixing ratios in planting. This is a factor that increases roughage yield and reduces crude protein rate and yield. In addition, in cases where grains are insufficient in terms of minerals in animal nutrition and are planted in mixtures with legumes, the rapid development and high yield effects of grains should be combined with the balanced nutrition potential of legumes in order to obtain better quality feed. In the research, which was conducted to determine the effect of vetch and triticale mixture ratios on yield and silage quality in Sakarya ecological conditions, plantings were carried out at different mixture ratios. In this study, 100% Vetch, 100% Triticale, 75% Vetch + 25% Triticale, 50% Vetch + 50% Triticale and 25% Vetch + 75% Triticale were planted in Sakarya conditions and the effect of the mixture ratios on grass yield was revealed. Additionally, silage was made from the mixtures obtained, and the effect of the applied mixture ratios on silage quality was determined. Field trials of the research were carried out in the trial areas of Sakarya University of Applied Sciences, Faculty of Agriculture. In the experiment, plant height, green and dry forage yield observations were taken. At the end of the maturation period, silage was made in 2 kilogram containers from each parcel as a silage quality criterion; silage dry matter ratio, silage pH, crude protein ratio, ADF and NDF observations were taken. By calculating the physical and sensory analysis and fleig point used to determine silage quality, the silages obtained from the mixtures were defined in detail by chemical, physical and sensory analysis and the fleig point. In the study, the highest green forage yield, hay yield, crude protein, ADF, NDF ratio and fleig point were obtained from 100% Vetch, 100% Triticale, 100% Vetch, 100% Triticale, 100% Triticale and 50% Vetch+50% Triticale mixtures, respectively. Due to the favorable outcomes in terms of forage yield and silage quality, 25% Vetch + 75% Triticale mixture ratio can be recommended to producers.

Keywords: Common vetch, Triticale, Mixture, Silage, Quality

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1. Introduction

The most extensively cultivated forage crop in our country is vetch, with a cultivated area of 288 thousand hectares (Anonymous, 1996). Except for narbon vetch, other vetch species tend to lodge due to the weakness of their stems. Lodging leads to a decrease in forage yield and quality, difficulty in harvesting, and leaf losses. To prevent lodging, mixed cropping is practiced with cereals (barley, oats, rye, and triticale) alongside vetch. As vetch develops, it wraps around with its tendrils, reducing yield losses and facilitating harvesting (Soya et al., 1996; Tan and Serin, 1996). Research indicates that mixed crops of vetch + cereals serve as an important forage source to compensate for the shortage of quality roughage during winter and can be easily cultivated (Iptas and Yılmaz, 1998). The type of cereals and legumes used in the mixture influences the yield and quality of the mixture. Furthermore, the most crucial factor determining forage yield and quality is the ratios of plants in the mixture (Carr et al., 2004).

Mixed cultivation of legumes and cereals offers several advantages. One of the most significant advantages is that mixed crops generally yield higher compared to sole cultivation of legumes (Ghanbari-Bonjar and Lee, 2003). Proper incorporation of vetch and cereal species into the mixture is crucial for ensuring abundant and balanced forage. Otherwise, in imbalanced mixtures, excessively competitive cereals can suppress vetch over time, reducing its ratios in the mixture (Moreira, 1989; Roberts et al., 1989). Mixed cropping provides increased resistance to diseases and helps control certain weeds (Sarunaite et al., 2010; Ghanbari-Bonjar and Lee, 2003). The primary objective of mixtures is to prevent vetch lodging. Therefore, studies have utilized different species and climatic conditions to determine optimal mixture ratios (Saglamtimur et al., 1986).

Common vetch (*Vicia sativa* L.) is a lean vetch plant with high energy content, rich in protein and minerals (Parissi et al., 2022), used for green fodder and roughage as well as green manure (Ozyazıcı and Manga, 2000; Ozyazıcı,



2022). It is an annual legume forage plant that is suitable for crop rotation with cereals and/or as a mixture with cereals (Munoz Santa et al., 2023). In recent years, triticale, used as a substitute for wheat and barley in animal husbandry (Yagbasanlar and Ulger, 1989), can be grown in combination with vetches (Soya et al., 1996; Hasar and Tukul, 1994). Iptas and Yilmaz (1998) reported that research indicates the best yield is obtained from mixtures of Hungarian vetch + triticale. In a study involving various vetch + cereal mixtures, they found that an average yield of 1388.0 kg da⁻¹ green forage, 316.0 kg da⁻¹ dry matter, and 56.4 kg da⁻¹ crude protein could be obtained from mixtures containing 2/3 Hungarian vetch + 1/3 Triticale. The mixture ratios significantly impact silage quality (Soya et al., 1991).

Silo feeds, used for the nutrition of ruminant animals, constitute a crucial source of roughage. Silage feeds hold significant economic importance, particularly in agriculturally advanced countries where a substantial portion of the feed volume is in the form of silage (Saricicek et al., 2002). Silage refers to the green and moisture-rich forage that undergoes fermentation (acidification) in an anaerobic environment and is stored as a result of the silage-making process (McDonald et al., 1991). Due to features such as palatability, long-lasting preservation without spoilage, and minimal susceptibility to adverse weather conditions, the use of silage has become widespread (Kilic, 1986; Filya, 2001; Acikgoz, 2001). Silages are composed of either grasses or leguminous plants, and these plants can be cultivated singly or in mixtures. It has been observed that silage obtained from grasses yields higher quality and is easier to ensile compared to leguminous plants. Leguminous forage crops are challenging to ensile due to their lower content of water-soluble carbohydrates (Pitt, 1990; Raques and Smith, 1966).

Both grasses and vetches are generally cultivated in our country for the purpose of obtaining forage and grain feed. The practice of making silage from the obtained vetch and grass forage is not yet widespread. Mixed cultivation of vetch and grasses, along with their evaluation as silage, could serve as an important source to meet the demand for high-quality feed.

This study was conducted with the aim of determining suitable mixture ratios for vetch and triticale mixed cultivation under Sakarya ecological conditions and assessing the silage quality at these mixture ratios.

2. Materials and Methods

The study was conducted at the Agricultural Sciences and Technologies Center of Sakarya Applied Sciences University in the Applied Research Field. The trial soils have a loamy texture. The soil pH was determined to be moderately alkaline (7.93), with a non-saline soil structure (0.12%), moderately calcareous (28.31%), and low organic matter (0.54%). In the research, vetch (*Vicia sativa* L.) (Yucel variety) and triticale (*x Triticosecale Wittmack*) (Okkan 54 variety) as a plant material were

used. Field trials were established according to a three-replicate randomized block design. Plot dimensions were set at a row length of 5 m and an inter-row distance of 20 cm, with 6 rows in each plot. One row on each side and 50 cm sections at the beginning of each row were discarded as border effects and the remaining portion was subjected to evaluation. The experiment included five different plantings: 100% Vetch, 75% Vetch + 25% Triticale, 50% Vetch + 50% Triticale, 25% Vetch + 75% Triticale, and 100% Triticale. To determine forage yield, plant height (cm), green forage yield (kg da⁻¹), and dry forage yield (kg da⁻¹) observations were taken during the grain maturity stage for vetch and milk stage for triticale (Anonymous, 2001).

Subsequently, 2 kilograms of samples were taken from each plot, cut into 1.5-2.0 cm pieces, placed in silage bags, vacuum-sealed, and closed. After a fermentation period of 60 days in laboratory conditions, matured silages were opened, and after discarding a 3-4 cm portion from the top of the silage containers, analyses were conducted on the remaining silage samples. Silage parameters such as dry matter content (%), silage pH (Budaklı Carpıcı, 2009), crude protein content (%) (Akyıldız, 1984), ADF (%), and NDF (%) were observed (Van Soest et al., 1991). Additionally, the quality class of the silage was calculated using the Fleig score method reported by Kilic (1986) [Fleig Score = 220 + (2 x % Dry matter - 15) - 40 x pH] (Ozata et al., 2012). Physical assessment of silages, including color, odor, and structure, was conducted by assigning scores according to the method specified by Alcicek and Ozkan (1997) (those in the range of 16-20 points were considered excellent-good (VG-G), 10-15 points were satisfactory (S), 5-9 points were medium (M), and 0-4 points were deemed low (L)).

2.1. Statistical Analysis

The obtained results were statistically analyzed using the Jump statistical analysis package program, and the averages were examined using the Duncan multiple comparison test (Anonymous, 2002).

3. Results and Discussion

3.1. Yield and Morphological Observation Values for Vetch-Triticale Mixtures

The plant height, green forage yield, dry matter content, and dry matter yield values obtained from vetch-triticale mixtures are presented in Table 1. The highest dry matter content was observed in 100% triticale with 44.13%. Similarly, in the study conducted by Kaplan et al. (2014), the dry matter content of the silage varied between 35.54% and 41.46%.

100% Triticale had a plant height of 83.22 cm, while in the 75% Vetch + 25% Triticale mixture, it was determined as 98.33 cm. Similarly, vetch had a plant height of 127.00 cm in sole planting, while in the 25% Vetch + 75% Triticale mixture; it was recorded as 142.67 cm (Table 1). As observed, mixed plantings positively influenced the plant height of both vetch and triticale. In a study conducted by Binici (2020) in Kahramanmaraş,

the highest vetch natural plant height was obtained from the 50% Wheat + 50% Vetch mixture with 72.20 cm, and the highest wheat plant height was 83.32 cm from the 75% Wheat + 25% Vetch mixture. In a study conducted by Egritas (2014) in Ordu ecological conditions, the longest cereal plant height, 108.25 cm, was obtained from the 50% Vetch + 50% Triticale mixture, both in pure and mixed plantings. Results from a study by Olgun (2018), on vetch-triticale mixtures in Kahramanmaras conditions, obtained the highest triticale plant height of 135.53 cm from the 50:17 triticale-vetch mixture, and the highest vetch plant height was 108.40 cm from the 50:33 triticale-vetch mixture. Acikgoz and Cakmakci (1986) also stated that the highest green herbage and dry matter yield was obtained from vetch + barley mixtures. The data obtained in other studies are in agreement with findings in line with our study.

The highest green forage yield in the experiment was obtained from 100% Vetch. The lowest green forage yield was obtained from the 75% Vetch + 25% Triticale mixture. Dry matter content was highest in 100% Triticale (44.13%) and lowest in 100% Vetch (13.87%), as expected. Dry forage yield was highest in 100% Vetch and the 25% Vetch + 75% Triticale mixture, parallel to the green forage yield (Table 1).

In his study, Olgun (2018) obtained the highest dry forage ratio in the triticale-vetch mixture with 30.54% from the 50 Vetch + 50 Triticale- mixtures. In mixed legume-cereal crops, Binici (2020) obtained the highest green forage yield of 2706.0 kg da⁻¹ from the 25% Vetch + 75% Barley mixture. Enayat et al. (2016), in a study

investigating the effects of common vetch and barley mixture ratios on forage yield, found an average green forage yield of 824.41 kg da⁻¹ and an average dry forage yield of 464.56 kg da⁻¹. Soya et al. (1991) obtained an average green forage yield of 1388.0 kg da⁻¹ from the 2/3 Hungarian vetch + 1/3 Triticale mixture. Demiroglu Topcu et al. (2020) determined the highest green forage yield in a pure plot with a 100-0 mixture ratio of 4250 kg da⁻¹. Olgun (2018) found a total green forage yield of 2979 kg da⁻¹ in the 50:50 triticale-vetch mixtures. Onal Ascı and Egritas (2017) reported the highest dry forage yield in the 50% Oat and 50% Vetch mixture at 8731.7 kg da⁻¹, while the lowest dry forage yield was obtained in plots with 100% Vetch planting (3627 kg da⁻¹). Binici (2020) obtained the highest dry forage yield of 1786.4 kg da⁻¹ from the 25% Vetch + 75% Wheat mixture. Enayat et al. (2016) determined an average dry forage yield of 464.56 kg da⁻¹ in a common vetch and barley mixture. Yucel and Avcı (2009) stated that triticale dry matter yield ranged from 1034 to 1252 kg da⁻¹ under Cukurova ecological conditions between 2004-2008. Albayrak et al. (2006), in their study on triticale, hay yield was 638.0-1892.5 kg/da. Demiroglu Topcu et al. (2020) obtained the highest dry forage yield of 717 kg da⁻¹ from 100% Vetch plots. While the dry matter content in our study is similar, it can be observed that our study has lower values in terms of green forage and dry forage yields. Yousif (2016) stated that dry forage yield ranged from 290.83 to 644.24 kg da⁻¹ in his research. This difference could be attributed to the use of different species and varieties, as well as location variations.

Table 1. Observation values and Duncan groups for Vetch-Triticale mixture ratios

Mixture Ratios	Triticale Plant Height (cm)	Vetch Plant Height (cm)	Green Forage Yield (kg da ⁻¹)	Dry Matter Content (%)	Dry Forage Yield (kg da ⁻¹)
100% Vetch	-	127.00b	852.78a	13.87e	295.99a
100% Triticale	83.22b	-	670.56bc	44.13a	118.34d
75% Vetch + 25% Triticale	98.33a	80.00c	662.78c	34.20d	226.65c
50% Vetch + 50% Triticale	92.33ab	83.00c	693.33bc	36.93c	255.95b
25% Vetch + 75% Triticale	93.44ab	142.67a	717.78b	38.73b	278.05a

^{a-d} Means with different letters in the same column are significantly different at P<0.05.

3.2. Observation Values for Vetch-Triticale Mixture Silages

The observation values obtained from the chemical analysis of mature silages are presented in Table 2. In the study, 100% Triticale had the highest ADF (acid detergent fiber) content (40.69%) and NDF (neutral detergent fiber) content (63.77%). The lowest ADF and NDF content was obtained from the mixture of 75% Vetch + 25% Triticale, with values of 34.54% and 50.47%, respectively. Since an ideal silage mixture is characterized by high protein content and low ADF and NDF content, it can be stated that the most suitable silage was obtained from the mixture of 75% Vetch + 25% Triticale (Table 2). In a study conducted by Egritas

(2014), the highest protein content (16.93%), lowest ADF (34.40%), and NDF (56.76%) were obtained from 100% Vetch plots. Yıldırım and Ozaslan Parlak (2016) reported that the highest NDF content (55.99%) was obtained from the mixture of 75% Triticale + 25% Peas, and the highest ADF content (36.23%) was obtained from the 100% Faba bean plot. Similarly, Seydosoglu et al. (2020) found that NDF and ADF content decreased as the legume ratio increased in mixed crops, with the highest NDF and ADF content obtained from 100% Barley plots and the lowest from 100% legume plots.

In the study, 100% Vetch had the highest protein content, while all other mixture ratios were in the same group (Table 2). Demiroglu Topcu et al. (2020) obtained the

highest protein content (23.1%) from the 100% Vetch plot (100-0). In a study investigating the nutrient content of winter silage feeds, the protein content of vetch silage was found to be 10.60-12.50% (Karakozak and Ayasan, 2010). Yıldırım and Ozaslan Parlak (2016) reported the highest protein content (21.47%) from 100% vetch and the lowest protein content (9.53%) from 100% Triticale plots.

The highest crude ash, Ca, K, Mg, P, and pH values of silage mixtures were obtained as follows: in 100% Vetch respectively, (20.72%), (1.40%), (5.28%), (0.40%),

(0.42%), and (4.92) (Table 2). Sahin et al. (2023) reported in their study that parallel to the increase in the clover ratio in silage, the pH of the silage also increased. They indicated that the lowest pH was obtained in 100% Triticale silage, while the highest pH was observed in 100% Vetch silage.

Yousif (2016) determined calcium at 0.68-1.14%, potassium at 0.642-0.864%, magnesium at 0.205-0.322%, phosphorus at 0.107-0.167%, and sodium at 0.007-0.032% in his study. Karakozak and Ayasan (2010) reported an ash content of 8.13-14.06% for vetch silage.

Table 2. Values obtained from Vetch-Triticale mixture silages with different mixing ratios and Duncan groups

Mixture Ratios	ADF (%)	NDF (%)	Crude Protein (%)	Crude Ash (%)	Ca (%)	K (%)	Mg (%)	P (%)	pH
100% Vetch	37.59b	44.40d	19.16a	20.72a	1.40a	5.28a	0.40a	0.42a	4.92a
100% Triticale	40.69a	63.77a	9.33b	11.26c	0.26c	3.78b	0.14cd	0.33b	4.22b
75% Vetch + 25% Triticale	34.54c	50.47c	11.19b	5.41d	0.49bc	3.34b	0.18bc	0.36ab	4.18b
50% Vetch + 50% Triticale	36.36bc	53.62bc	10.89b	5.67d	0.50b	3.47b	0.13d	0.33b	4.06b
25% Vetch + 75% Triticale	38.14b	56.32b	10.08b	13.47b	0.53b	3.32b	0.19b	0.22c	4.31b

^{a-d} Means with different letters in the same column are significantly different at P<0.05.

3.3. Fleig Scores of Silages Obtained from Vetch-Triticale Mixtures

The evaluation based on Fleig scores indicates that silage obtained from a 50% Vetch + 50% Triticale mixture is considered good, while silage from a 25% Vetch + 75% Triticale mixture is deemed satisfactory. Silage obtained from a 75% Vetch + 25% Triticale mixture and 100% Triticale is evaluated as medium, whereas silage from 100% Vetch is considered low (Table 3).

In their study, Sahin et al. (2023) obtained the lowest dry matter (DM) content from 100% Vetch (22.96%) and the highest DM content from 100% Triticale (29.87%). In a study conducted by Karadeniz et al. (2020) under ecological conditions in Mardin, they determined the quality characteristics of silage obtained by mixing different ratios of Grass pea + Triticale. The same study

reported the highest DM content at 33.3% from 100% Triticale and the lowest at 28.5% from 100% Grass pea, which aligns with the DM ratios obtained in our study.

Karadeniz et al. (2020) reported in their study that 100% Triticale and 20% Grass pea + 80% Triticale mixtures had the highest Fleig scores. Sahin et al. (2023) found the highest Fleig score in 100% Triticale silage (101.34), noting that an increase in the vetch ratio in mixtures resulted in a decrease in Fleig scores. They reported the lowest Fleig score in 100% Vetch silage (79.32). Dogan and Terzioğlu (2010) investigated the effects of mixture ratios on silage quality in forage pea-barley mixtures in Van conditions, determining that the silage was of medium quality according to the Fleig scoring. The results obtained in this study are supportive of findings in other research.

Table 3. Fleig scores of vetch triticale silage mixtures

Mixture Ratios	Dry Matter (%)	pH	Fleig point	Assessment
100% Vetch	0.14	4.92	8.61	Low
100% Triticale	0.46	4.22	36.99	Medium
75% Vetch + 25% Triticale	0.34	4.18	38.62	Medium
50% Vetch + 50% Triticale	0.37	4.06	43.35	Good
25% Vetch + 75% Triticale	0.39	4.31	33.52	Satisfactory

3.4. Physical Analysis Values of Vetch Triticale Silage Mixtures

The evaluations based on the physical and sensory analyses of the obtained silage are presented in Table 4. In the physical analysis of silage, an assessment is made based on scores given for color, odor, and structure. Accordingly, 100% Triticale silage is considered very good, all other mixed silages are regarded as satisfactory, and 100% Vetch silage is evaluated as medium and

satisfactory (Table 4).

High scores are observed to be obtained from the odor and color characteristics of 100% Triticale silage, and as the ratio of triticale in the mixture increases, the overall score also increases (Table 4).

Bulgurlu and Ergul (1978) reported that in legume and cereal mixed silage, legumes easily deteriorated in color and structure, negatively affecting the silage quality.

Table 4. Vetch triticale silage mixtures physical analysis scores

Mixture Ratios	Physical Analysis Scores	Assessment
100% Vetch	9.54	Medium/Satisfactory
100% Triticale	15.75	Very Good
75% Vetch + 25% Triticale	12.62	Satisfactory
50% Vetch + 50% Triticale	13.04	Satisfactory
25% Vetch + 75% Triticale	14.75	Satisfactory

In conclusion, upon evaluating the data obtained from the mixtures, it has been determined that planting both vetch and triticale together in a mixed cultivation results in better plant development. The mixture of 25% Vetch + 75% Triticale stands out in terms of both green and dry forage yield. Silages were assessed in three groups based on chemical content, Fleig score, and physical analysis criteria. It was observed that the mixtures of 25% Vetch + 75% Triticale, 50% Vetch + 50% Triticale, and 100% Triticale silages were prominent in these categories. It can be said that producing forage crops in a legume and cereal forage mixture provides a significant advantage for achieving a balanced ration to meet the roughage needs of livestock.

4. Conclusion

In terms of animal nutrition, it is observed that standalone cereal forage crops may not sufficiently meet the needs of animals. However, the cultivation of legumes and cereals together can provide a significant amount of protein and carbohydrates to meet the nutritional requirements of animals. In addition, making silage as a mixture of graminea forage crops and legume forage crops gave better results in terms of nutrient content than silage alone. As the ratios of legumes increases in the mixtures, the protein content also increases, positively impacting fermentation. On the other hand, an increase in the amount of cereals contributes to a higher level of easily digestible carbohydrates, enhancing silage quality. The examined mixture of 25% Vetch + 75% Triticale has been shown to yield high green and dry forage from a unit area, and the silage obtained from this mixture is of good quality. Therefore, due to the favorable outcomes in terms of both forage yield and silage quality, this mixture ratio can be recommended to producers.

Author Contributions

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

	M.Ö.	M.K	A.G.
C	100		
D	100		
S	100		
DCP		50	50
DAI	100		
L		50	50
W	50	25	25
SR	20	20	20
PM		50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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FRUIT QUALITY AND BIOCHEMICAL CHARACTERISTICS OF DIFFERENT STRAWBERRY TREE (*Arbutus unedo* L.) GENOTYPES GROWN IN GİRESUN

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Abstract: This study was conducted to examine fruit quality, chemical and biochemical properties of 20 strawberry tree (*Arbutus unedo* L.) genotypes that naturally spread in Bulancak district of Giresun province. In this context, fruit size, pH, SSC (Soluble Solid Content), color indices, specific sugars, Vitamin C, citric and malic acids were detected in the fruit samples of the evaluated genotypes. Fruit weight varied between 2.33 and 4.69 g, fruit width 16.85 and 20.62 mm, fruit length 14.52 and 17.93 mm, pH 3.36 and 3.88, SSC 11.60 and 19.60%. The range of the results of glucose, sucrose, fructose and total sugars were 2.32-8.34 g 100 g⁻¹, 0.02-1.93 g 100 g⁻¹, 3.08-17.93 g 100 g⁻¹ and 7.17-27.18 g 100 g⁻¹, respectively. The amount of vitamin C contained in the fruits of strawberry tree was determined as 13.53-126.60 mg 100 g⁻¹, the amount of citric acid was 0.16-0.69% and the amount of malic acid was 0.27- 1.30%.

Keywords: Strawberry tree, *Arbutus* spp., Fructose, Vitamin C, Fruit quality

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1. Introduction

Türkiye, in terms of its geographical location, is considered as the origin of many fruit species cultivated worldwide. The favorable ecological conditions contribute significantly to the presence of numerous species and varieties in the country. Out of 138 fruit species globally adopted, 80 can be cultivated in Türkiye, along with numerous wild fruit species known for their high nutritional value (Özbek, 1988; Sakaldaş, 2012). One such species naturally occurring in the Turkish flora is the strawberry tree, also known as strawberry tree (*Arbutus unedo* L.) which is also referred to as Dal çileği, Çilek ağacı, Enderek, and Sandal ağacı in Anatolia (Karadeniz, 2004). Belonging to the Heath family (Ericaceae), *Arbutus unedo* L. is utilized both as a fruit and an ornamental plant (Karadeniz et al., 1996; Pekdemir, 2010).

Recognized in some countries as the strawberry tree due to the resemblance of its fruits to strawberries, its native habitat includes Greece, Lebanon, Ireland, and Southern Europe, which encompasses Anatolia (Anonymous, 2003a, b, c; Anşın and Özkan, 1993; Karadeniz and Şişman, 2004). It has a distribution across the Mediterranean Region, Northwest, and Central America, with 12 species, among which *Arbutus unedo* L. and *Arbutus andrachne* L. are the most significant (Torres et al., 2002; Anonymous, 2016a, b; İslam and Pehlivan, 2016). Some commercially valuable cultivars include

Compacta, Elfin King, and Rubra (Christman, 2011; İslam and Pehlivan, 2016).

Although strawberry tree has a wide geographical distribution area, it grows together with oaks, wild olive trees, shrubs and many other tree and shrub species in red pine forests and maquis vegetation in regions where the Mediterranean climate prevails (Karadeniz et al., 1996; Sakaldaş, 2012).

In addition to being used as table food, the fruits of strawberry tree are used in jelly, marmalade and cake decoration, as well as in the production of some wines and liqueurs in European countries (Zenginbal and Gündoğdu, 2016; Şanlıdere Aloğlu et al., 2018). Additionally, the trees of this species are employed in landscaping, and their branches and leaves are valued in flower arrangements (İslam and Pehlivan, 2016). Due to its hard wood texture, it is also used as handicraft material and fuel (Onursal and Gözlekçi, 2007).

The fruits of strawberry tree possess significant potential in terms of carbohydrates, organic acids, vitamin C, phenolic compounds, flavonoid content, and antioxidant capacity (Ates et al., 2022; Balta et al., 2023). They contain approximately 14% sugar, high levels of vitamin C (15-8 mg) and tannins, and various compounds such as tannins leaves, sucrose, arbutin, methyl arbutin, and urson in the leaves, and tannins in the roots (Karadeniz, 2004).

Being a rich source of dietary fiber, strawberry tree fruits



exhibit protective effects against various diseases, including cancer and cardiovascular diseases, due to their abundant antioxidants. They strengthen the body, provide protection against microbes, and prevent illnesses. Moreover, they aid in the healing of kidney and bladder inflammations, alleviate gastrointestinal sluggishness, benefit liver insufficiency, assist in the expulsion of gallstones, and fortify nerves. In addition to its effects on lowering high blood pressure and relieving arteriosclerosis, it gives freshness to the skin and its fruits can be consumed by diabetics (Karadeniz, 2004; Zenginbal and Gündoğdu, 2016; Şanlıdere Aoloğlu et al., 2018)

The aim of this study was determining fruit quality and some biochemical properties of fruit samples taken from 20 arbutus genotypes selected from the naturally growing strawberry tree population in Bulancak District of Giresun province, Türkiye.

2. Materials and Methods

2.1. Plant Material

The materials used in this study were naturally grown populations of the strawberry tree (*Arbutus unedo* L.) with 20 different genotypes, located in the village of Pazarsuyu, Bulancak district, Giresun province. Randomly 500 g fruit samples were taken during the period when the fruits of these naturally growing plants turned red and the taste of the fruits was at its peak. The selected trees were both labeled and marked on their trunks with spray paint for identification purposes. The collected fruit samples were placed in storage containers, labeled, and promptly transported to the Pomology Laboratories of the Department of Horticulture at Ordu University Faculty of Agriculture, maintaining the cold chain to prevent deterioration. Various parameters such as fruit weight (g), fruit width (mm), fruit length (mm), color measurement, pH, and soluble solid content (%) were determined in the strawberry tree fruits. Genotypes collected from Bulancak are coded between B1-B20.

2.2. Fruit External and Internal Characteristics

Ten fruits were randomly selected from each plant, and their average fruit weight was determined by weighing them on a scale with a precision of 0.01 mg. The fruit width was measured using a digital caliper by assessing the widest distance perpendicular to the central axis of the fruits. Additionally, the fruit height was measured by determining the distance between the style tip and the fruit stalk using the same caliper. Ten fruit samples from the identified strawberry tree plants were homogenized for one minute using a hand blender after the addition of 40 ml of distilled water. The prepared fruit juice was then filtered, and a drop of the filtered juice was measured using a portable digital refractometer. The amount of soluble solid content (SSC) was determined by calculating the Brix value with the formula (equation 1) given below.

$$SSC (\%) = \frac{B \times V}{M} \quad (1)$$

B= brix degree determined in diluted sample (%), V= dilution volume of the sample (mL), M= weight of sample (g)

To measure the acidity of the prepared fruit juice, the pH-meter electrode was immersed in 10 ml of fruit juice. After waiting until the value stabilized, the reading was recorded as pH value.

Using a Konica-Minolta colorimeter, measurements were conducted on fruit peels with 2 replicates, and for each replicate, 5 fruits were measured for L^* , a^* , and b^* values. The L^* value represents the brightness, with 0 indicating black and 100 indicating white. 'a' indicates redness (-a for green), and 'b' indicates yellowness (-b for blue)."

2.3. Extraction of Sugars and Liquid Chromatographic Analysis of Sugars

Harvested fruits were frozen at -21 °C until sugar analysis. Glucose, sucrose, fructose and total sugar contents of fruit samples thawed at room temperature were determined using HPLC (HP-1100 series) RID (Refractive Index Detector) and Shim-Pack HRC NH2 (300x7.8 mm, 5µm) column according to the extraction method developed by Miron and Scahffer (1991). Sugar contents in the samples were established using an external standard and determined qualitatively and quantitatively based on calibration curves and retention time of the standard.

2.4. Extraction and Liquid Chromatographic Analysis Organic Acid and Ascorbic Acid

Harvested fruits were frozen at -21 °C until organic acid analysis performed. Fruit samples thawed at room temperature were determined by HPLC technique (HP-1100 series) using a UV detector and HPX 87H (300x7.8 mm, 5µm) column, according to the method developed by Bozan et al. (1997).

2.5. Statistical Analysis

The data were analyzed using the JMP 16.0 software (trial). Principal components and cluster analysis were performed based on physical and chemical properties of investigated strawberry tree genotypes.

3. Results and Discussion

3.1 Fruit External and Internal Quality

3.1.1. Fruit external quality

In strawberry tree genotypes; fruit weight, fruit width, fruit length, and fruit color are given in Table 1. Fruit weight values of the genotypes varied between 2.33 g and 4.69 g. Among the 20 selected genotypes, the largest fruits were found in type B20 with 4.69 g, and the smallest fruits were found in types B13 and B14 with 2.33 g. Among the genotypes, the highest fruit width was found in type B20 with 20.62 mm, and the lowest was in type B13 with 16.85 mm. Similarly, the genotype with the longest fruit length was determined as type B20 with 17.93 mm, and the shortest was determined as type B15 with 14.52 mm (Table 1).

Table 1. External and internal fruit quality of the strawberry tree genotypes

Genotypes	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	pH	SSC (%)	L*	a*	b*
B-1	3.59	19.23	16.61	3.71	16.40	36.26	24.62	33.42
B-2	2.96	17.50	15.98	3.80	16.80	31.35	34.18	31.56
B-3	3.81	18.38	15.25	3.73	14.00	30.55	32.10	31.89
B-4	3.15	18.07	16.70	3.56	17.60	32.21	30.01	35.54
B-5	3.71	18.79	16.11	3.68	19.60	35.39	28.45	31.93
B-6	3.61	19.23	16.72	3.88	16.80	30.38	28.68	36.44
B-7	3.85	19.55	16.78	3.57	15.20	34.24	32.82	31.54
B-8	4.54	20.48	17.51	3.61	19.20	37.15	33.90	35.42
B-9	3.48	19.34	16.38	3.67	14.80	32.63	37.38	28.77
B-10	2.95	18.50	15.65	3.36	14.40	37.90	31.67	36.51
B-11	2.92	19.80	16.66	3.88	13.20	30.52	34.78	34.50
B-12	3.72	20.26	17.14	3.84	13.20	35.51	35.38	34.39
B-13	2.33	16.85	14.70	3.78	14.80	30.45	34.69	27.86
B-14	2.33	17.62	14.81	3.86	11.60	34.10	35.68	34.41
B-15	3.41	17.14	14.52	3.76	14.40	33.13	34.25	30.50
B-16	2.75	18.18	15.67	3.77	15.20	34.29	30.97	33.57
B-17	3.60	19.55	16.45	3.67	16.80	30.20	36.40	30.46
B-18	2.98	19.36	16.83	3.75	15.60	34.13	31.51	35.07
B-19	4.20	20.32	17.41	3.82	17.20	30.86	34.84	31.87
B-20	4.69	20.62	17.93	3.66	15.20	30.87	34.88	30.01

In their study conducted in the central district of Giresun, Karadeniz and Şişman (2004) determined the fruit weight as 10.71 g, the fruit width as 27.96 mm and the fruit length as 24.37 mm. Yarılgaç and İslam (2007) found that strawberry tree types growing naturally in Ünye and its surroundings have fruit weights between 5.25 g and 10.30 g, fruit widths between 16.10 mm and 24.23 mm, and fruit lengths between 16.42 mm and 22.16 mm; Çelikel et al. (2008) found that the fruit weights of 5 promising types they selected from the local strawberry tree population growing in the Central Black Sea Region were 11.08, 8.06, 6.95, 6.30 and 6.17 g, respectively; Pekdemir, (2010) reported that the strawberry tree grown in Bulancak and Espiye districts of Giresun province has fruit weights between 2.28 g and 11.00 g, fruit width between 16.51 mm and 28.05 mm, and fruit length between 13.06 mm and 22.03 mm; in their study in Zonguldak province, Zenginbal and Gündoğdu (2016) determined fruit weight as 3.39 g to 7.25 g, fruit width as 17.75 mm to 23.18 mm, fruit length as 17.57 mm to 23.18 mm; in their study conducted on the Marmara island, İslam and Pehlivan (2016) reported that fruit weights were between 1.14 g and 8.19 g, fruit width was between 12.81 mm and 25.64 mm, and fruit length was between 10.87 mm and 27.64 mm. Sakaldaş (2012) found that strawberry trees selected from two regions in Çanakkale had a fruit width of 17.39 mm and a fruit length of 16.48 mm in the Atikhisar region; in the Umurböy region, they determined the fruit width as

16.57 mm and the fruit length as 15.69 mm. The fruit weight, width and length results we obtained in our study appear to be close to the literature reports. Climatic conditions and genetic factors can affect fruit weight, fruit width and length. Furthermore, this high variation can be explained by the rich genetic diversity of strawberry trees resulting from natural seed dispersal in the Black Sea region.

As a result of the examinations, it was determined by the color measurement values that the strawberry tree genotypes differed in size and appearance, but all the collected fruits were ripe, they generally had a red color in the color evaluations, and especially the B9, B17 and B13 genotypes were more clearly red than the others. In shell color measurements, L*, a* and b* values were measured as 37.90-30.20, 36.40-24.62, 36.51-27.86, respectively (Table 1). It can be thought that this variation in coloration is due to the high genetic diversity due to the genotypes being propagated by seeds. In a previous study conducted in the Black Sea region, it was reported that the skin color was dark-light red and the fruit flesh color was light orange (Toy, 2019).

3.1.2. Fruit internal quality

In strawberry tree genotypes, pH contents range between 3.36 and 3.88, with the highest pH value of 3.88 observed in B6 and B11 types, and the lowest pH value of 3.36 in B10 type. In a study by Karadeniz and Şişman (2004), the pH value in a strawberry tree type grown in the central district of Giresun was reported as 3.5;

Yarılgaç and İslam (2007) found pH values ranging from 3.70 to 4.01 in naturally grown strawberry tree types in Ünye and its surroundings. Koca et al. (2008) reported pH values between 3.80 and 3.99 in a study conducted in the Black Sea Region. Pekdemir (2010) determined pH contents of strawberry grown in the Bulancak and Espiye districts of Giresun province to be in the range of 3.64 to 4.10. The pH results obtained in our study align with the literature findings.

The SSC in genotypes varies between 11.60% and 19.60%, with the highest SSC value of 19.60% found in B5 type and the lowest SSC value of 11.60% in B14 type (Table 1). Karadeniz and Şişman (2004) reported an SSC of 22.9% in a strawberry tree type grown in the central district of Giresun. Yarılgaç and İslam (2007) found SSC values ranging from 16.62% to 24.02% in naturally grown strawberry tree types in Ünye and its surroundings. Koca et al. (2008) reported a range of 20.50% to 25.80% for soluble solid content in a study conducted in the Black Sea Region. Pekdemir (2010) reported SSC values ranging from 24.0% to 31.0% in strawberry tree grown in the Bulancak and Espiye districts of Giresun province. Sakaldaş (2012) found SSC contents of strawberry tree selected from two different regions in Çanakkale to be an average of 20.47% in Atikhisar and 23.74% in Umurbey. İslam and Pehlivan (2016) reported SSC values ranging from 12.00% to

24.00% in a study conducted in Marmara Island. Zenginbal and Gündoğdu (2016) stated an SSC ratio of 25.50% in a study conducted in the Western Black Sea Region. The SSC ratios obtained in this study were found to be lower than those reported by previous researchers. This situation can be explained mainly by genotype differences or climatic differences (Ates et al., 2022; Kurnaz et al., 2023).

3.1.3. Biochemical compounds

3.1.3.1. Sugar composition of strawberry tree genotypes

The sugar composition results of the selected strawberry tree genotypes' fruit juices are presented in Table 2. The most prevalent sugars found in the fruit juice were determined to be fructose, glucose, and sucrose. Among the selected strawberry tree genotypes, the highest glucose content was 8.34 g/100 g in B2 type, while the lowest was 2.32 g/100 g in B15 type. In a study conducted by Ayaz et al. (2000), the glucose content in strawberry tree fruits was reported as 21.50% of the dry weight. Gündoğdu et al. (2018) found the glucose content in strawberry tree fruits in the western part of Türkiye to be 6.10 g/100 g. Sagbas et al. (2020) reported glucose content ranging from 3.85 to 6.07 g/100 g. Ait Lhaj et al. (2021) determined the glucose content of selected strawberry tree from various regions in Morocco to be between 11.6 and 15.2 g/100 g.

Table 2. Sugar compositions (g 100g⁻¹) of the strawberry tree genotypes

Genotypes	Sucrose (g 100g ⁻¹)	Glucose (g 100g ⁻¹)	Fructose (g 100g ⁻¹)	Total Sugars (g 100g ⁻¹)
B-1	0.20	6.75	8.40	15.35
B-2	0.48	8.34	14.00	22.82
B-3	0.27	6.65	12.32	19.24
B-4	0.25	6.83	9.82	16.90
B-5	0.02	4.21	8.83	13.06
B-6	0.40	7.08	14.15	21.63
B-7	0.35	6.81	9.67	16.83
B-8	0.25	7.71	13.69	21.65
B-9	0.42	5.00	4.85	10.27
B-10	0.21	7.00	11.80	19.01
B-11	0.43	5.35	10.54	16.32
B-12	0.72	4.32	8.30	13.34
B-13	0.22	6.05	7.48	13.75
B-14	1.19	8.06	17.93	27.18
B-15	1.22	2.32	3.63	7.17
B-16	1.43	3.72	3.08	8.23
B-17	0.43	5.93	11.72	18.08
B-18	1.93	8.08	12.47	22.48
B-19	1.58	7.09	15.37	24.04
B-20	1.56	8.04	17.13	26.73

The fructose content in the fruits ranged from 3.08 g/100 g in B16 type to the highest value of 17.93 g/100 g in B14 type. Ayaz et al. (2000) reported fructose content as 27.80% of the dry weight of strawberry tree fruits. Gündoğdu et al. (2018) determined fructose content as

11.63 g/100 g in soluble sugars. Sagbas et al. (2020) found fructose content to range from 6.09 to 10.56 g/100 g. Ait Lhaj et al. (2021) reported fructose content in selected strawberry tree from various regions in Morocco as between 8.7 and 13.1 g/100 g.

Sucrose levels in the strawberry tree types ranged from 0.02 g/100 g in B5 type to the highest value of 1.93 g/100 g in B18 type. Ayaz et al. (2000) identified and determined sucrose sugar in strawberry tree fruits as 21.50% of the dry weight. Koca et al. (2008) reported sucrose levels ranging from 0.00 to 28.02 g/100 g. Gündoğdu et al. (2018) found sucrose content in fruits to be 1.44 g/100 g. Sagbas et al. (2020) identified sucrose content ranging from 0.78 to 1.56 g/100 g. The literature findings align with our study results, supporting the observed sucrose levels. Ait Lhaj et al. (2021) determined sucrose content in selected strawberry tree from various regions in Morocco to be between 4.2 and 8.1 g/100 g.

Total sugar contents in the selected strawberry tree types exhibited a variation between 7.17 and 27.18 g/100 g, with the highest total sugar content found in B14 type and the lowest in B15 type. Alarco-E-Silva et al. (2001) reported that strawberry tree fruits have a high sugar capacity, accounting for 42% of the fruit. Koca et al. (2008) found reducing sugar levels in the range of 108.82 to 182.80. The sugar content results in strawberry tree from previous studies align with our literature findings, supporting and validating our results.

3.1.3.2. Organic acid composition of strawberry tree genotypes

Organic acids play a significant role in fruits and are primarily composed of citric, malic, and tartaric acids in most fruit types. These organic acids play a crucial role in preserving the quality of products derived from fruits. Fruit ripening, aging, taste, and various other characteristics are significantly influenced by the content of organic acids (Saradhuldhath and Paull, 2007; Etienne

et al., 2013). The organic acid content in the fruit juice of strawberry tree genotypes was determined, and the amounts of citric, L-ascorbic, and malic acids in the fruit juice were identified (Table 3). The C vitamin (L-ascorbic acid) content of the examined strawberry tree types ranged from the highest of 126.60 mg/100 g in B6 type to the lowest of 13.53 mg/100 g in B12 type. Koca et al. (2008) determined the ascorbic acid content to be in the range of 223.60 mg to 395.20 mg per 100 g of fruit in their study. Çelikel et al. (2008) reported that the C vitamin content of selected strawberry tree genotypes from the Central Black Sea Region ranged from 97.83 to 280.00 mg/100 g. Ruiz-Rodriguez et al. (2011) identified an average vitamin C content of 202.60 mg/100g in strawberry tree from two different regions in Spain. Sakaldaş (2012) detected vitamin C content in strawberry tree from two locations in Çanakkale as 154.65 mg/100g in Atikhisar region and an average of 143.50 mg/100g in Umurbey region. Ulloa et al. (2015) determined the ascorbic acid content as 18.85 mg/100 g in strawberry tree grown in Portugal. Gündoğdu et al. (2018) reported an average vitamin C content of 56.22 g/100g in strawberry tree genotypes naturally grown in the western part of Türkiye, while Sagbas et al. (2020) reported vitamin C content ranging from 58 to 93 mg/100 g FW in strawberry tree genotypes. The results of our study on ascorbic acid content align with the literature findings. While the C vitamin content is mainly determined by the genotype, factors such as harvest date, growing region, etc., also significantly affect the vitamin C content (Ruiz-Rodríguez et al., 2011; Çelik et al. 2019 Ates, 2023).

Table 3. Organic acid compositions of the strawberry tree genotypes

Genotypes	L-Ascorbic acid (mg 100 g ⁻¹)	Citric acid (%)	Malic acid (%)
B-1	58.68	0.32	0.71
B-2	67.00	0.50	0.51
B-3	39.55	0.19	0.55
B-4	35.24	0.31	0.62
B-5	16.08	0.23	0.54
B-6	126.60	0.21	0.90
B-7	50.51	0.52	0.77
B-8	41.10	0.55	1.09
B-9	32.14	0.19	0.56
B-10	61.12	0.69	1.30
B-11	50.89	0.42	0.94
B-12	13.53	0.18	0.27
B-13	64.13	0.16	0.93
B-14	16.04	0.34	0.65
B-15	57.57	0.37	0.49
B-16	38.70	0.36	0.73
B-17	38.34	0.38	0.77
B-18	14.96	0.20	0.76
B-19	101.61	0.38	0.55
B-20	60.94	0.29	0.42

Malic and citric acids have been reported as the main organic acids contributing to the pleasant sour taste of strawberry tree fruits in previous studies (Vidrih et al., 2013). In our study, the citric acid content of strawberry tree types ranged from 0.16% to 0.69%, with B10 containing the highest citric acid and B13 the lowest (Table 3). Ayaz et al. (2000) reported the presence of citric acid in strawberry tree fruits in their study. Gündoğdu et al. (2018) investigated phenolic compounds, biochemical characteristics, and pomological features in strawberry tree genotypes naturally grown in the western part of Türkiye, determining citric acid content in the range of 0.25 to 0.87 g/100 g. Sagbas et al. (2020) found citric acid content in strawberry tree genotypes' fruit juice ranging from 1.03 to 0.44 g/100 g FW. Regarding malic acid levels, the strawberry tree types analyzed in our study showed B10 as the type with the highest malic acid content at 1.30% and B12 as the type with the lowest malic acid content at 0.27% (Table 3). Ayaz et al. (2000) determined the malic acid content, classified under the non-volatile acids group, as 0.84 mg/g in strawberry tree fruits. Gündoğdu et al. (2018) investigated phenolic compounds, biochemical characteristics, and pomological features in strawberry tree genotypes naturally grown in the western part of Türkiye, determining malic acid as the main organic acid and measuring its content in the range of 0.67 to 2.33

g/100 g. Sagbas et al. (2020) found malic acid content in strawberry tree genotypes' fruit juice ranging from 1.12 to 0.37 g/100 g FW. Plant genotypes (Mikulic Petkovsek et al., 2007) and environmental conditions (Wu et al., 2007) affect the organic acid content of fruits. Additionally, horticultural practices such as irrigation, pruning, and soil type have been reported to influence organic acid content (Hudina et al., 2006).

3.1.4. Principal components analysis

Out of the 14 principal components, 5 PCs had eigenvalue higher than 1.0 these components explained 78.9% of total variation. PC1 was mainly related to fruit width, fruit height, glucose, fructose and total sugars, explaining 26.1% of total variability. PC2, which explained 19.0% of total variability, was related to pH, L, sucrose, citric acid and malic acid. Explaining 15.0% of total variability, PC3 was related to fruit weight (Figure 1).

According to cluster analysis results, strawberry genotypes divided into two main group. The first group included five strawberry genotypes (B-9, B-12, B-13, B-15 and B-16). The second group divide into two sub-cluster. First sub-cluster consisted of two strawberry genotypes (B-8 and B-10). The second sub-cluster included 13 strawberry genotypes (B-1, B-2, B-3, B-4, B-5, B-6, B-7, B-11, B-14, B-17, B-18, B-19 and B-20) (Figure 2).

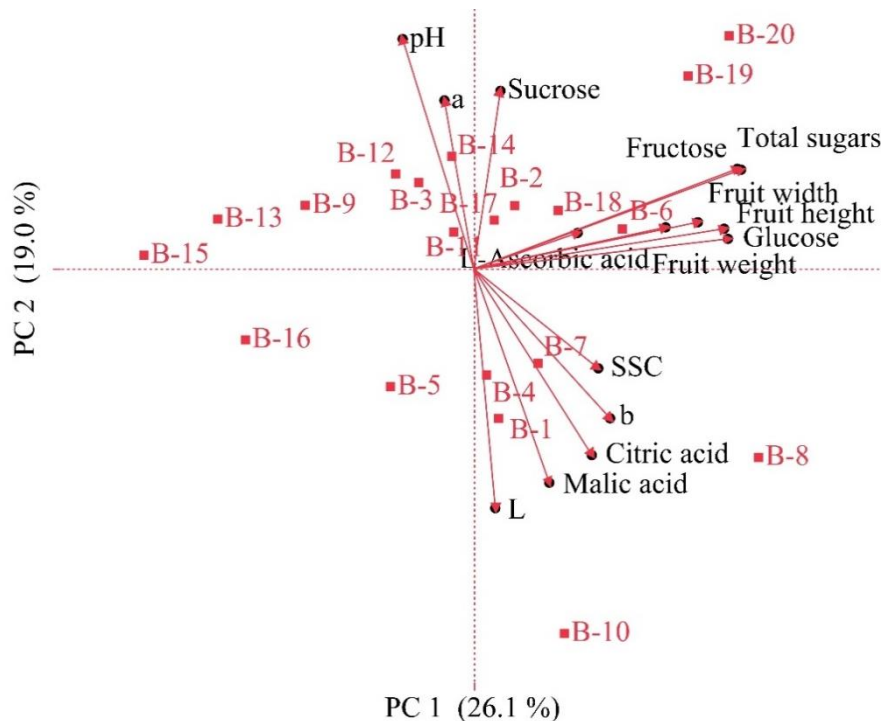


Figure 1. Component plot of the first two principal components in the strawberry genotypes

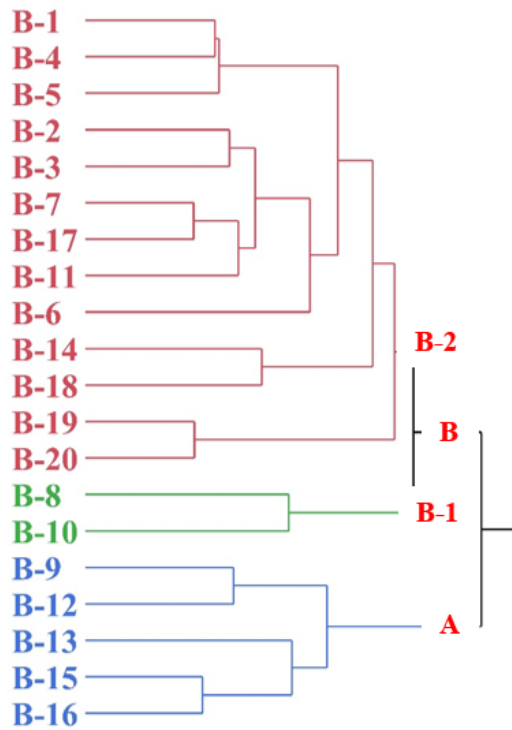


Figure 2. Dendrogram grouping of strawberry genotypes based on fruit quality characteristics

4. Conclusion

The strawberry tree (*Arbutus unedo* L.) and its fruits are not widely recognized in terms of horticultural characteristics. However, there are limited studies suggesting that the fruit content possesses important health-related properties. Based on the results obtained in our study, it is considered to have significant contents beneficial for human health, and it could serve as an important natural resource in the market. The genotype-based horticultural characteristics and fruit contents obtained in this study could make a substantial contribution to the development of the food and pharmaceutical sectors. In addition, it can make significant contributions to the development of both the food and pharmaceutical industries with its unique taste and rich bioactive content. Moreover, the observed wide variation in the examined traits may be valuable for future consideration by plant breeders as a genetic resource.

Author Contributions

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

	S.Ç.	M.Y.
C	10	90
D		100
S		100
DCP	80	20
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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THE EFFECT OF TREATED WASTEWATER LEVELS ON THE ROOTING OF BLACKBERRY (*Rubus fruticosus* L.) GREEN CUTTINGS

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Abstract: The world population is growing, leading to a gradual decrease in available water supplies. Reusing wastewater is seen as beneficial for environmental conservation and mitigating water contamination. Recycled wastewater includes essential nutrients for agricultural growth, and its reuse can lower the need for fresh water in dry areas. This study examined the impact of diluted treated wastewater at various ratios on the roots and shoot growth of blackberry green cuttings. For this purpose, Jumbo blackberry green cuttings located at the Bilecik Şeyh Edebali University Agricultural Practice and Research Center were used in 2023. In September, cuttings were taken and treated with a control (0 ppm IBA) and a 4000 ppm dose of indole-3-butyric acid (IBA). The cuttings were then planted in rooting tables with perlite and bottom heat (22±2 °C). From the time of planting, the cuttings were irrigated with treated wastewater from the university's wastewater treatment facility, diluted in five different doses (0, 25, 50, 75, 100 %). In the cuttings removed from the rooting medium after 90 days, the following were determined: survival rate (%), rooting rate (%), callus formation rate (%), number of roots (per cutting), root length (cm), root diameter (mm), fresh and dry root weight (g), chlorophyll a (µg/g DW), chlorophyll b (µg/g DW), and total chlorophyll content (µg/g DW). At the end of the trial, the positive effects of treated wastewater on rooting had been determined. The rooting rate was determined to be best in the Control (0 ppm IBA)+TWW100 application (73%). The research suggests that blackberry cuttings can be rooted using treated wastewater without the need for IBA


Keywords: Chlorophyll, IBA, Jumbo, Rooting rate

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1. Introduction

Water is acknowledged as the most crucial resource for sustainable agricultural development globally (Davies and Simonovic, 2011; Ungureanu et al., 2020). It is a crucial resource for supporting economic growth and social well-being. Factors such as fast worldwide population growth, changes in land use, the expansion of productive activities including agriculture, industry, and tourism, and urban development have contributed to the deterioration of water supplies (Liu et al., 2017; Zhang et al., 2017). Recently, there has been a consistent rise in the demand for water resources. Global water usage has increased by an average of 1% annually from 1980 to the present (Velasco et al., 2018). The growth is projected to persist until 2050, with an anticipated surge of 20-30% above present levels in the next several years (Bouwer, 2002).

The rising need for water resources, coupled with the incapacity to fulfill this need, can be eased by utilizing wastewater for irrigation, thus reducing strain on freshwater resources (Toze, 2006), and can also reduce the need for fertilizers due to their nutrient content (Angelakis, 1999). Both treated and untreated

wastewater is utilized for irrigation in certain arid and semi-arid regions for various reasons. Research indicates that regions irrigated with wastewater see heightened soil biological activity and nitrogen cycling (Filip et al., 1999; Speir, 2002; Chen et al., 2008).

Wastewater can decrease heavy metal levels based on its composition (Charerntanyarak, 1999; Brown et al., 2000; Rae and Gibb, 2003; Lesage et al., 2007) and microbial pathogens (Zhang and Farahbakhsh, 2007; Reinoso et al., 2008), as well as increase organic and mineral components (Mujeriego and Asano, 1999; Toze, 2006; Muga and Mihelcic, 2008).

The blackberry plant, scientifically known as *Rubus fruticosus*, belongs to the *Rosaceae* family and is part of the aggregate fruit group (Padmanabhan et al., 2016). Its adaptability in different environments and its sweet yet somewhat tangy flavor have caused a rise in popularity in recent times.

Blackberry fruit is a rich source of carbohydrates, protein, ascorbic acid, sugars, carotenoids, minerals, and vitamins (Zia et al., 2014). Blackberry plants are commercially propagated using cuttings from breeding plants (Lopez-Medina and Moore, 1997; Bray et al., 2003;



Takeda et al., 2011).

Various irrigation methods are used in blackberry cultivation. Blackberries intended for fresh consumption are typically irrigated with drip systems, whereas those for processed consumption are mainly watered with overhead sprinklers, mobile pipes, or huge gun systems (Strik and Finn, 2012).

Global warming has caused more hot weather, resulting in drought as a major environmental stress that greatly hinders plant growth and development in many areas. Therefore, the need for water resources is increasing. Wastewater is now being utilized as a solution to address the challenges of global water scarcity.

Worldwide studies have reported examples of wastewater reuse in the irrigation of table grapes (Petousi et al., 2019), olives (Petousi et al., 2015), and even vegetables (Christou et al., 2017; Farhadkhani et al., 2018; Libutti et al., 2018; Mehmood et al., 2019). However, no studies have investigated the use of treated wastewater for irrigating blackberry cuttings.

This study examined the impact of treated wastewater,

diluted at different ratios, on the roots and shoot growth of blackberry green cuttings. The study investigated the feasibility of utilizing treated wastewater in agriculture and its effects on rooting. It also determined how propagation through cuttings responded to these waters.

2. Materials and Methods

This study was carried out in 2023 at the Bilecik Şeyh Edebali University Agricultural Application and Research Center, within a high tunnel rooting system. The material used was the Jumbo blackberry variety obtained from the research center. The specific origin of the Jumbo blackberry variety is uncertain, but it has been grown in America since 1920 and has shown positive results in adaptation tests undertaken in our nation (Akbulut et al., 2003; Cangi and İslam, 2003; Gerçekcioğlu et al., 2003). The treated wastewater used in the study was obtained from the Bilecik Şeyh Edebali University wastewater treatment facility and diluted according to the doses used in the study. The chemical values of the treated wastewater are provided in Table 1.

Table 1. Chemical analysis results of the treated wastewater used in the study

Chemical properties of treated wastewater ¹	Average values	Limit value ²
Total Suspended Solids (mg l ⁻¹)	20.35	70
Chemical Oxygen Demand (mg l ⁻¹)	58.84	180
Biological Oxygen Demand (mg l ⁻¹)	34.00	50
pH	8.42	6-9

¹ Conducted within the scope of the Ministry of Environment, Urbanization and Climate Change qualification certificate and TÜRKAK.

² Limit values, water pollution control regulation.

The green cuttings were taken on September 15th, a time identified by Edizer (2011) as the most suitable for vegetative multiplication in the Jumbo blackberry variety, as stated in his research on assessing the vegetative propagating potential of this variety. 15-20 cm cuttings from one-year-old branches were treated with a 0.3% fungicide (Benlate) and let to dry for about ten minutes before planting.

In the experiment, along with a control treatment, five different levels of treated wastewater (0%, 25%, 50%,

75%, and 100%) were used in conjunction with the 4000 ppm IBA dose, which was determined to be the best rooting rate for green cuttings (Edizer, 2011) (Table 2). The basal sections of the cuttings were immersed in the IBA solution utilizing the quick dip technique (Zenginbal and Eşitken, 2016), subsequently planted in rooting tables that contain perlite with bottom heat provision (22±2 °C). Irrigation was conducted at the designated water levels at the time of planting.

Table 2. The IBA dose applied in the study and the concentrations of treated wastewater

Applications	Definition of treated wastewater (TWW) concentration
Control (0 ppm IBA)+TWW0	4000 ppm IBA+TWW0 100% pure water was used
Control (0 ppm IBA)+TWW25	4000 ppm IBA +TWW25 25% treated wastewater and 75% pure water was used
Control (0 ppm IBA)+TWW50	4000 ppm IBA +TWW50 50% treated wastewater and 50% pure water was used
Control (0 ppm IBA)+TWW75	4000 ppm IBA +TWW75 75% treated wastewater and 25% pure water was used
Control (0 ppm IBA)+TWW100	4000 ppm IBA+TWW100 100% treated wastewater was used

TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

Blackberry cuttings were placed on propagation rooting tables with perlite for 90 days. After the study ended, the cuttings were removed to assess the following parameters: survival rate (%), callus formation rate (%), number of roots (per cutting), root length (cm), root thickness (mm), fresh and dry root weight (g), chlorophyll a (g l⁻¹), chlorophyll b (g l⁻¹), and total chlorophyll content (g l⁻¹).

The trial was conducted according to a randomized block trial design with 3 replications, and each replication consisted of 15 cuttings. Following the analysis of variance, the means of the treatments were compared using the Least Significant Difference (LSD) multiple comparison test. The statistical analyses were performed using the MSTAT-C software package (Michigan State University v. 2.10).

3. Results and Discussion

Statistically significant differences were found in all categories except for the ratio of live cuttings and callus formation when analyzing the data acquired from the use

of treated wastewater on blackberry cuttings. Survival was determined by counting callused and rooted cuttings. Figure 1 shows that the survival rate of cuttings and the rate of callus formation were comparable. There was no statistical difference between the treatments, except for when 4000 ppm IBA+TWW100 was applied. The control group exhibited a 100% survival rate and callus ratio at all concentrations of treated wastewater (Figure 1). Edizer (2011) conducted a study on blackberry green cuttings in September and discovered that the survival rate of cuttings and callus formation was comparable at a 4000 ppm IBA dose. They were reported to have achieved a 100% success rate in both the control group and the group treated with a 4000 ppm dose. Our investigation showed a 100% success rate in survival cuttings and callus formation while using the control application. However, a decrease in success rate was noted in applications other than the 25% treated wastewater (4000 ppm IBA+TWW25) at the 4000 ppm dose.

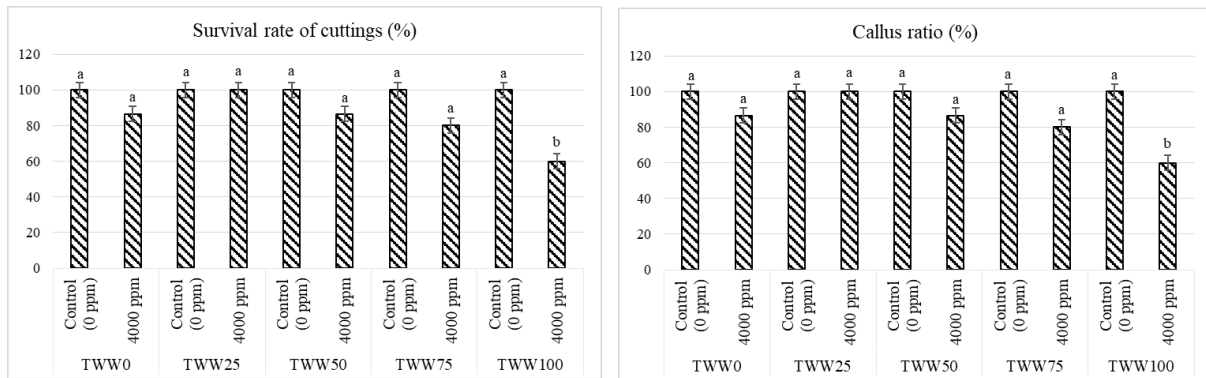


Figure 1. The effect of treated wastewater on the survival rate(%) and callus ratio (%) of Jumbo blackberry cuttings. TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

The effects of treated wastewater applications on the rooting rate, root number, root length, root diameter, root fresh and dry weight of blackberry cuttings are presented in Tables 3 and 4. The highest rooting rate average was found in the Control group (0 ppm) at 67.21%. After analyzing the mean concentrations of treated wastewater, it was discovered that concentrations of 50%, 75%, and 100% are statistically equivalent, with the optimal value identified as 75% (71.00%) (Table 3). The findings from the control group, 4000 ppm IBA + TWW50, and 4000 ppm IBA + TWW75 applications are statistically similar when analyzing the interaction table for rooting rate and treated wastewater. The control group achieved the maximum rooting rate of 73% with the application of 100% wastewater (Control (0 ppm IBA)+TWW100). (Table 4). As mentioned in previous studies, it has been observed that wastewater has an effect on rooting. Yıldız et al. (2009) reported a 24% rooting rate as a result of applying 6000 ppm IBA,

Roussos et al. (2020) achieved an 85% rooting rate following the application of 2000 ppm IBA, and Edizer (2011) reported a 100% rooting rate in green cuttings after applying 4000 ppm IBA. The study results showed that the rooting rate of 35% achieved with the treatment of 4000 ppm IBA+TWW0 was low. This is believed to be caused by temperature and environmental factors, as mentioned in the literature (Cigdem et al., 2022).

The analysis of the mean values for the applications for the number of roots revealed that the combination of a 4000 ppm IBA dose and 100% treated wastewater yielded the most favorable outcomes (Table 3). The analysis of the interaction table revealed that the number of roots per cutting ranged from 3.83 to 10.03. The Control (0 ppm IBA)+TWW75 application has the fewest roots (3.83 per cutting), whereas the 4000 ppm IBA+TWW100 application has the most roots (10.03 per cutting). Treated wastewater has been found to have a beneficial impact on root growth at the 4000 ppm IBA

concentration (Table 4).

The most favorable results for root length and root diameter averages were found to be 3.21 cm and 0.67 mm, respectively, in the Control (0 ppm IBA) group. Upon examining the average concentrations of treated wastewater, it was found that the best root length was obtained at 75% concentration (TWW75), while the best root diameter was obtained from both 25% (TWW25) and 75% (TWW75) concentrations (Table 3). The best root length was determined in the Control (0 ppm IBA)+TWW75 interaction, while the lowest root length was found in the 4000 ppm IBA+TWW25 interaction (Table 4). The root diameter interaction with treated wastewater showed that the Control (0 ppm IBA)+TWW0 and Control (0 ppm IBA)+TWW25 applications had statistically similar diameters of 0.84–0.82 mm. Reductions were observed in the interaction between treated wastewater and the 4000 ppm IBA dose (Table 4). The beneficial impacts of treated wastewater combined with 4000 ppm IBA on rooting rate and root quantity have resulted in a reduction in root length and diameter. In *Helianthus annuus* (Fozia et al., 2008), *Phaseolus vulgaris* (Bhardwaj et al., 2009), and *Pisum sativum* (Hattab et al., 2009) plants, a decrease in root length was also observed, which is reported to likely result from the accumulation of toxic elements in the root zone. Kocak et al. (2005) reported that the nutrients contained in wastewater become toxic to plants after irrigation with wastewater.

The control (0 ppm IBA) treatment yielded superior

results in terms of root fresh and dry weights compared to the 4000 ppm IBA dose. The concentrations of treated wastewater had no statistically significant effect on root fresh weight. For root dry weight, it was determined that the 75% (TWW75) concentration yielded the best result. Upon examining the interaction between root fresh weight and treated wastewater, it was determined that the control (0 ppm IBA)+TWW50 and 4000 ppm IBA +TWW25 applications, respectively, yielded the best results. For root dry weight, it has been observed that the Control (0 ppm IBA)+TWW0 application was statistically the best. Baskaran et al. (2009) reported that diluted sugar factory wastewater maintained optimum nutrient levels, which in turn increased the dry weight of *V. radiata* plants. Plant biomass decreased when exposed to concentrated sugar mill wastewater. Marwari and Khan (2012) found that irrigating plants with 20-30% polluted water led to a reduction in both fresh and dry biomass. Dagianta et al. (2014) reported that the application of wastewater along with fertilization reduced the dry matter content of the biomass in peppers. Anwar et al. (2016) noted that the biomass of mint, coriander, and fenugreek was negatively affected when irrigated with wastewater. Ganjegunte et al. (2017) reported that there was no significant difference in biomass production of grasses irrigated with wastewater and freshwater within a specific year. It has been determined that the data obtained from these studies is consistent with our findings.

Table 3. Average values of the examined features

Applications	RR	RN	RL	RD	RFW	RDW
Control (0 ppm IBA)	67.21 ^a	6.41 ^b	3.21 ^a	0.67 ^a	0.41 ^a	0.08 ^a
4000 ppm IBA	53.05 ^b	8.41 ^a	2.87 ^b	0.55 ^b	0.27 ^b	0.06 ^b
	RR	RN	RL	RD	RFW	RDW
TWW0	49.56 ^b	7.22 ^b	2.72 ^c	0.61 ^b	0.28	0.09 ^b
TWW25	51.00 ^b	7.64 ^{ab}	2.84 ^c	0.72 ^a	0.35	0.03 ^e
TWW50	60.00 ^{ab}	7.44 ^b	3.20 ^b	0.52 ^c	0.41	0.05 ^d
TWW75	71.00 ^a	6.17 ^c	3.46 ^a	0.69 ^a	0.28	0.13 ^a
TWW100	69.09 ^a	8.58 ^a	2.98 ^{bc}	0.50 ^c	0.40	0.07 ^c

^{a-c} Means with different letters in the same column are significantly different at P<0.05. RR= Rooting rate (%), RN= Root number (per cutting), RL= Root length (cm), RD= Root diameter (mm), RFW= Root fresh weight (g), RDW= Root dry weight (g). TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

Table 4. Average values of doses and interactions of the examined traits

Applications	RR	RN	RL	RD	RFW	RDW
Control (0 ppm IBA)+TWW0	63.33 ab	9.44 a	2.93 d	0.84 a	0.53 ab	0.16 a
Control (0 ppm IBA)+TWW25	66.00 ab	5.62 d	3.22 a-d	0.82 ab	0.11 de	0.02 i
Control (0 ppm IBA)+TWW50	60.00 ab	6.02 cd	3.40 ab	0.45 e	0.69 a	0.06 e
Control (0 ppm IBA)+TWW75	66.00 ab	3.83 e	3.55 a	0.66 cd	0.29 cd	0.11 c
Control (0 ppm IBA)+TWW100	73.00 a	7.14 bc	2.95 d	0.61 cd	0.47 abc	0.10 d
4000 ppm IBA+TWW0	35.00 c	5.00 de	2.52 e	0.36 e	0.03 e	0.01 j
4000 ppm IBA +TWW25	46.00 bc	9.65 a	2.45 e	0.65 cd	0.58 a	0.04 f
4000 ppm IBA +TWW50	60.00 ab	8.86 a	3.01 cd	0.57 d	0.13 de	0.03 h
4000 ppm IBA +TWW75	66.00 ab	8.50 ab	3.36 abc	0.72 bc	0.27 cde	0.15 b
4000 ppm IBA+TWW100	66.00 bc	10.03 a	3.02 bcd	0.39 e	0.34 bcd	0.02 g

^{a-i} Means with different letters in the same column are significantly different at $P < 0.05$. RR= rooting rate (%), RN= root number (per cutting), RL= root length (cm), RD= root diameter (mm), RFW= root fresh weight (g), RDW= root dry weight (g). TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

The total chlorophyll concentration of plants is a crucial indicator of their physiological state and photosynthetic efficiency. The chlorophyll contents are shown in Figure 2. In the control (0 ppm IBA) dose application, it was observed that the total chlorophyll amount increased as the concentration of treated wastewater increased, while in the 4000 ppm IBA dose application, a decrease is observed up to the TWW100 concentration. The highest total chlorophyll content was achieved with the 4000 ppm IBA+TWW100 combination (Figure 2). Chlorophyll a content showed a consistent rise at all dosages and concentrations, except for the control (0 ppm IBA) +TWW100 application. The highest chlorophyll b level was found in the 4000 ppm IBA+TWW0 treatment. The increase in chlorophyll content in the cuttings' leaves when treated with wastewater is believed to be caused by the nutrients present in the wastewater, as indicated by the results. Nutrients act as building blocks for

proteins and enzymes that play a crucial role in the proper formation of pigment biosynthesis. Liu et al. (2002) observed a decrease in chlorophyll levels when seedlings were irrigated with wastewater. Manisha and Angoorbala (2013) obtained a decrease in chlorophyll content with increasing concentrations of wastewater. Faizan et al. (2014) reported higher chlorophyll a, chlorophyll b, and total chlorophyll content in okra irrigated with treated wastewater. Hassena et al. (2018) determined that young olive plants irrigated with treated wastewater showed an improvement in growth, soluble sugars, photosynthetic rate, and in the content of chlorophyll a, b, and total chlorophyll. The use of treated wastewater is thought not only to reduce the need for fertilizers due to its watering purpose but also because of its nutritive properties. Moreover, it offers a solution to reduce environmental pollution (Seleiman et al., 2021).

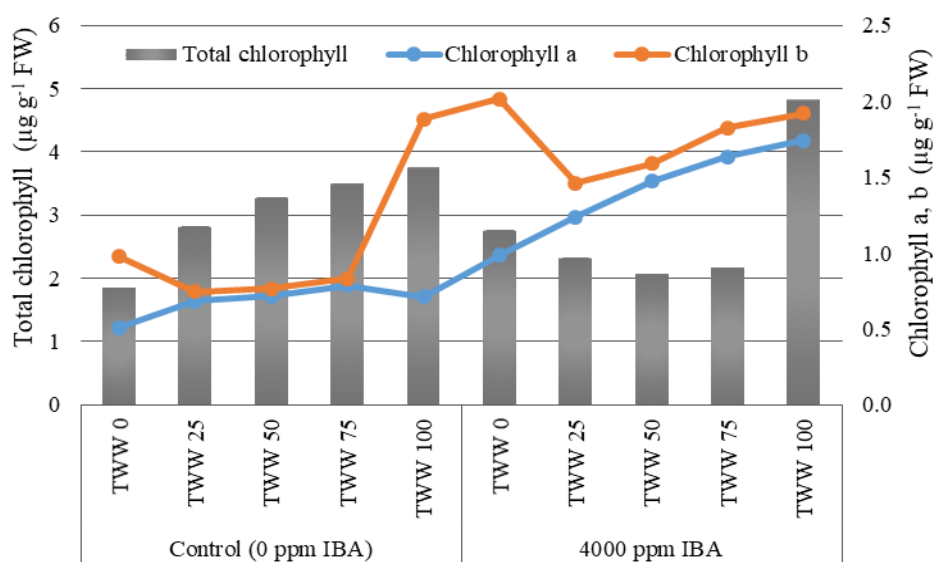


Figure 2. The effect of treated wastewater on chlorophyll a ($\mu\text{g g}^{-1}\text{FW}$), chlorophyll b ($\mu\text{g g}^{-1}\text{FW}$), and total chlorophyll ($\mu\text{g g}^{-1}\text{FW}$) content. TWW0= 100 % pure water, TWW25= 25% treated wastewater and 75 % pure water, TWW50= 50% treated wastewater and 50 % pure water, TWW75= 75% treated wastewater and 25 % pure water, TWW100= 100% treated wastewater.

4. Conclusion

Treated wastewater is used to increase efficiency in terms of irrigating crops and increasing biomass in arid regions. The study shows that treated wastewater can be used for rooting of the green cuttings. The control dose (0 ppm IBA) produces better outcomes in several rooting features compared to IBA applications, which are crucial for rooting.

An advantage in fertilizing has been identified, while also avoiding harm to the environment and plants. It has been discovered that the correct irrigation dose differs depending on the species thus; distinct investigations are required for each variety.

Author Contributions

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

	A.C.	M.K.	S.O.E.
C	20	40	40
D		40	60
S			100
DCP	40	40	20
DAI		80	20
L	30	35	35
W	10	50	40
CR		40	60
SR		50	50
PM	30	40	30

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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EVALUATION OF AGRICULTURAL MECHANIZATION AS AN INDICATOR OF AGRICULTURAL DEVELOPMENT: A COMPARATIVE STUDY FOR TÜRKİYE AND EGYPT

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Abstract: Nowadays, one of the main goals of agriculture is to meet the needs of human communities in all countries through more efficient and high-quality production in agricultural areas. The incorporation of technological advancements, particularly agricultural mechanization, has become imperative for enhancing agricultural production. In recent years, the growing need and inclination to minimize reliance on human labor have heightened the significance of agricultural mechanization, its continual development, and its integration into agricultural operations. The progress of agriculture is intricately tied to the adoption of production technologies within the agricultural sector. The heightened integration of new and contemporary technologies in agriculture can elevate agricultural productivity and income while concurrently lowering production costs. The degree of a country's agricultural development is inherently linked to the utilization of production technologies within its agricultural practices. This research aims to study the existing state of agricultural mechanization in Türkiye and Egypt by identifying and measuring important indicators of the mechanization level of farming, focusing on conducting a comparative analysis between the two countries. Türkiye has a total cultivated area of approximately 23 million hectares, whereas Egypt's cultivated area is around 3 million hectares. Tractors equipped with mechanical power supplies, constituting a fundamental energy source in agriculture, in this study are estimated to be approximately 1.354.912 and 135.100 in Türkiye and Egypt, respectively. The results showed that the levels of mechanization in Türkiye and Egypt were represented by engine power per hectare (1.28 kW/hectare and 0.93 kW/hectare), the number of tractors per 1000 hectares (58.91 tractor /1.000 hectare, and 45 tractor /1.000 hectare) and the cultivated area per tractor (16.97 ha/tractor and 22.21 ha/tractor), respectively. Additionally, the average tractor power in Egypt and Türkiye reached 33-41 kW and 44-52 kW, respectively.


Keywords: Agriculture, Mechanization level, Tractors, Agricultural machinery

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1. Introduction

The global population stood at 7.5 billion in 2017, with projections indicating an increase to 8.5 billion by 2030 and 9.7 billion by 2050. In order to provide the population's food needs, the agricultural sector assumes a pivotal role in addressing the nutritional requirements of the populace. It substantially contributes to national income, employment, and foreign trade. Consequently, the imperative to enhance productivity within this sector becomes pronounced (FAO, 2017).

There are two types of technology utilized in the agriculture industry. These are mechanical and biological technology. Biotechnology encompasses several breeding practices, including the production of seeds, fertilizers and pharmaceuticals. It is conceivable to create things that are more robust and healthy than high-productivity items, especially with this technology. Mechanical technology typically encompasses mechanization,

incorporating elements such as tractors, tractor-utilized tools and machinery, irrigation equipment, and livestock machinery. The implementation of mechanization in agricultural enterprises results in efficiencies, yielding savings in labor, time and production costs (Tarmakbir, 2018).

Agricultural mechanization constitutes a sector within agricultural machinery, with the overarching goal of enhancing the vitality of agricultural areas, augmenting the diversity of agricultural production and optimizing the utilization of agricultural products. Positioned as a sub-sector within the manufacturing sector, agricultural mechanization is characterized by its involvement in the production, manufacturing, development, marketing, sales and management of a diverse array of mechanical designs (Anonymous, 2023a).

The primary indicators delineating the extent of agricultural mechanization within a country encompass the quantitative and qualitative assessment of the tractor



fleet, its year-by-year development, its correlation with agricultural machinery, as well as the density and power levels in unit farming (Evcim and Ertuğrul, 2017).

Mechanical technologies in agriculture have played a pivotal role in reshaping the global farming landscape. These technologies have not only led to an expansion in farm size but have also perpetuated the ongoing trend of displacing or substituting farm labor (McNulty and Grace, 2009).

Agricultural mechanization entails the substitution of human labor across the value chains of crops, livestock and aquaculture with the utilization of animal or mechanical power (Daum and Kirui, 2021). While agricultural motorization concentrates on the use of mechanical power, which can be powered by fossil or renewable energy (FAO and AUC, 2018).

In developed nations, research indicates that emerging technologies, such as agricultural robotics, will persist in displacing and substituting farm labor (Schmitz and Moss, 2015). Moreover, specific studies indicate that while the need for manual labor diminishes with mechanization, this reduction is observed primarily in specific stages of agricultural production, namely land preparation, transplanting, and harvesting (Chandran, 2017).

Mechanization initiatives have predominantly concentrated on large machinery, particularly four-wheeler tractors, along with associated equipment such as tillers, drill machines, rotavators, harvesters, driers, balers, and more. Recent evaluations of these schemes are beginning to surface in literature, with a notable emphasis on output indicators like the quantity of imported and distributed equipment (Daum and Birner, 2017).

Agricultural mechanization has been characterized in various ways, with the most encompassing and pertinent definition being the one that encompasses all facets of farming and processing technologies. This ranges from elementary hand tools to more advanced, and motorized equipment, as reported by the Food and Agriculture Organization (FAO, 2016). Mechanization serves to alleviate and diminish strenuous labor, address labor shortages, enhance farm labor productivity, improve the efficiency and timeliness of agricultural operations, optimize resource utilization, facilitate market access, and contribute to the mitigation of climate-related hazards (Sims and Kienzle, 2017).

Baudron et al. (2015) stated that the agricultural mechanization process encompasses multiple facets, ranging from the identification of farm operations suitable for mechanization to the adaptation or production of appropriate machinery.

Agriculture plays a pivotal role in both Türkiye and Egypt. Türkiye is a nation with a population of approximately 80 million people, and 81 provinces. The total utilized agricultural land in the country is approximately 38,3 million hectares, with 15,6 million hectares dedicated to cereals and other crops (TÜİK,

2017a). Noteworthy production areas for crops harvested by combine harvesters include 7,7 million hectares for wheat, 2,4 million hectares for barley, 0,8 million hectares for sunflower, 0,6 million hectares for corn, and 0,03 million hectares for soybeans (TÜİK, 2017b), while Egypt, with a total land area of approximately one million km². The country boasts a cultivated area of 3,6 million hectares, with around 2,7 million hectares concentrated in the Delta Region, benefiting from surface irrigation. Additionally, 0,88 million hectares consist of newly reclaimed soils, utilizing pressurized irrigation methods such as sprinkler and pivot irrigation (Peter and Sewilam, 2016).

The agricultural sector in Türkiye is a significant economic and social domain, exerting influence on nutrition, employment, national income, and the provision of raw materials to the industrial sector (Sümer et al., 2004; Anonymous, 2023b). The Egyptian economy is presently undergoing a process of liberalization and privatization, with a pronounced impact on the agricultural sector, which constitutes a pivotal aspect of the nation's economic framework. Egypt's economy has been heavily dependent on the agricultural sector. Approximately 55% of the population derives their livelihood from agriculture, which serves as a source of employment for about 34% of the total labor force. Furthermore, agriculture contributes around 20% to the gross domestic product (GDP), approximately 20%, of total exports and foreign exchange earnings (Kadah et al., 2018; World Bank, 2019).

Despite Türkiye surpassing the global average in terms of the criteria defining current agricultural mechanization, there remains an imperative to elevate both the existing production levels and productivity. This is particularly crucial to meet the increasing demand for agricultural production. Achieving a higher degree of effectiveness in planning the agricultural mechanization levels across regions in Türkiye involves diversifying the tractor and agricultural machinery fleet, as suggested by Altuntas and Demirtola (2004).

Soil cultivation equipment and machinery are extensively utilized in Türkiye. Additionally, the projection coefficients, along with other pertinent information, were determined for machines involved in sowing, planting, fertilizing, harvesting, and spraying, as well as tractor and trailer units equipped for silage, mowing, and baling operations (Baran et al., 2019).

The aim of this study was to determine the level of agricultural mechanization in Türkiye and Egypt considering the existing status of agricultural potentials in both countries.

2. Materials and Methods

The material of this study consists of the number of agricultural tractors according to powers, the data obtained from the Turkish Statistical Institute (2023) for Türkiye and the Ministry of Agriculture and Land Reclamation (2019) for Egypt. Additionally, the data on

surface area and cultivated area for both countries has been obtained from the Food and Agriculture Organization of the United Nations Statistics (2023).

Mechanization level signifies the proportion of total tensional powers relative to the under-cultivated area. This index provides insights into the extent to which mechanical forces are applied to the cultivated land, offering a valuable indicator of the overall mechanization intensity in a given area.

The criteria for determining the level of mechanization for Türkiye and Egypt as shown in the following indicators:

- A. The average of tractor powers (kW).
- B. The number of tractors per 1000 hectares (tractor/1.000 hectare).
- C. The cultivated area per tractor (ha /tractor).
- D. Mechanization level (Power availability).

According to Pishbin (2013), the level of mechanization is determined as shown in the following equations 1, 2 and 3;

$$ML = \frac{Pm \times Cf}{A} = \frac{\text{Total real power}}{\text{cultivated area}} \quad (1)$$

Where:

ML = mechanization level (kW/ha).

pm = Total power of tractors (kW).

Cf = Convey coefficient (0,5 or 0,75 for waste and useful

instruments respectively).

A = Total cultivated area (ha).

The total power of existing tractors (kW) = (2)
Average nominal of one tractor × Number of working tractors

The total real power of tractors = Total power of existing tractors × conversion coefficient (0.75) (3)

Egypt takes a broad view of food security, recognizing that with limited arable land and water resources, it will never be self-sufficient in grains, vegetable oil, and animal proteins 40% of Egypt's imports are food and agricultural products. The agricultural vision for Türkiye in 2023 delineates ambitious objectives, with the primary goal being the transformation of Türkiye into a nation capable of supplying its population with ample, high-quality, and safe food. Additionally, the vision aims to enhance Türkiye's position as a net exporter of agricultural products, strengthen its competitive edge, and establish leadership in the field of agriculture both within its regional context and globally.

Table 1 presents a comparative analysis of the land area in Türkiye and Egypt for the year 2021; encompassing both total agricultural lands and the specific area designated as arable land with 1.000 hectares (FAO, 2023).

Table 1. Comparison between Türkiye and Egypt in terms of agricultural land area in 2021

Items	Area (1000 ha)	
	Türkiye	Egypt
Country area	78.535	100.145
Agricultural land	38.089	4.031
Cultivated area	23.000	3.000
Cropland	23.473	4.031
Arable land	19.881	3.077
Permanent crops	3.591	954

In Türkiye, there exist 13 manufacturers specializing in tractors and over 1.000 enterprises engaged in the production of agricultural machinery. The progressive rise in the exportation of tractors, agricultural equipment, and machinery from Türkiye is playing a significant role in bolstering the country's economy (Akdemir, 2013). In Egypt, there are approximately 9 companies that assemble and manufacture some agricultural equipment and machinery (MALR, 2019).

3. Results and Discussion

Agricultural mechanization plays a strategic role in improving agricultural production and productivity in developing countries. Tractor is the main important indicator taken into consideration in the activities of agricultural areas for determining the mechanization level in all countries.

Initially, upon looking at the number of tractors in both

Türkiye and Egypt, it is observed that since the 1980s, Türkiye's tractor market has experienced rapid growth alongside advancements in agricultural production. The total number of tractors has surged by more than 3,5 times. As a developing country, statistical reports from 2022 indicate that there are 1.526.769 registered tractors in Türkiye, according to the Ministry of Agriculture and Forestry (Anonymous, 2023c) as shown in (Figure 1).

In contrast, Figure 2 shows the total number of tractors for agricultural purposes, including private, public sector, and government sectors in Egypt during the period from 2011 to 2019 as reported by CAPMAS (2020).

Table 2 presents the number of tractors in Türkiye for the years 2014 and 2022, categorized by power groups (Anonymous, 2023c). The data reveals an increase in the total number of tractors from 1.243.300 in 2014 to 1.526.769 in 2022, indicating a growth trend over the

specified period. Similarly, Table 3 outlines the number of tractors in Egypt from 2014 to 2018, categorized by their power (MALR, 2019). The total count stood at approximately 127.704 in 2014 and increased to 136.144

in 2018. This information provides insights into the tractor distribution and changes in Egypt over the specified years.

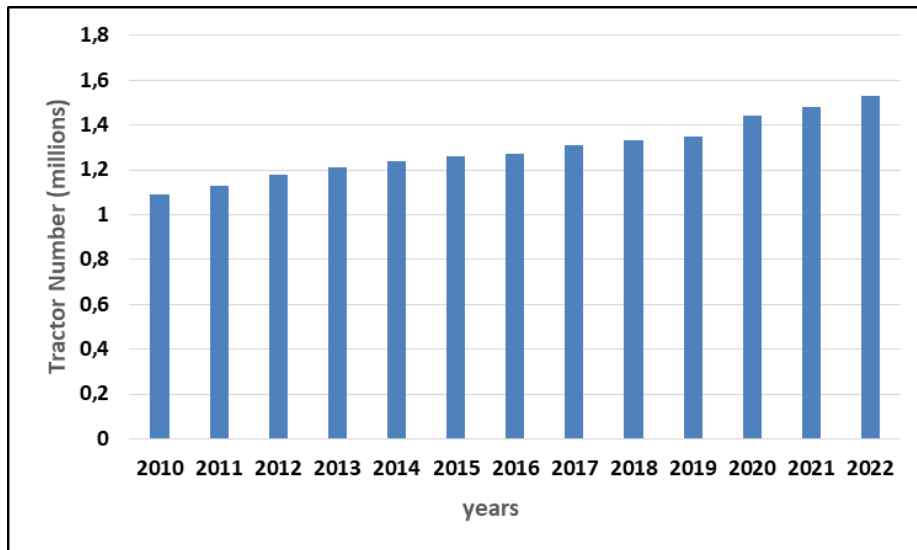


Figure 1. Tractor number changes by year, Türkiye.

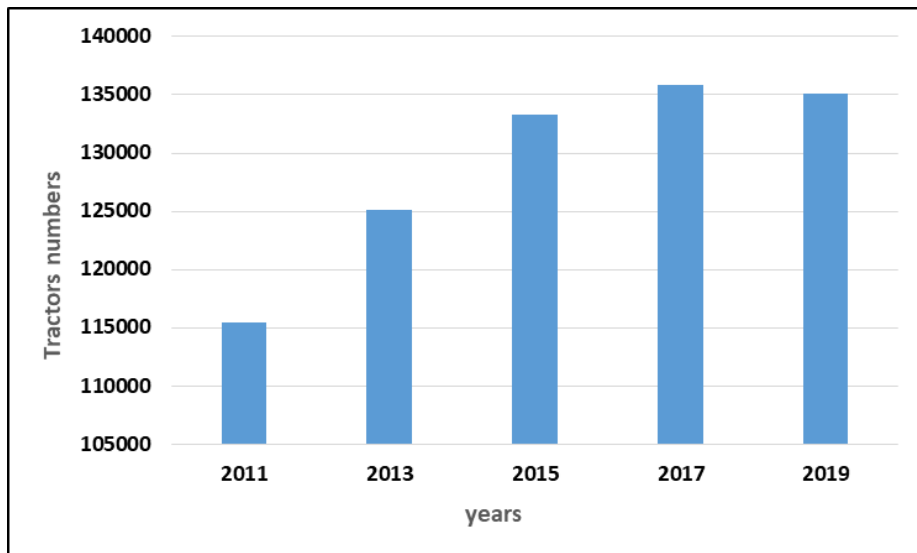


Figure 2. The development of the number of tractors in the agricultural activity, Egypt.

Table 2. Numbers of agricultural tractors according to powers, Türkiye (2014-2022).

Year	Total	One axle						Two axle				
		kW						Change* (%)	38.5-52.5	Change* (%)	52.5 +	Change* (%)
		0.75-3.7	4.5-6.7	0.75-7.5	8.25-18	18.75-25.5	26.5-37.5					
2014	1.243.300	14.383	51.492	6.247	20.906	69.223	493.914	--	461.399	--	125.536	--
2015	1.260.358	14.856	54.604	6.252	21.181	68.074	491.828	0.42	468.060	1.42	135.297	7.78
2016	1.273.531	15.736	57.131	6.448	21.274	66.825	489.621	0.45	475.665	1.62	140.699	3.99
2017	1.306.736	16.589	59.061	6.432	20.527	65.866	492.343	0.55	493.660	3.64	152.133	8.13
2018	1.332.139	17.129	60.707	6.554	20.886	66.104	493.134	0.16	505.087	2.26	162.425	6.77
2019	1.354.912	17.512	62.178	6.589	20.513	65.496	495.375	0.45	513.035	1.55	174.105	7.19
2020	1.442.909	19.416	73.782	6.969	20.944	68.157	517.899	4.50	544.909	6.21	190.677	9.52
2021	1.481.461	20.517	79.658	6.853	20.841	68.730	523.718	1.11	555.536	1.91	205.488	7.77
2022	1.526.769	20.008	84.568	6.384	20.212	68.045	532.393	1.63	570.629	2.64	224.408	9.21
Change* (%)	22.79	39.10	64.23	2.19	-3.32	-1.70	7.79	--	23.67	--	78.75	--

Table 3. Numbers of agricultural tractors according to power categories, Egypt (2014-2018)

Year	Total	kW						
		0,75-26,25	26,25-37,5	Change* (%)	38,25-52,5	Change* (%)	52,5 +	Change* (%)
2014	127.704	10.358	14.817	--	74.635	--	27.894	--
2015	133.298	12.421	16.600	10,74	74.339	0,39	29.938	6,83
2016	135.090	11.095	18.018	7,86	74.765	0,57	31.212	4,08
2017	136.683	9.385	19.682	8,45	72.775	2,73	34.841	10,41
2018	136.144	9.236	20.080	1,98	73.278	0,69	33.550	3,85
Change* (%)	6,61	-10,83	35,52	-	-1,82	-	20,28	-

In Türkiye, the examination of single-axle tractors within the 0.75-3.7 kW range reveals a consistent increase from 2014 to 2022. Furthermore, there is a continuous upward trend in single-axle tractors with more than 3.7 kW. The quantity of double-axle tractors in the 0.75-7.5 kW range remains notably low. Notably, the number of tractors in the 26.5-37.5 kW range has risen from 493.914 in 2014 to 532.393 in 2022. Similarly, the tractor group within the 38.5-52.5 kW range has experienced continuous growth. Particularly noteworthy is the recent increase in tractors with a power exceeding 52.5 kW in the later years of the observed period.

When comparing the number of single-axle tractors in Türkiye between 2014 and 2022, there was a notable 39.10% surge in the 0.75-3.7 kW category and a substantial 64.23% increase in tractors with over 3.7 kW. As for double-axle tractors, there was a modest 2.19% rise in the 0.75-7.5 kW category, a 3.32% decline in the 8.25-18 kW category, and a 1.7% reduction in the 18.75-25.5 kW category. On the contrary, there was a growth rate of 23.67% in the 38.5-52.5 kW categories and a substantial increase of 78.75% in the category exceeding 52.5 kW.

In Egypt, the analysis of tractors within the 0.75-26.25 kW range indicates a decline from 2014 to 2018. However, there is a notable increase in tractors falling within the 26.25-37.5 kW range. In contrast, the quantity of tractors within the 38.25-52.5 kW range has decreased, dropping from 74.635 in 2014 to 73.278 in 2018. Additionally, there is a conspicuous rise in the number of tractors with a power exceeding 52.5 kW during the specified period.

Analyzing the tractor data in Egypt from 2014 to 2018 indicates a decline of 10.83% in the 0.75-26.25 kW category, juxtaposed with a notable increase of 35.52% in tractors within the 26.25-37.5 kW power range. Additionally, there was a modest 1.82% decrease in the 38.25-52.5 kW category, while the category of tractors with more than 52.5 kW experienced a straightforward growth rate of approximately 20.28%.

To effectively compare the mechanization status between Türkiye and Egypt, it is imperative to have a comprehensive understanding of the mechanization indicators in each nation.

By using the above indicators to determine the level of mechanization for Türkiye and Egypt. It was found that

Türkiye's agricultural mechanization indicators were 58.91 tractor/1.000 hectare, 16.97 ha/tractor, and the level of mechanization was 1.28 kW/hectare. At the same time, Çiçek and Sümer (2017) reported that the average power per unit area in Türkiye was 1.97 kW/ha, based on data from 2014. Subsequently, Saygili and Çakmak (2021) utilized data from 2020 and asserted that the mechanization level in Türkiye had reached 1.69 kW/ha. In addition, Koçtürk and Avcioğlu (2007) found, in their study, that the level of agricultural mechanization across various regions in Türkiye was 1.75 kW/ha.

In this context, Egypt's agricultural mechanization indicators were 45 tractor/1.000 hectare, 22.21 ha/tractor, and the level of mechanization was 0.93 kW/hectare, respectively. The average tractor power was 44-52 kW and 33-41 kW, respectively.

When assessing the recent advancements in level mechanization, it becomes evident that Türkiye has experienced a more pronounced increase compared to Egypt as shown in (Figures 3, 4, and 5).

Although the surface area of Egypt is larger than the surface area of Türkiye, Türkiye has a larger cultivated area. This indicates that the number of tractors used in Türkiye is higher than the number of tractors used in Egyptian agricultural lands as shown in (Figure 6).

Türkiye is home to a fleet of more than 17 thousand combine harvesters, actively engaged in harvesting cereal, corn, soybean, and sunflower crops across a vast expanse exceeding 10 million hectares, in Egypt, a substantial number of combine harvesters have been employed for comprehensive wheat harvesting and threshing. Upon analyzing the evolution in the quantity of combine harvesters over the past decade in Türkiye, there has been a notable 28% increase. Given that cereals constitute the primary agricultural products and serve as raw materials for various industries, these transformations carry significant implications, particularly in reducing dependence on imports. As a prevalent usage model, the adoption of shared combined usage among entrepreneurs is becoming more widespread. Nevertheless, the degree of mechanization in harvesting processes in Egypt has not attained a significant level as shown in (Figure 7) with the comparison with Türkiye.

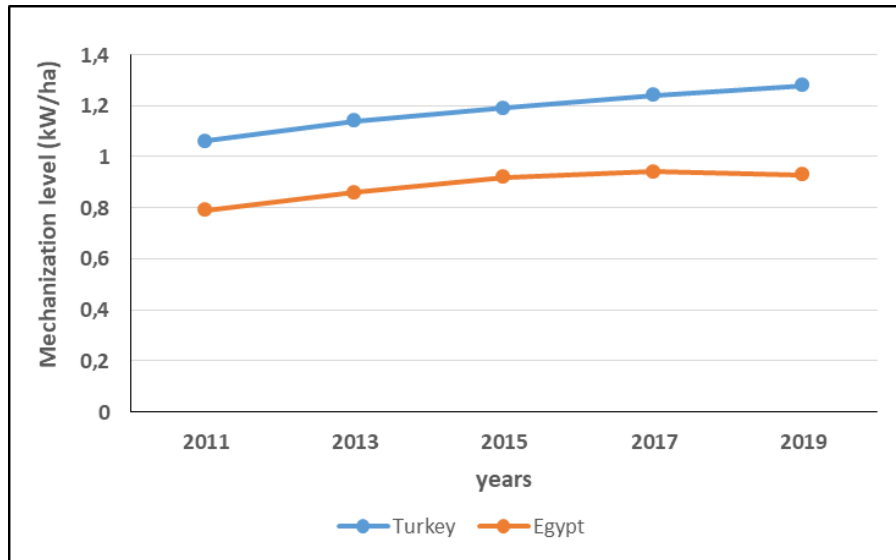


Figure 3. Mechanization level in Türkiye and Egypt.

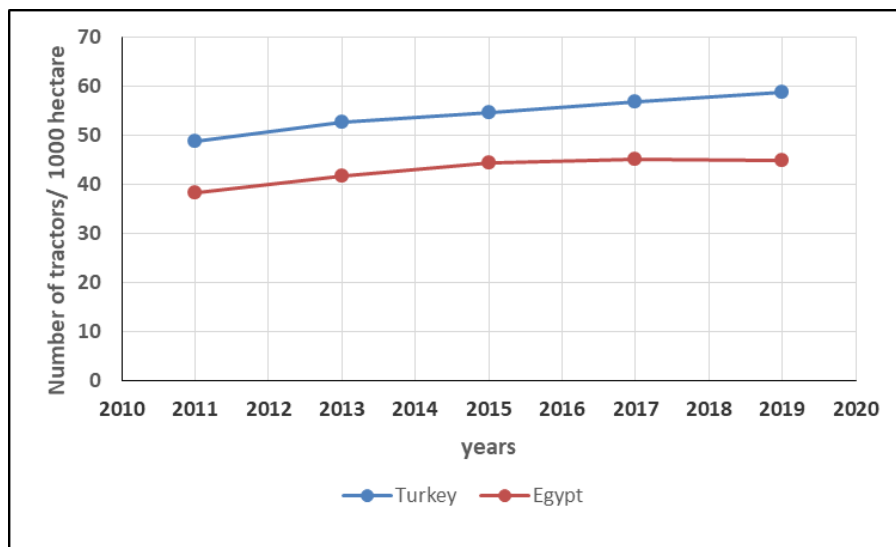


Figure 4. Comparison between the number of tractors per 1000 hectare for Türkiye and Egypt.

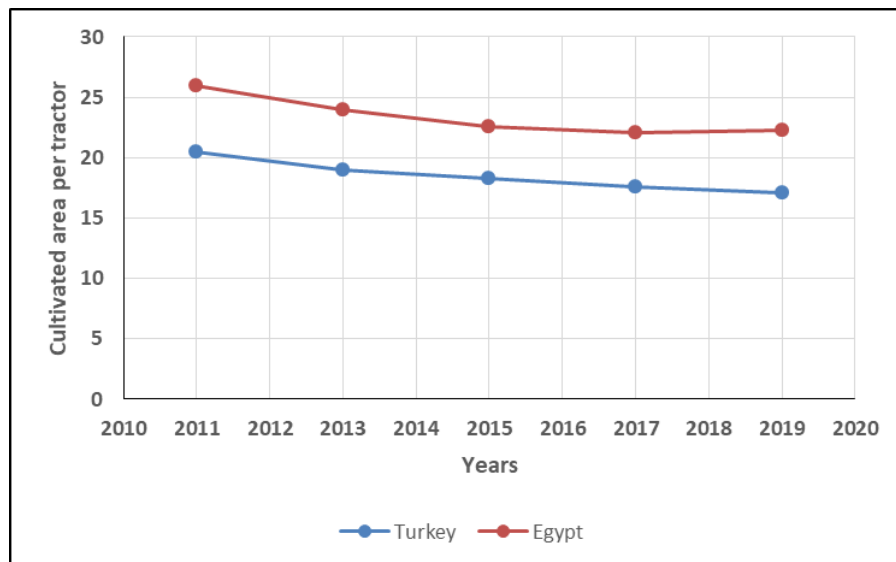


Figure 5. Comparison between the cultivated area per tractor for Türkiye and Egypt

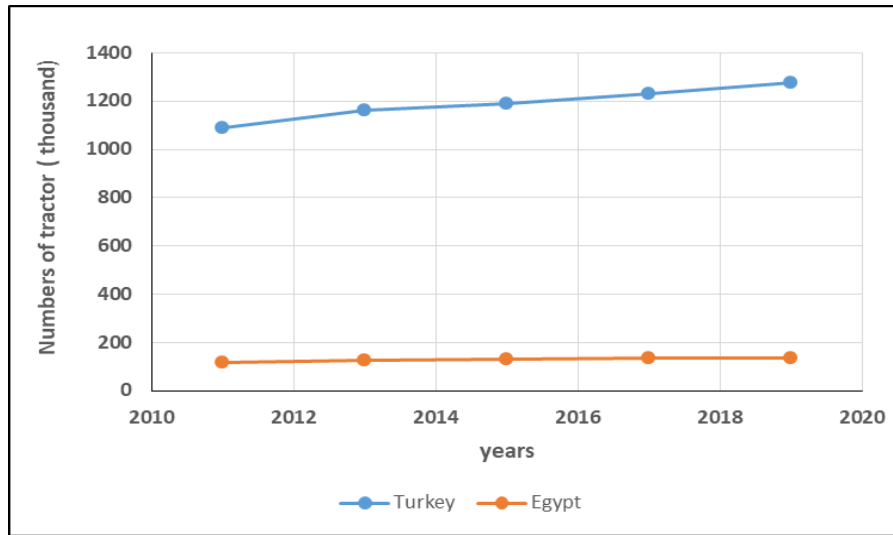


Figure 6. The number of tractors in Türkiye and Egypt.

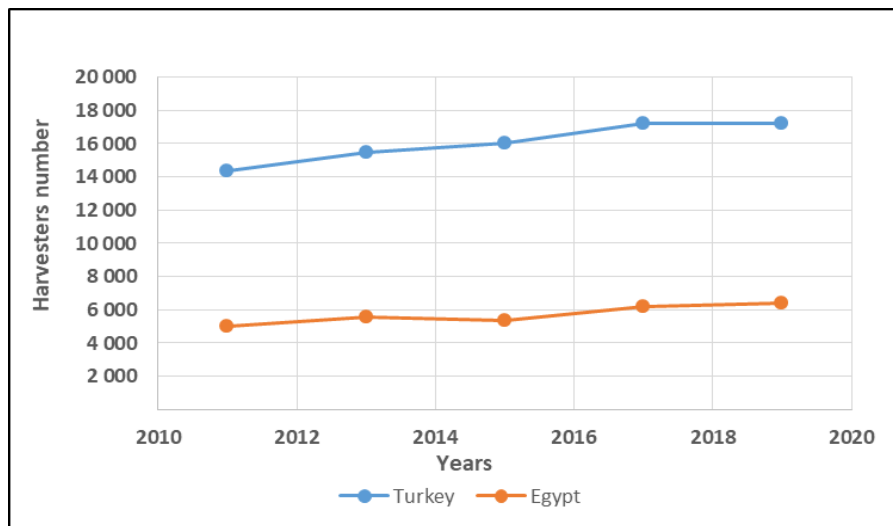


Figure 7. The comparison of harvester numbers between Türkiye and Egypt.

Table 4 outlines Türkiye's agricultural machinery and equipment exports from 2014 to 2021, along with their proportional contribution to the total exports. The data indicates a consistent upward trend in these exports over the ten years. Despite a notable 8.69% decline in 2020, following their global emergence in December 2019,

there was a more moderate decrease of 2.66% in 2021. The percentage share of agricultural machinery and equipment in Türkiye's exports, initially at 0.47% in 2010, rose to 0.75% in 2020, and then slightly decreased to 0.73% in 2021 (Trade Map, 2023).

Table 4. Exports of agricultural machinery and equipment in Türkiye (2010-2021)

Year	Total Export (1000 USD)	Total Agricultural Machinery and Equipment Exports (1000 USD)	Rate %
2014	166.504.862	1.022.732	0,61
2015	143.844.066	945.804	0,66
2016	142.606.247	860.669	0,60
2017	156.992.940	951.580	0,61
2018	167.923.862	1.127.056	0,67
2019	180.870.841	1.243.226	0,69
2020	169.657.940	1.264.995	0,75
2021	225.264.314	1.646.014	0,73

Table 5 illustrates the production and export figures for tractors in Türkiye over the years 2014 to 2022 (Tarmakbir, 2022). According to the data, tractor production has seen a noticeable increase, from 66.922 units in 2014 to 82.500 units in 2022. These findings indicate that Türkiye's exportation of tractors makes a substantial and impactful contribution to the overall

economic indicators. When analyzing Egypt's tractors and agricultural machinery exports, it is apparent that these products are not locally manufactured for export but are instead acquired through imports from foreign countries. It was found that the number of machines and equipment used in the field of agriculture in Türkiye has increased in recent years compared to Egypt.

Table 5. Number of tractor production, exports, and their corresponding values in thousands of USD in Türkiye (2014 - 2022)

Years	Tractor Production (Units)	Tractor Exports (Units)	Value (1000 USD)
2014	66.922	17.739	434.241
2015	69.978	17.471	374.472
2016	71.955	15.766	338.701
2017	76.071	14.544	320.937
2018	52.357	19.282	423.603
2019	34.393	23.401	481.298
2020	58.710	21.762	417.211
2021	89.000	23.135	524.757
2022	82.500	26.492	584.664
Change* (%)	23,28	49,34	---

*Calculated values

4. Conclusions

The indicators of agricultural mechanization were determined for Türkiye and Egypt to evaluate the status of agricultural mechanization in both countries.

In Türkiye, there has been a notable increase in the percentage of tractors with power exceeding 26.5 kW, rising from 7.79% to 23.67%. Conversely, in Egypt, tractors with the same power have experienced a decline from 35.52% to 1.82%. Additionally, it is important to clarify that the rate of increase in the number of tractors with a power of more than 52.5 kW was on the rise in Türkiye when compared to Egypt.

Türkiye is undergoing a significant transformation in both the production and export of agricultural tractors. The rate of change in tractor production from 2014 to 2022 amounted to approximately 24%. Furthermore, there was a substantial increase in the export of tractors to other countries, with the rate of change reaching approximately 50% over these years. The data reflects a dynamic shift in Türkiye's agricultural machinery sector, indicating noteworthy developments in both domestic production and international trade compared to Egypt.

In this study, it was found that the level of mechanization in Türkiye is much higher than in Egypt. The number of tractors used in Türkiye is greater than the tractors used in Egypt.

The average power of the tractor, the number of tractors per 1.000 hectares, and the cultivated area per tractor in both countries were (44-52 kW and 33-41 kW), (58.91 tractors /1.000 ha and 45 tractors/1.000 ha), (16.97 ha/tractor and 22.21 ha/tractor) in Türkiye and Egypt, respectively. It was found that Türkiye has made significant advancements in the field of agricultural mechanization compared to Egypt.

Author Contributions

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

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

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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EFFECTS OF BREEDING PRACTICES ON REPRODUCTIVE EFFICIENCY OF EWES AND FATTENING PERFORMANCE OF LAMBS

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Abstract: In this study, the data obtained by questionnaire from small ruminant farms in Tekkeköy district of Samsun province were evaluated. Among the enterprises in the study, 50% of them breed Karayaka sheep, and 33.4% of them breed Bafra sheep. It was determined that 95.8% of the farmers applied flushing before ram siring, 83.3% of the farmers applied feed supplements to the ewes after birth, 95.8% of the farmers applied additional vitamin supplements to the lambs, and 83.3% of the farmers used salt-containing licking stones for the lambs. In the study, internal and external parasites were applied to the lambs to be fattened in all of the farms, and all of them were not released to pasture until sale. Winter lambs were fattened in 91.7% of the farms. The average age of the breeders is 50, and the number of ram sires per year is 1.08 on the farms. The average age of lambs sent to slaughter is 4 months, and the average carcass weight at slaughter is 19.15 kg. Feed supplementation to the ewes after birth and application of salt-containing licking stones to the lambs had a positive effect on the average carcass weight of the fattened lambs. In this study, it was determined that winter lambs were preferred more than summer lambs. According to this result, by fattening winter lambs in this period when there is no pasture opportunity, both seasonal meat needs can be met, and it provides the opportunity to sell at high prices in the winter period. In this case, the small ruminant farms will earn more income.


Keywords: Karayaka sheep, Bafra sheep, Winter lamb, Flushing, Fattening, Profitability

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1. Introduction

Nutrition has been one of the most important needs of humanity since its existence. The most important of the building blocks of nutrition is the need for protein. Animal products come first in meeting this need. Sheep have met many needs of mankind with their meat, milk, fleece, and skin in every period of history. Although the importance of sheep breeding varies according to countries, it is practiced in many regions of the world and in all regions of our country. Sheep breeding is a livestock breeding branch based mostly on pasture for structural and economic reasons (Çiçek et al., 2022). Sheep breeding is carried out more widely in regions with large meadows and pastures and arid climatic conditions in large and small flocks to the extent of climatic and natural conditions and technical and economic possibilities. The most economical and income-generating livestock for these regions is sheep breeding. It is resistant to adverse environmental conditions and diseases, easy to manage, low-cost, and safe in terms of production. They make good use of vegetation that cattle cannot utilize (Akçapınar, 2000). Sheep farming enterprises in Türkiye are small family farms, settled

village flocks, highland livestock farming, and nomadic livestock farming enterprises (Kaymakçı, 2006). The most important condition for successful sheep breeding is to determine the direction and type of breeding according to the geographical and economic conditions of the region and to choose the appropriate sheep breed or type for the purpose (Gül et al., 2022).

The total sheep population in the Black Sea region is 2.261.719 heads, and there are 33 sheep breeds in 18 provinces. In the region, Karayaka, Akkaraman, and Merino breeds are the most common, and Tuj, Zom, Hamdani, and Koçeri breeds are the least common. Half-fat sheep breeds have adapted to the climate of the region. Thin-tailed sheep breeds are common in the west of the region, and fat-tailed sheep breeds are common in the eastern provinces (Kandemir and Taşkın, 2022). In Samsun province, 16 sheep breeds have been identified (7 of these breeds are combined: 3 are fertility, 2 are meat, 2 are milk, 1 is fleece, and 1 is a meat-dairy breed). The total sheep population of the province is 246,918 heads and has a share of 10.92% in the region (Kandemir and Taşkın, 2022).

The biggest potential in sheep meat production is for the



lambs to be fattened. Determining the technical and economic conditions of lamb fattening and putting them into practice will positively affect the required increase in meat production. This situation is closely related to the number of lambs per ewe that can reach marketing age and the meat production abilities of lambs. Measures such as the live weight and live weight gains of lambs at various periods are meat yield measures. Economic meat production per lamb is largely influenced by breed, age, sex, and some environmental factors (Bozgüllü and Macit, 2022; Uğurlu et al., 2022). Among the general reasons that prevent the production of more meat from lambs, studies have shown that malnutrition is the main environmental factor that causes low yield (Bozgüllü and Macit, 2022; Uğurlu et al., 2022). For this reason, intensive fattening is applied in order to reach the desired level of meat yield and quality in butchery animals in a short time and with the least cost.

When it comes to lamb fattening in our country, male lambs are generally used as material. Females are generally not fattened for meat production (Altın et al., 2005). The fattening performance of male animals is always better than that of female animals, and they provide more and faster live weight gain than females. At the same time, the fattening of the live weight of males is higher than that of females. The meat of male animals is darker and less fatty than the meat of female animals. Because of these characteristics, it is recommended to use male animals for fattening (Yılmaz et al., 2007; Mercan et al., 2022). In addition, female lambs fatten faster than males (Yılmaz et al., 2007).

In intensive fattening, which is one of the lamb fattening methods, lambs are fed immediately after weaning. Approximately 20 kg of carcass can be obtained from lambs that are fed with high-energy (65-70% total digestible nutrients, 16-17% crude protein) rations until 4-5 months of age. Considering the research, as the animal ages, its energy requirement increases, and the amount of feed consumed for high live weight gain increases. As the lambs get older, the feed utilization rate deteriorates, the feed cost increases, and the profitability of fattening decreases. Prolonging the fattening period may cause fatty carcasses and the production of fatty carcasses that are undesirable in terms of market demands and may negatively affect the economics of fattening (Tekel et al., 2007).

In the studies conducted in Türkiye in recent years, it has been observed that lambs of both local breeds and crossbred lambs reached 36–40 kg of live weight, i.e., 17–20 kg of carcass, in 4-5-month-old lambs as a result of 2-3 months of fattening after 2-2.5 months of suckling period (Tekel et al., 2007; Uğurlu et al., 2022).

Different lamb-fattening techniques are applied in different regions of our country. The factors that reveal these differences are the climatic factors of the regions, the structure of the genetic material, and meat consumption habits. The lamb fattening techniques include milk lamb fattening, intensive lamb fattening

following weaning, pasture lamb fattening, and lamb fattening (Koçak, 2009).

The first aim of this study was to determine the effects of breeding practices on the reproductive efficiency and lamb fattening performance of ewes. Also second aim of this study was to examine the lamb breeding model in Tekkeköy district of Samsun province and to determine the effects of breeding practices on early lamb production.

2. Materials and Methods

Tekkeköy district is 14 km from Samsun city center and 7 km from Samsun Çarşamba international airport. The Black Sea transit road passes through the district, and it is possible to reach Samsun city center by divided road. There are 63 neighborhoods connected to the district. The Tekkeköy population is 53,247, according to the population registration system. There are Çarşamba district in the east and south of the district, Canik district in the west, Black Sea in the north, and Asarcık district in the southwest. The climate of the Central Black Sea is dominant in the district. Summers are hot and humid, winters are mild, and spring and fall are rainy. The hottest month is August, with an average temperature of 25 °C; the coldest month is January, with an average temperature of 7 °C. Agriculture and animal husbandry are among the important sources of income in the district. One-third of the district's territory is the continuation of the fertile delta plain deposited by Yeşilirmak and is suitable for irrigated agriculture.

As seen in Table 1, Tekkeköy district has an important place in the sheep population of Samsun province, with a rate of 10.33%. The sheep breeds in Tekkeköy district are mostly Karayaka and Bafra sheep, with a small amount of Sakız breed sheep. Karayaka breed: It is a local sheep breed with a thin tail, low milk and fertility, and coarse and mixed fleece, which is raised mostly on the coastline in the Black Sea region. Bafra sheep was obtained by crossbreeding (75% Sakız and 25% Karayaka blood) of Sakız and Karayaka breeds with high milk and reproductive efficiency in Amasya Gökhöyük Agricultural Enterprise Directorate in order to produce high quality and a high amount of lamb meat.

In the animal information system TURKVET records of the Tekkeköy District Directorate of Agriculture and Forestry, 4522 lambs were registered in January 2022. When the number of small ruminants slaughtered at Tekkeköy Municipality Slaughterhouse in 2021 is examined in the table below, it is seen that the average slaughter carcass weight is 18.8 kg. Almost all of the breeders engaged in ovine breeding in Tekkeköy district utilize or sell their lambs within Tekkeköy.

Data on lambs slaughtered in the Tekkeköy Municipality Slaughterhouse in 2021 are given in Table 2. Also, Tekkeköy District Directorate of Agriculture and Forestry Farmer Registration System 2021 agricultural area data are given in Table 3.

Table 1. Small ruminant livestock in Samsun districts (TUIK, 2020)

Districts	Sheep (head)	Goat (head)	Total
Alaçam	16.343	1.064	17.407
Asarcık	2.105	240	2.345
Atakum	8.372	721	9.093
Ayvacık	2.741	300	3.041
Bafra	47.102	11.010	58.112
Canik	8.145	290	8.435
Çarşamba	13.438	0	13.438
Havza	21.660	4.050	25.710
İlkadım	4.990	228	5.218
Kavak	11.798	770	12.568
Ladik	18.435	365	18.800
Ondokuzmayıs	4.08	30	4.113
Salıpazarı	2.212	50	2.262
Tekkeköy	24.025	359	24.384
Terme	3.294	0	3.294
Vezirköprü	36.238	6.225	42.463
Yakakent	7.609	1.495	9.104
Total	232.590	27.197	259.787

Table 2. Data on lambs slaughtered in the Tekkeköy Municipality Slaughterhouse in 2021

Features	Data
Lambs slaughtered (n)	5112
Total carcass weight (kg)	96.088
Average carcass weight (kg)	18.8
Female animal (n)	63

Table 3. Tekkeköy District Directorate of Agriculture and Forestry Farmer Registration System agricultural area data for 2021 (da)

Land types	Field
Agricultural area (excluding pasture)	160.283
Vegetable	1.675
Fruit	96.194
Lying fallow	2.100
Unused agricultural land	16.035
Permanent grassland-moorland	3.181

2.1. Statistical Analysis

In the study, the results of one-to-one surveys conducted with 24 breeders in 15 neighborhoods within the borders of Tekkeköy district and data on 3096 ovine animals were used. The survey was conducted face-to-face with sheep breeders within the borders of Tekkeköy district. The survey results were obtained by asking questions about the issues that will directly or indirectly affect sheep breeding and lamb fattening in Tekkeköy

district. The villages and enterprises included in the survey study breeders who sent the lambs obtained in sheep breeding and lamb fattening to slaughter without sending them to pasture were included in the survey. In the evaluation of the data obtained from the questionnaires, frequency analysis and chi-square independent tests were performed using the OMU-licensed SPSS 20.0 version statistical model.

3. Results

3.1. Distribution of Sheep Breeds and Breeding Practices in Enterprises

The results of the survey were statistically evaluated, and the distribution of sheep breeds and the results obtained are given in Table 4. When Table 4 is analyzed, the largest share belongs to the Karayaka breed with a rate of 50%, followed by Bafra sheep with 33.3%. In this respect, it was determined that Karayaka sheep is the breed preferred more by the people in the study area.

Table 4. Distribution of sheep breeds in the enterprises included in the survey in Tekkeköy district

Breeds	Frequency (n)	%
Karayaka	12	50.0
Karayaka other breed hybrids	2	8.3
Bafra	8	33.4
Sakız	2	8.3
Total	24	100.0

In the study, it was determined that the average age of the breeders was 50 years (Table 5). As can be seen in Table 5, the average number of ewes used in the holdings was 129, the average number of ram sires per year was 1.08, the number of lambs fattened was 11, the age at which lambs started to eat concentrate feed was 30 days, and the amount of concentrate feed consumed by lambs until slaughter was 11,187.5 kg. In addition, the average age at slaughter was 4 months, and the average carcass weight of lambs sent to slaughter was 19.15 kg. The carcass weight findings obtained in the study were similar to those found in different literatures (Duman and Ulutaş, 2018 (14.10 kg); Akçapınar et al., 2002 (18.55 kg)).

Table 5. Some data on breeding practices

Features	n	Med.	Min.	Max.	Mean	St. Deviation
Age of grower	24	48	23	82	50.00	12.36
Number of breeding sheep	24	110	50	265	129.00	61.82
Number of ram sires per year	24	1	1	2	1.08	0.28
Number of lambs fattened	24	100	30	250	11.88	63.19
Age/day of lambs starting to eat concentrate feed	24	30	20	45	30.42	5.30
The amount of concentrate feed consumed by lambs until slaughter/kg	24	9250	2000	25.000	11.187.5	6575.4
Age at slaughter of lambs (months)	24	4.25	4	5	4.48	0.50
Average carcass weight/kg of lambs sent to slaughter	24	19.25	17	22	19.15	1.43

3.2. Flushing Practice in Flocks and Postpartum Feed Supplementation for Mother Ewes

The results of the questionnaire data evaluated in terms of feed supplementation for ewes after birth are given in Table 6. When Table 6 is analyzed, it is seen that 83.3% of the breeders give supplementary feed to their ewes after birth. When Table 6 is analyzed, 95.8% of the surveyed breeders apply flushing to their flocks before ram siring.

This has a positive effect on the pregnancy rate and lamb yield in flocks.

The results of the questionnaire data evaluated in terms of the application of additional vitamin supplements for lambs are given in Table 6. It was found that 95.8% (23 farms) and 4.2% (1 farm) of the lambs were supplemented with vitamin supplements.

Table 6. Data on sheep breeding and lamb feeding practices

Flushing application before ram siring	n	%	Supplementary vitamin supplementation to lambs	n	%
Yes	23	95.8	Yes	23	95.8
No	1	4.2	No	1	4.2
Supplementary feed supplementation to ewes after birth			Supplementary salt supplementation to lambs		
Yes	20	83.3	Yes	20	83.3
No	4	16.7	No	4	16.7
Internal and external parasite application to lambs			Release of lambs to pasture during fattening		
Yes	24	100.00	Yes	0	0.00
No	0	0.00	No	24	100.00
Preference of winter-summer lambs					
Winter lambs	22	91.7			
Summer lambs	2	8.3			

When the reflection of this practice is analyzed in Table 7, lambs with a carcass weight between 18 and 22 kg were obtained with supplementary feed supplementation for the ewes after birth. This practice had positive effects on lamb development. In addition, supplementary feeding to the ewe ewes ensured that the lambs were well fed during the lactation period and improved the carcass weight. As can be seen in Table 6, 23 out of 24 respondents take vitamin supplements. This shows that the lambs received adequate amounts of various vitamins needed during the development period, and as a result, they continued their lives without any negative effects on their growth and development. In the next stage, it is seen that the lambs showed good development in the fattening period and gave satisfactory results in terms of

live weight gain.

According to the answers given to the question about the use of licking stones, 20 out of 24 producers (83.3%) stated that they gave licking stones to their lambs. As shown in Table 7, where the effects of licking stone supplementation on the carcass weight of lambs were evaluated, positive effects of additional mineral supplementation for lambs were observed on the average carcass yield between 18 and 22 kg.

The internal-external parasite control practices applied to the fattened lambs are shown in Table 6. In Table 6, it is seen that all of the breeders who participated in the questionnaire paid attention to internal-external parasite control. It shows that the positive effects of this control method on both feed utilization rate and carcass yield are

adopted by the breeders.

The release of lambs to pasture during the period until sale is shown in Table 6. All of the breeders who participated in the survey stated that lambs were not released to pasture when they were fattened. The reason for this is the concern that the lambs going to the pasture will consume extra energy in the pasture, which will negatively affect the carcass yield. Another important factor in the success of sheep breeders in Tekkeköy is this practice. Lambs gain live weight rapidly in the pen by both sucking their mothers and consuming fattening feed. With this practice, fattening is completed in a shorter time, and better live carcass weights are obtained. The preference of the breeders for winter or summer lambs in the selection of female lambs for breeding is shown in Table 6. While 22 (91.7%) of the 24

respondents preferred winter lambs, 2 (8.3%) preferred summer lambs. The general reason for this situation is that summer lambs are more affected by the heat, and most of the births coincide with the winter months.

3.3. The Effect of Feed Supplementation for Ewes and Licking Stone (containing salt) Supplementation for Lambs on Average Carcass Weight After Birth

The carcass averages obtained by the breeders who did and did not make postpartum feed supplementation in ewes and who did and did not make licking stone application to lambs are given in Table 7. When Table 7 is analyzed, it is seen that there are 20 breeders who reported that these practices affected the carcass weight and 4 breeders who reported that they did not.

Table 7. The effect of feed supplementation for ewes and licking stone (containing salt) supplementation for lambs on average carcass weight after birth

Average carcass weight/kg	Yes		No	
	n	%	n	%
17.00	0	-	3	100.00
18.00	6	85.7	1	14.3
19.00	2	100.00	0	-
19.50	1	100.00	0	-
20.00	7	100.00	0	-
21.00	3	100.00	0	-
22.00	1	100.00	0	-
Total	20		4	

4. Discussion

Karayaka sheep are resistant to the harsh climate and poor pasture conditions of the hills and plateaus in the Black Sea region and local diseases (Cam and Kuran, 2004). It has an important place among domestic sheep in terms of meat production and meat quality (Sen et al., 2011). Karayaka lambs have high meat quality due to the mosaic distribution of fat between muscle fibers (Ulutas et al., 2010). As a result of the study, it can be said that Karayaka sheep is more preferred in terms of meat quality and meeting the meat needs of the region.

Feeding before and during pregnancy in ewes is important for the development of the offspring. Maternal mineral status in ewes, including manganese (Mn), is critical for both fetal growth and newborn lamb health. Adequate levels of minerals should be provided to the pregnant animal to support embryonic and fetal development and prevent losses during pregnancy (Toghdory et al., 2023). In the study, it can be said that sheep breeders in Tekkeköy are more conscious in terms of postnatal feed supplementation and vitamin and mineral applications to lambs.

Breeding practices and environmental effects have important effects on reproductive efficiency and offspring development in sheep breeding. It has been reported in many studies that, especially in pre-shearing body conditions, supplementary feeding and flushing

practices have a positive effect on the concentration of estrus, ovulation rate, fertility rate, early embryo mortality, and lamb survival rate (Hafez et al., 2011; Naqvi et al., 2011; Scaramuzzi et al., 2006). In Yozgat province, it was reported that 8% of the breeders practiced flushing before vaccination (Tüfekci, 2020). It has been reported that flushing application in Kivırcık ewes aggregates estrus and increases lamb yield (Öziş Altınçekiç et al., 2018). In this study, the rate of flushing was high (95.8%). This result was similar to the results of many studies (Hafez et al., 2011; Naqvi et al., 2011; Öziş Altınçekiç et al., 2018; Scaramuzzi et al., 2006).

Vitamins play an important role in the regularity of vital activities in living organisms. It is recommended to be added to feeds for the activity of the female reproductive system, the functions of hormones and enzymes, and the prevention of fouling, early lamb mortality, and lamb development (Yeşil and Sarıözkan, 2017). It has been reported that organic Mn consumption during pregnancy in ewes causes a significant increase in plasma Mn concentration in ewes and lambs. It was also reported to significantly increase glucose, insulin, and superoxide dismutase levels and total protein and albumin concentrations in both ewes and lambs. In general, organic Mn nutrition has been reported to improve blood biochemical and hematological factors in ewes and newborn lambs (Toghdory et al., 2023). In this study, it

was determined that the rate of vitamin supplementation in lambs was high.

Lambs fed with organic or inorganic trace mineral supplementation showed higher dry matter intake and growth rate and better feed conversion efficiency ($P<0.05$). It was also reported that the triglyceride ($P<0.01$) concentration was lower and the vitamin B12 concentration was higher in the mineral-supplemented groups. It was stated that feeding with organic trace elements improved growth performance (Samarin et al., 2022). In cases of low growth performance in lambs, deficiencies of several minerals, especially Co, Cu, and Se, should be considered (Helmer et al., 2021). In another study, the rate of oral vitamin and mineral administration as a health protection practice in the flock was found to be 20.2% (Şahin and Olfaz, 2019). In this study, vitamin administration was 95.8% (Table 6). It is seen that vitamin and mineral supplementation is adopted by breeders for the development of lambs and their resistance to diseases.

Tüfekci (2020) reported that all of the breeders in Yozgat province used salt-containing licking stones. In another study, 79.80% used licking stones as health protection practices in the herd (Şahin and Olfaz, 2019). In a study conducted in Elazığ province, 73.1% of the breeders' added salt and minerals to the feed (Köseman et al., 2022). Weakening, growth retardation, anemia, wool discoloration, disease, and mortality were observed in four- and six-month-old lambs due to mineral deficiencies (Helmer et al., 2021). The findings obtained in this study are consistent with those reported in the literature (Şahin and Olfaz, 2019; Tüfekci, 2020; Köseman et al., 2022). The widespread use of licking stones in enterprises shows that the awareness of breeders about feeding has increased.

In a study, Tuj male lambs were vaccinated against internal and external parasites, followed by a one-week feed acclimatization period, and then divided into three groups with 13 lambs in each group. These groups were given ad libitum concentrate feed and 300 g of medium-quality dry meadow grass daily for each lamb, and clean water was kept in front of them during the experiment. At the end of the study, the difference between the groups was found to be significant ($P<0.05$) in terms of anterior shank circumference measured at the middle and end of the experiment in the sixty-day fattening group (Adıgüzel Işık et al., 2023). In ovine animals, internal and external parasites can negatively affect feed utilization and reduce meat yield. For this reason, internal and external parasite treatment should be performed before fattening. In many fattening studies, internal and external parasite applications were performed (Alshamiry et al., 2023; Güngör et al., 2023; Ölmez et al., 2023). The disease rate due to external parasites in Ordu province was 15.9% (Alkan and Türkmen, 2021). Diseases due to internal and external parasites can be seen in different provinces in the Black Sea region. The effect of internal and external parasite

control in the fight against disease agents is important. In this respect, according to the results of the research, it is seen that the breeders in Tekkeköy district adopt internal and external parasite control practices to protect herd health.

In another study, it was reported that lambs were put up for sale in the first 6 months and fed on pasture (Çiçek et al., 2022). In Karayaka ewes, the intensive fattening method is preferred more than semi-intensive and extensive fattening methods in 2-3-month-old lambs (Pala and Gülşen, 2021). Natural lamb rearing is applied in 89.9% of the holdings in Elazığ province (Köseman et al., 2022) and 96.77% in Malatya province (Şeker et al., 2021). In Yozgat province, 24.5% of the holdings gave supplementary feeding to lambs (Tüfekci, 2020). The results of the study are similar to those reported in the literature (Köseman et al., 2022; Şeker et al., 2021; Tüfekci, 2020). In the study, fattened lambs were not released to pasture, and both natural rearing and supplementary feeding were performed.

In a study conducted in Karayaka ewes, it was reported that birth and slaughter performances of lambs born in winter and autumn were similar, but the weaning weight of lambs born in autumn was higher ($P\leq 0.05$) (Sen et al., 2013). In a study investigating the effect of breeding season on meat quality, it was reported that winter lambs had higher carcass quality than lambs born in spring, summer, and autumn (Yalcintan et al., 2017). The fact that live weight gain and meat quality are better in winter lambs, as reported in the literature (Sen et al., 2013; Yalcintan et al., 2017), may cause breeders to prefer winter lamb fattening.

Lambing season (winter and summer) has a significant ($P\leq 0.005$) effect on birth and weaning weights (Ürüşan and Emsen, 2010). In a study on the fattening performance of Kivırcık and Karya ewes, fattening age was determined as the 4th week after birth, fattening period as 10 weeks, and daily live weight gain during fattening was 246 g for males and 185 g for females (Altın et al., 2005). In another study, in terms of fattening performance, the live weights of lambs fed on pasture (33 kg) were higher than those fed in a confined environment (28 kg) from the 15th week to the 32nd week after weaning. Rations supplemented with rumen-protected conjugated linoleic acid are recommended for indoor feeding (Bittante et al., 2021). It has been reported that slaughtering Karayaka lambs or crossbred male lambs in winter may have a positive effect on hot carcass weight without affecting fattening performance (Çam et al., 2007). According to the reports in the literature, it is seen that additional supplements and winter fattening are preferred in closed-system fattening. The age of starting fattening, vitamin and licking stone applications, and winter lamb fattening applied in this study are similar to the literature (Altın et al., 2005; Çam et al., 2007).

It was reported that 90.86% of the farms in Malatya province did not make supplementary feeding but added additional salt and minerals to the feed (Şeker et al.,

2021). In this study, the finding that supplementary feed supplementation to broodstock affected the average carcass weight of lambs was different from that reported by Şeker et al. (2021). This situation shows that the importance of feed supplementation in terms of mother body reserves and offspring nutrition is recognized by breeders.

Minerals have an important place in body functioning. Especially in the bone and skeletal systems, many minerals influence the activation of enzymes. The positive effects of licking stones and mineral supplements are reported in sheep farms where pasture-dependent feeding is done (Tuncer, 2018). The result reported in the study is like that reported by Tuncer (2018).

5. Conclusion

As a result of the study, it was determined that approximately 96.0% of the breeders practiced flushing. This practice had a positive effect on reproductive efficiency, birth weight of lambs, and carcass weight obtained because of fattening. Supplementary feed, vitamins, and licking stone supplementation for ewes after birth had a positive effect on the development and live weight gain of lambs and had a positive effect on carcass weights. While 3 of the holdings that obtained 17 kg of carcass weight did not supplement feed, all 7 holdings that obtained 20 kg of carcass weight supplemented feed. Similar results were observed in the enterprises that added licking stones. When the results obtained were evaluated in general, lamb carcass weight was positively affected in the enterprises applying flushing, giving additional feed, vitamins, and licking stones to the broodstock. Most of the farms in the Tekkeköy region do not send their lambs to pasture until slaughter, but they bring their lambs to slaughter in the early winter period and market them with high carcasses and high prices by adding the previously reported positive practices.

Author Contributions

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

	H.T.Ç.	M.O.	T.D.
C	100		
D	100		
S	50	50	
DCP		50	50
DAI	50	30	20
L	50	20	30
W	50	20	30
CR	50	50	
SR	100		
PM	20	50	30
FA		50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Permission to conduct the study was obtained with the decision of the Ondokuz Mayıs University Social Sciences and Humanities Research Ethics Committee (approval date: August 26, 2022, protocol code: 2022-707).

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DETERMINATION OF ERGONOMIC FACTORS OF THE BACKPACK BLOWERS USED IN THE WINDROW OF HAZELNUTS

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Abstract: Ergonomics plays an important role in the design and use of agricultural machinery to protect the health and safety of workers. Lack of ergonomic design negatively affects worker discomfort and safety. Therefore, ergonomic design should ensure that workers can work comfortably, maintain correct posture, and minimize the risk of injury. Especially workers using backpack machines are exposed to physical fatigue, posture problems, risk of injury, and ergonomic effects due to vibration and noise. Considering all these, this study was conducted to determine the ergonomic factors (ergonomic analysis of the operator's working posture, operator's fatigue values, noise level, and dust concentration) that the operator is exposed to as a result of different body positions and moving the air diverter hose while using the backpack type blowers used to windrow the kernel+husked nuts falling on the orchard ground during the hazelnut harvest period. According to this, in all three ergonomic risk methods, the operator's body posture was found to be risky, and ergonomic adjustments were needed. Again, the operator's fatigue value, noise level, and dust concentration were obtained as 10.43 kcal min⁻¹, 91.70 dB(A)-106.20 dB(A) (average 98.95 dB(A)), 19.241-21.390 mg m⁻³-air (average 20.315 mg m⁻³-air), respectively. The identification of ergonomic factors is also very important to improve the user experience, prevent occupational accidents, and improve machine performance in general. With the identification of these factors, optimized solutions for the safety and comfort of users will be put forward by adopting a human-centered approach in machine design.

Keywords: Ergonomic, Backpack blower, Hazelnut harvester

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1. Introduction

The main purpose of ergonomics is to design and organize work or living environments where people and technology come together so that users can work effectively, efficiently, and safely. Working conditions need to be improved and harmonized with the employee. Accordingly, it will be possible to increase the performance of the employee by ensuring the health, safety, and comfort of the employee. Since agricultural activities have a dynamic structure, it is more difficult to systematically analyze the ergonomic factors to which employees in the agricultural sector are exposed compared to other sectors (Mert, 2014; Kir, 2015; Sauk et al., 2022).

Hazelnut harvesting, which has an important place in the agriculture of our country, is done manually, but it is also done mechanically with the widespread use of hazelnut harvesting machines developed by local manufacturers recently. Although the use of human labor requirement has decreased somewhat with the development of mechanization, the use of the labor force continues during hazelnut harvesting (Beyhan and Sauk, 2018). Three different harvesting methods are generally applied in the mechanical harvesting of hazelnuts. The first of these is the harvesting of the hazelnut kernel+husked

nuts into windrow with the help of a backpack-type blower after orchard cleaning by aspiration harvesting machines. The second one is orchard cleaning followed by harvesting with aspiration harvesting machines and finally harvesting the hazelnuts with mechanical sweepers by sweeping the hazelnuts into a windrow and harvesting the hazelnuts with machines with mechanical harvesting units. With these methods, hazelnut harvesting is completed in a short time and costs can be reduced significantly (Biondi et al., 1992; Beyhan and Yıldız, 1996; Tous, 2001; Monarca et al. 2005; Fanigliulo and Tomasone, 2008).

Backpack-type leaf blowers are generally used for orchard maintenance and cleaning works. However, in recent years, in our country, it has been used both to obtain a clean orchard ground before harvesting by cleaning the foreign material residues on the ground before the hazelnuts fall on the orchard ground and to windrow the hazelnuts on the orchard ground during the harvest period. By windrow the hazelnut, the harvesting process is shortened and the working efficiency is increased. Thus, a fast and effective hazelnut harvest helps to save time and labor, reduce soil contamination, and obtain a cleaner and healthier product (Beyhan, 1992; Bernardi et al., 2017), However, in addition to all



these advantages, carrying these machines on the back may cause ergonomic problems in long-term use. As the weight of the loads carried on the back increases, there is an increasing forward bending and strain on the knees, and similarly, as the load begins to descend from the shoulder to the waist, the forward bending of the trunk increases. The World Labor Organization (ILO) and individual countries have "Maximum Transportable Load" decisions and practices for this type of back-carrying work (Anonymous, 2016). It has been reported that the use of back-carrying type machines increases the body load of employees, will cause musculoskeletal disorders (MSSR) in long-term work, the vibration created by the machine spreads along the entire back, hands, and arms, which develops physiological and biomechanical stress, and also causes pain in the palm and wrist areas of the hand grip (Kumar et al., 2011; Halim et al., 2019; Ajid, 2021; Aygün et al., 2021).

Ergonomics plays an important role in the design and use of agricultural machinery to protect employee health and safety. Employees using this type of machinery are exposed to physical fatigue, posture problems, risk of injury, and ergonomic effects due to vibration and noise. In addition, the lack of ergonomic design also negatively affects the discomfort and safety of the worker. Therefore, ergonomic design should ensure that workers can work comfortably, maintain correct posture, and minimize the risk of injury. Considering all these, backpack blowers have started to be widely used by farmers in the region in recent years to windrow the hazelnuts that fall on the orchard ground during the hazelnut harvest period. These factors aim to provide an optimized solution for the safety and comfort of users by adopting a human-centered approach to machine design. These factors aim to provide an optimized solution for the safety and comfort of users by adopting a human-centered approach to machine design.

2. Materials and Methods

2.1. Materials

The study was conducted in a farmer's orchard located in Çarşamba district of Samsun province (41°17'59"N 36°43'15"E). The hazelnut orchard where the trials were conducted has Palaz and Çakıldak hazelnut varieties which are widely grown in the region. During the trials, the air temperature was 29.20 °C, relative humidity was 58.21% and wind speed was 0.50-0.65 m s⁻¹.

The backpack blower used in the study consists of six main units: engine, blowing unit, air routing system (blowing hose), control and control unit, carrying system (such as handle and shoulder straps), and fuel tank. Technical specifications of the machine are given in Table 1. These units are the basic components of backpack blowers. However, depending on the model and mark, the design and features of these units may vary.

Noise measurements of the machine were made with a handheld device model TES 1351B, IEC61672-1 Class 2, with a measurement range of 35-130 dB(A) and a

sensitivity of 0.1 dB. Before each measurement, the noise meter was calibrated with a CEM SC-05 model 23 calibrator with a signal of 94 dB(A) at 1000 Hz. Operator fatigue values were determined using a Geonate model On the Rhythm 410 program clock and chest strap. Dust concentration was measured using an AeroTrak APC 9303-01 handheld particle meter with a particle size range of 0.3, 2.5, and 5 µm.

Table 1. Technical specifications of the backpack blower used in the trials

Mark	Stihl BR 700
Cylinder volume (cc)	64.80
Power (kW)	2.80
Weight (kg)	10.80
Air flow rate (m ³ h ⁻¹)	1550
Blowing speed (m s ⁻¹)	74
Blowing power (N)	35

2.2. Method

To determine the ergonomic factors of the backpack-type blower, experimental plots were formed in the orchard with an average of 4.90 m between rows and 4.85 m above rows, each plot containing 10 hazelnut brushes ("ocak" in Turkish). The experiments were carried out with three replications according to the randomized plots experimental design under the condition of 189 kg da⁻¹ average orchard yield as milling dry hazelnut with 10% moisture content (y.b.). In each plot, the hazelnuts with kernel+husked nuts in the ocak, between the rows and above the rows were blown between the rows to form a windrow. During the studies, a 46-year-old male operator, 172 cm tall and 94 kg in weight, was used. Images from the hazelnut orchard where the experiments were carried out and during the operation of the operator are given in Figure 1.

2.2.1. Determination of operator's ergonomic risk analysis

The tasks performed by the operator during the windrow of hazelnuts were examined, and work postures were documented. No intervention was made to the operator during the experiments. The body postures of the operator during work were determined using the ergonomic risk assessment methods of REBA, OWAS, and RULA, and risk scores and action plans were identified. In the work environment, various methods are used to determine the ergonomic risk levels based on the nature of the tasks. For tasks predominantly involving the upper body (hand, wrist, elbow, upper arm, shoulder, and neck) performed repetitively in agricultural activities, RULA (Rapid Upper Limb Assessment) is commonly used. Tasks are grouped, and based on the percentage of total working time that each posture covers, OWAS (Ovako Working Posture Analyzing System) is preferred. For the evaluation of dynamic and static body postures, the REBA (Rapid Entire Body Assessment) method is widely used.



Figure 1. General view of the hazelnut orchard during the trials.

As a result of the analysis, the physical load scores of the workers were determined, and an ergonomic risk assessment was conducted (Eminoğlu and Koç, 2018; Sauk et al., 2022). ErgoFellow 2.0 software package was used for the examination of images.

2.2.2. Determination of operator fatigue values

During the experiments, the operator's heart rate values were measured using telemetry (remote measurement) (Eminoğlu et al., 2012). In this method, the transmitter part of the device was attached to the operator's chest throughout both work and rest periods. The heart rate, maximum heart rate (BPM), total energy consumption (kcal), and the amount of burned fat (g) were recorded. The chest strap is attached to the operator's body as seen in Figure 2.



Figure 2. Attachment method of the chest band.

The total energy consumed by the operator in each plot was measured, and the energy consumption per minute was obtained by dividing the total time spent during the windrow of the kernel+husked nuts in the plot with a backpack blower. Before the start of the trials, the operator's heart rate was measured with the help of a chest strap, and after each plot was blown and windrow, the trials were interrupted until the operator's heart rate reached the initial heart rate. The energy consumption of the operator was compared with the recommended limit value of 10 kcal min⁻¹ for seasonal workers. Using the energy consumption, the rest intervals of the operator were determined with the help of the following Equation 1 (Sabancı et al., 2012).

$$RTR = [(EC - 4 \text{ kcal}) / EC].100 \quad (1)$$

where; RTR= Rest time rate (%) and EC= Energy consumption (kcal min⁻¹).

2.2.3. Determination of the noise level

The noise level of the machine was measured for five minutes with the microphone of the measuring device at the operator's ear level. The noise level was measured in dB(A) and the instrument was calibrated before each measurement. The noise level exposed during the operation of the machine was taken as the warning limit of 85 dB(A) and the hazard limit of 90 dB(A), which are

recommended for the health of people under the influence of different levels of noise according to the noise control regulation and compared with these values (Sauk, 2016).

2.2.4. Determination of dust concentration

To determine the dust concentration values of the backpack-type blower, measurements were made for 30 minutes with the dust measuring device at a height of 1.5 m above the ground. The measurement points were determined by the purpose of the study, within the working area of the operator. The average of the dust concentration values obtained are given. As a result of the measurements; PM_{0.3} (0.3 µm), PM_{2.5} (2.5 µm), and PM_{5.0} (5 µm) dust concentrations were determined in µg m⁻³-air. The dust levels generated in the environment during the operation of the machine were compared considering the dust concentration threshold limit of 3 µg m⁻³-air value specified in occupational health and safety legislation and regulations (Sauk, 2016).

3. Results and Discussion

3.1. Ergonomic Risk Analysis of the Operator

Risk scores and action plans of the operator's body postures during work using REBA, OWAS, and RULA ergonomic risk methods are given in Table 2.

Table 2. Operator's risk scores and action plan according to ergonomic risk analysis methods

Ergonomic risk method	Obtained risk score	Action plan
REBA	7	Medium risk, precautions required Working postures have clear detrimental effects on the
OWAS	Category 3	musculoskeletal system. Ergonomic adjustment for these postures is needed as early as possible.
RULA	6	Medium risk, change required soon

As can be seen from Table 2, in all three ergonomic risk methods, the operator's body posture was found to be risky, and ergonomic adjustments were needed. Carrying the blower on the back increases the physical load of the operator. Considering the REBA, OWAS, and RULA scores obtained in the back carrying, it is predicted that it may lead to MSSR in long-term working conditions. The observations and results from the measurements show that the windrow of hazelnuts with the backpack blower

takes place under non-ergonomic conditions. The weight of the blower forces the operator to keep himself in a position of balance. In this case, the load not only increases the strain on the knees over time but also increases the forward tilt of the trunk when the load starts to descend from the shoulder to the waist. Carrying the machines on the back for a long time is the most difficult situation for the operator. To minimize this kind of physical strain, the operator should take a break from working at certain intervals.

3.2. Operator Fatigue Values

Before the start of the study, the operator's heart rate per minute was 78 BPM, maximum heart rate was 82 BPM, energy consumption was 2.2 kcal min⁻¹ and the amount of fat burned was 0.21 g. The number of heartbeats, total energy consumption, amount of fat burned, weight of hazelnuts (kernel+husked nuts), and windrow times measured in the experimental plots where fatigue tests were performed are given in Table 3.

Table 3. Parameters of operator fatigue values measured in trial plots

Parameters	Average values
Weight of hazelnuts in the plot (kg)	44.92
Windrow making time (sec)	725
Heart rate (BPM)	118
Total energy consumption (kcal)	126
Amount of fat burned (g)	6.20

As seen in Table 3, during the windrow of 44.92 kg of hazelnuts in the experimental plot with a backpack blower, the operator's heart rate, total energy consumption, amount of fat burned, and windrow time were 118 BPM, 126 kcal, 6.20 g and 725 seconds, respectively. During the windrow of the hazelnuts in the plot, the energy expended by the operator per minute was 10.43 kcal min⁻¹. Again, the energy value that the operator will need to windrow 1 kg of hazelnuts is 2.80 kcal kg⁻¹-nut. According to the energy consumption of the operator in 725 seconds, the resting time ratio is approximately 61.64%. In other words, in 1 hour period, the operator must devote approximately 36.98 minutes to resting.

3.3. Operator's Exposed Noise Level

The minimum, maximum, and average noise level values of the backpack blower were 91.70 dB(A), 106.20 dB(A), and 98.95 dB(A), respectively. Since sounds with noise levels above 85 dB(A) have effects such as temporary and permanent hearing loss, the International Labor Organization (ILO) has accepted 85 dB(A) as the warning limit and 90 dB(A) as the dangerous limit (Sabancı et al., 2012). Accordingly, it is not suitable for the operator to work with this machine continuously for 8 hours a day. The operator can work for a maximum of 3-4 hours continuously.

3.4. Operator's Exposed Dust Concentration

The lowest and highest measurement range of dust

concentrations in the environment during the windrow of hazelnuts with a backpack blower according to PM_{0.3}, PM_{2.5}, and PM_{5.0} diameter groups are given in Table 4. Dust concentration values were converted to mg m⁻³-air.

Table 4. Variation of the dust concentrations created by the machines in the environment according to the lowest and highest measurement range according to diameter groups

Particle Diameter (µm)	Dust concentration (mg m ⁻³ -air)	
	Lowest	Highest
PM _{0.3}	7.501	8.218
PM _{2.5}	6.927	7.448
PM _{5.0}	4.813	5.724
PM ₁₀	19.241	21.390

As seen in Table 4, the lowest and highest values of the dust concentrations emitted by the machine to the environment according to PM_{0.3}, PM_{2.5}, and PM_{5.0} µm diameter groups were measured between 7.501-8.218 mg m⁻³-air, 6.927-7.448 mg m⁻³-air, and 4.813-5.724 mg m⁻³-air, respectively. Total (PM₁₀; particle diameter less than 10 µm) dust concentration values emitted by the machine ranged between 19.241-21.390 mg m⁻³-air (average 20.315 mg m⁻³-air). These values are above the threshold limit of 3 mg m⁻³-air for respirable dust in the ACGIH TLV standard. As a result, dust concentrations are above the threshold limit value and are at a level to adversely affect the health of workers.

4. Conclusion

Ergonomic analysis of the working postures, fatigue values, noise level, and dust concentration values of the backpack-type blowers used to windrow the hazelnuts that fall on the orchard ground during the hazelnut harvest period was determined as a result of different body positions and moving the air diverter hose while using the blowers in the orchard on the operator's back. Accordingly, the use of the backpack blower had the highest ergonomic risk scores affecting worker health. Among the ergonomic risk factors, improper body posture, repetitive movements, and ergonomic inadequacy of the tools used are important factors that cause MSSR. Considering that these factors are the movements that are frequently performed during the windrow of hazelnuts, it can be said that the backpack blower causes MSSR. From this point of view, if the existing machines are to be used, it is necessary to have a sufficient number of employees so that employees can be rotated among them. However, it can be said that this will bring an additional burden to the harvesting cost. According to the energy expended by the operator to windrow the hazelnuts with the backpack blower, working with this machine is classified as heavy work. The use of a backpack blower can cause different fatigue levels in people depending on many factors. The operator

should take regular breaks, exercise regularly, eat a balanced and healthy diet, have good sleeping habits, and avoid stress. In addition, the weight of the machine and its handling systems will affect the user's comfort in handling and using it, so they should prefer machines with a lightweight and ergonomic design. Implementing these measures can help prevent fatigue in the work environment.

The noise level may vary depending on the type of engine used, power level, make, and model specifications. Gasoline-powered models generally have a higher noise level, so the operator may need to wear hearing protection such as business headsets or earplugs. It is preferable to use equipment with low noise levels (such as models powered by electric motors). Training and raising awareness among workers on noise handling and protection is also important.

The high dust concentration created by the backpack-type blower in the environment is due to the working principle of the machine. During the circulation of the air guide hose of the machine on the orchard ground, particles such as leaves, small branches, small stones, soil, and coarse dust together with hazelnuts with kernel+husked nuts are mixed into the air during blowing from the orchard ground and dust is formed. Working in such an environment may involve potential risks of damage to the respiratory tract and skin. When working with this type of machine, the operator should wear dust masks or respiratory protection masks, goggles to prevent dust contact with the eyes, and appropriate clothing (such as long sleeves and leggings) to protect the skin.

In conclusion, with the increasing level of mechanization, the necessity of investigating the hazards and risks of new systems in terms of occupational health and safety and preventing the identified risks through continuous improvement has become evident. In such researches, examining the working conditions from an ergonomic point of view, putting forward and implementing improvement suggestions will play an important role in reducing occupational accidents as well as protecting employee health.

Author Contributions

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

	H.S.	K.M.U.
C	55	45
D	100	
S	100	
DCP	70	30
DAI	100	
L	60	40
W	60	40
CR	60	40
SR	60	40
PM	60	40
FA	60	40

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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EFFECTS OF SALICYLIC ACID SEED PRIMING ON GERMINATION OF LENTIL (*Lens culinaris* Medik.) EXPOSED TO SALT STRESS

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Abstract: Lentil (*Lens culinaris* Medik.) is an essential crop globally, particularly in Türkiye, West Asia, Southern Europe, India, and Africa. Lentil is a valuable food source, rich in proteins, carbohydrates, minerals, and vitamins. However, lentil production faces challenges due to salinity stress, which hampers water uptake and causes toxic effects on plants. The study aimed to investigate the effects of salicylic acid (SA) seed priming and varying salt (NaCl) concentrations on the germination and development of lentil seeds. The research was conducted in the Field Crops Biotechnology Laboratory, University of Dicle, Faculty of Agriculture, Türkiye. In the research, control, two salt (NaCl), four salicylic acid (SA) doses and their combinations were used on *Lens culinaris* variety called İlke. Germination percentage, energy, rate index, mean germination time, peak value, and vigor index were calculated. The results demonstrated that SA had a significant impact on improving lentil germination under salt stress conditions. Specifically, lower concentrations of NaCl combined with SA showed positive effects on germination and growth parameters compared to higher NaCl concentrations. The highest germination percentage, energy, and rate index values were in the control and 0.25 SA dose treatment. Salicylic acid doses under 50 NaCl concentration exhibited higher germination percentage, energy, and rate index values than under 100 NaCl. Increasing SA and salt doses negatively affected lentil seed germination. This finding is significant for lentil cultivation in salt-affected areas, offering a potential strategy to improve crop resilience and yield in challenging environmental conditions.


Keywords: Lentil, *Lens culinaris* Medik., Salt stress, Salicylic acid, Germination

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1. Introduction

Lentil is a legume crop with 2n=14 chromosomes and an annual growth cycle. It is an important crop in Türkiye, following peas and chickpeas (Ladizinsky, 1979; Jha and Halder, 2016; Sehgal et al., 2021). In Türkiye, lentils are produced on 81,741 hectares of land, yielding 353,631 tons of product (FAO, 2019). Lentils are also cultivated in West Asia, Southern Europe, India, and Africa.

Lentils are a nutritious food source high in carbohydrates, protein, minerals, and vitamins (Migliozzi et al., 2015). The protein content varies depending on growing conditions, techniques, and varietal characteristics, but it averages 22.7-31.9% (Toklu et al., 2009). Lentils are essential for human and animal nutrition, especially for those who consume vegan and vegetarian products.

Plants are exposed to biotic and abiotic stress factors (Georgieva and Vassileva, 2023). Biotic stress conditions refer to the impact of bacteria, fungi, plants, and animals, while abiotic stress conditions include radiation, drought, salinity, and variations in temperature and light intensity. Reactive oxygen species (ROS) are produced and accumulated by plants under different stress conditions

(Guo et al., 2023). Disruptions in metabolic events lead to oxidative stress, which causes damage to DNA, enzyme inactivation, and lipid peroxidation (Tounekti et al., 2013).

Legumes are highly sensitive to salinity stress, which can reduce yield (Farooq et al., 2017). Salinity stress can reduce plant quality and yield and negatively affect soil structure. Salinity can increase due to evaporation at high temperatures and uncontrolled irrigation. Physiological drought is caused by salinity stress, which prevents water uptake from plant roots and can have a toxic effect on glycophytic plants due to the accumulation of salt ions (Tester and Davenport, 2003). Salinity stress can reduce the germination rate and cause yield losses by preventing germination (Ondrasek et al., 2022). According to AL-Tawaha et al. (2013), salinity hurts the growth and development of lentils. Munns (2002) found that plant cells lose water and shrink in volume shortly after salt is applied.

Several growth regulators have been shown in numerous studies to effectively prevent salt stress. Studies, including the use of salicylic acid, a phenolic compound that belongs to the hydroxyl group. Salicylic acid helps



prevent oxidative damage caused by water stress from salt accumulation in plant roots and protects protein structure (Kaydan et al., 2007; Vicente and Plasencia, 2011). Choudhary et al. (2021), Idrees et al. (2011) and Lee et al. (2010) found that applying salicylic acid increased germination rates by preventing salt stress. Jain and Srivastava (1981) and Ramanujam et al. (1998) reported that salicylic acid, when applied at low concentrations, accelerates vegetative growth, and increases nodule formation, flowering, and pod number, particularly in legumes, resulting in a positive effect on grain yield. Additionally, Senaratna et al. (2000) and Kaydan and Yagmur (2006) found that salicylic acid applications in beans and tomatoes increase plant tolerance to drought and frost stress.

There is study aimed was to investigate the effects of different salicylic acid seed priming and salt concentrations on the germination and development of lentil.

2. Materials and Methods

The research was conducted in the Field Crops Biotechnology Laboratory, Faculty of Agriculture, Dicle University.

In the research, control, 2 salt (NaCl), 4 salicylic acid (SA) doses and their combinations were used on *Lens culinaris* variety called İlke (Table 1).

Table 1. Salt (NaCl), Salicylic acid (SA) and NaCl + SA combinations

Treatment	Combinations	
Control	50 NaCl +	0.25
NaCl (mM /L)		0.50
50		0.75
100		1.0
SA (mM/L)	100 NaCl +	0.25
0.25		0.50
0.50		0.75
0,75		1.0
1.0		

SA= salicylic acid, NaCl= salt, NaCl + SA= salt + salicylic acid.

The experiment followed the ISTA (1996) rules. To ensure surface sterilization of seeds, they were shaken in a 5% sodium hypochlorite solution for three minutes and then washed with distilled water. Excess water from the surface-sterilized seeds was removed with sterile filter papers. The experiment was arranged in randomized plots with three replications, using 40 lentil seeds for each treatment.

For salicylic acid pretreatment, seeds were soaked for 24 h in solutions of different concentrations (0.25, 0.50, 0.75, 1.0 mM/L). For the salt treatment, irrigation water with different salt concentrations (50 and 100 mM NaCl/L) was used. The seeds were soaked in distilled water for the control treatment. The Petri dishes were then incubated in a germination cabinet at 25±2 °C

(Doruk Kahraman and Topal, 2024). The germination paper was changed every two days for the duration of the experiment. Germinating seeds were defined as those with a length of the radicle of more than 2 mm (Doruk Kahraman and Topal, 2024). Measurements of the radicle and shoot traits were made after the seeds had been transferred to containers of 20 x 5 x 10 cm on the fourth day. Radicle and shoot lengths were measured with a ruler in cm, and radicle and shoot fresh weights were measured with a precision balance with an accuracy of 0.0001 g.

Germination percentage (GP) (%) (Equation 1), germination energy (GE) (%) (Equation 2), germination rate index (GRI) (Equation 3), mean germination time (MGT) (Equation 4), peak value (PV) (Equation 5), and vigour index (VI) (Equation 6) values were calculated using the following formulas:

$$GP = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds tested}} \times 100 \quad (1)$$

$$GE = \frac{\text{No. of seeds germinated on day (4. day)}}{\text{Total number of seeds tested}} \times 100 \quad (2)$$

$$GRI = \left(\frac{G1}{1}\right) + \left(\frac{G2}{2}\right) + \left(\frac{G3}{3}\right) + \dots + \left(\frac{Gi}{i}\right) \quad (3)$$

$$MGT = \frac{\sum Fx}{\sum (F * x +, \dots)} \quad (4)$$

$$PV = \frac{\text{Highest seed germinated}}{\text{Number of days}} \quad (5)$$

$$VI = (\text{Average root length} + \text{Average hypocotyle length}) * GP \quad (6)$$

2.1. Statistical Analysis

The data obtained from the study were subjected to ANOVA analysis in the JMP 14 PRO statistical program. Differences between means were determined according to 0.05 in the Duncan test.

3. Results and Discussion

The effects of NaCl, salicylic acid and their combinations on *Lens culinaris* Medik (İlke cv.) germinated seeds were observed during for seven days (Figure 1). In the control group, 17 out of 40 seeds were recorded as germinated on the first day, and the germination of 36 seeds was completed on the 3rd day of the experiment, and 4 seeds weren't germinated during the experiment. The highest number of germinated seeds on the first day was observed at 0.25 SA dose and 0.50 SA dose. Under 50 NaCl concentration, 5 germinated seeds were recorded on the first day, and the highest number of germinated seeds was reached on the 5th day with 13.7 seeds. Under 100 NaCl concentration, seed germination occurred on the first day, but no germinated seeds were observed on the following days. SA treatment doses at 100 NaCl concentration regressed germination compared to the 50 NaCl concentration and significantly reduced the number

of germinating seeds.

The effects of NaCl, salicylic acid and their combinations on the germination percentage, germination energy and germination rate index of *Lens culinaris* Medik (İlke cv.) seeds were significant (Table 2, Figure 2).

The highest germination percentage, energy and rate index values were in the control (90.0%, 90.00% and 76.53, respectively). 100 NaCl concentration was exhibited the lowest germination percentage, energy and rate index values (3.33%, 2.50% and 1.32%, respectively). The values for germination percentage, energy and rate index under 50 NaCl concentration were 34.2%, 30.0% and 22.8%, respectively. 50 NaCl concentration showed lower germination values than the

control, SA, and salt + SA combinations, although not as much as 100 NaCl.

The values for germination percentage, energy and rate index for the 0.25 SA dose treatment (89.2%, 89.2% and 76.5%, respectively) were higher than those for the 1.0 SA dose treatment (80.8%, 78.3% and 54.3%, respectively). Salicylic acid doses, under 50 NaCl concentration exhibited higher germination percentage, energy and rate index values than under 100 NaCl. 0.25 SA dose, under 50 NaCl and 100 NaCl concentrations exhibited higher germination percentage, energy and rate index values than other doses. It was determined that increasing SA and salt doses negatively affected lentil germination.

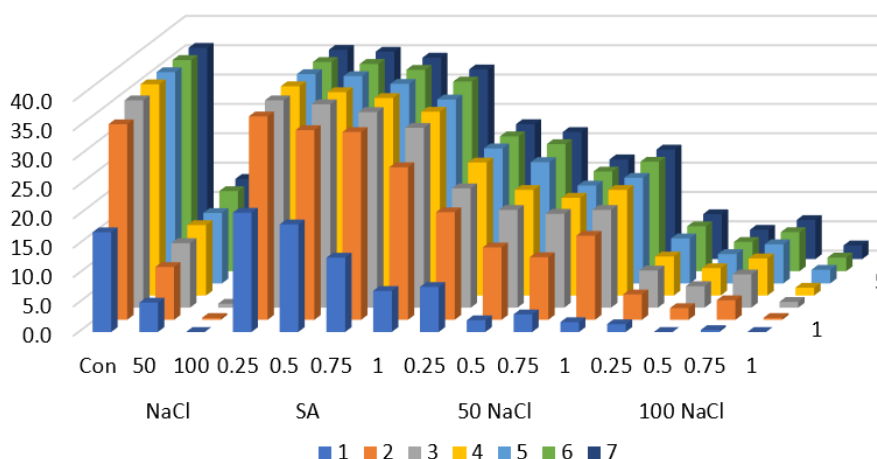


Figure 1. Effect of salt and SA treatments for germinated seeds and days.

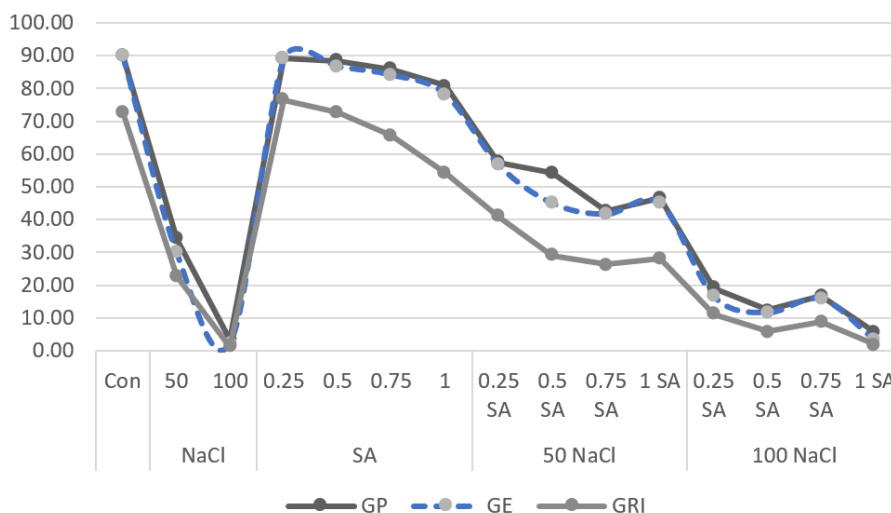


Figure 2. Effect of salt and SA treatments on germination percentage (GP), germination energy (GE), and germination rate index (GRI).

Table 2. Anova analysis for germination traits

Source	DF	GP	GE	GRI	MGT	PV
Treatment	14	3169.31**	3215.32**	2258.71**	0.293428**	14.3092**
Error	30	57.36	45.97	18.11	0.034870	0.3222
C. Total	44					

GP= Germination percentage %, GE= Germination energy %, GRI= germination rate index, MGT= Mean germination time, PV= Peak Value, VI= Vigour index %, *= P<0.05, **= P<0.001.

Ekmekci et al. (2005) reported that the decrease in germination rate with increasing salt dose was due to the toxicity of Na and Cl ions and the increase in osmotic pressure, which prevented the entry of water into the seed needed for germination. Uyanik et al. (2014) and Foolad and Lin (1997) reported that some metabolic disorders and inhibition of germination regulatory protein synthesis in plants exposed to salt stress caused a decrease in germination rate. It was also found by Mahdavi and Sanavy (2007), Fallahi et al. (2015), Gheidary et al. (2017), Haileselasie and Gselasie (2012), Tsegay and Gebreslassie (2014) and others that germination rate decreased with increasing salinity.

Mean Germination Time (MGT) and Peak Value (PV) were given in Table 2, Figure 3. MGT ranged from 4.0 to 5.5 days across all treatments. Mean germination time was more than 4.5 days for 100 NaCl concentration and all SA doses under 100 NaCl concentration. The highest mean germination time (5.26 days) was observed in 1.0 SA doses under 100 NaCl concentration. The control

group and salicylic acid doses had the highest peak values. Specifically, 0.25 SA (5.94) and 1.0 SA (6.27) had the highest peak values. Altuner et al. (2022) found that salt applications decreased the germination index in all varieties and decreased to the lowest level in 150 mM salt applications. Additionally, the average germination time increased with increasing salt doses. Similarly, Petrović et al. (2016) and Anaya et al. (2018) reported negative effects of increasing salt stress on germination in legumes. It is worth noting that SA can act as a signaling molecule, activating defense mechanisms in seeds that help them tolerate salt stress. High concentrations of SA can inhibit germination or have no significant effect, although it can indirectly lead to improved water uptake and faster germination in salt-stressed environments. The effectiveness of SA can vary depending on the type of seed, the level of salt stress, and other environmental factors.

Radicle length, hypocotyl length and vigor index were given in Table 3 and Figure 4.

Table 3. Analysis of variance for radicle and hypocotyl length, radicle and hypocotyl fresh weight and vigor index

Variation Sources	DF	Radicle fresh weight	Hypocotyl fresh weight	DF	Radicle length	Hypocotyl length	Vigor Index
Treatment	4	0.000090	0.000073	9	11.5764**	5.29765**	2655.53**
Error	10	0.000061	0.000025	18	0.4355	0.06657	45.31
C. Total	14			27			
Treatment		Radicle fresh weight (g)	Hypocotyl fresh weight (g)				
Control		0.058	0.041				
0.25 SA		0.058	0.031				
0.50 SA		0.054	0.028				
0.75 SA		0.066	0.031				
1SA		0.066	0.035				

SA= Salicylic acid, *= P<0.05, **= P<0.001.

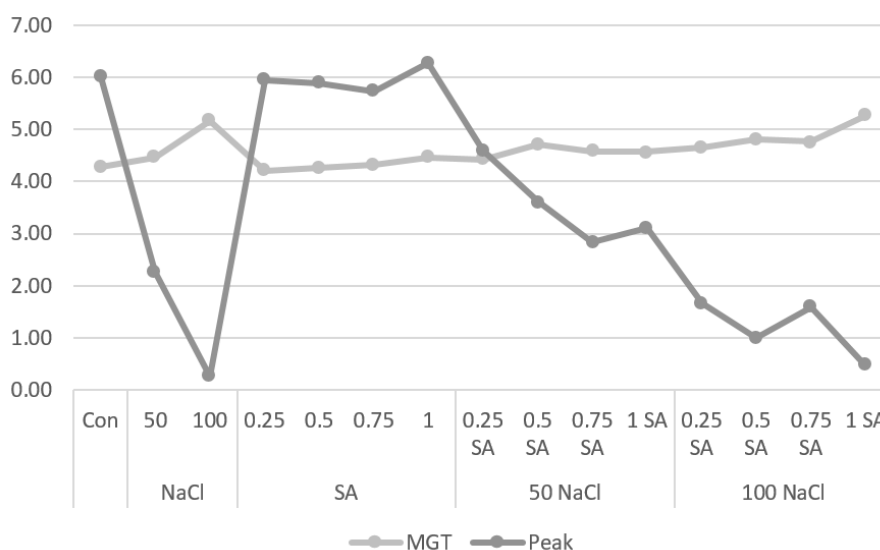


Figure 3. Effect of salt and SA treatments on radicle length, hypocotyl length, and vigor index on 8th days.

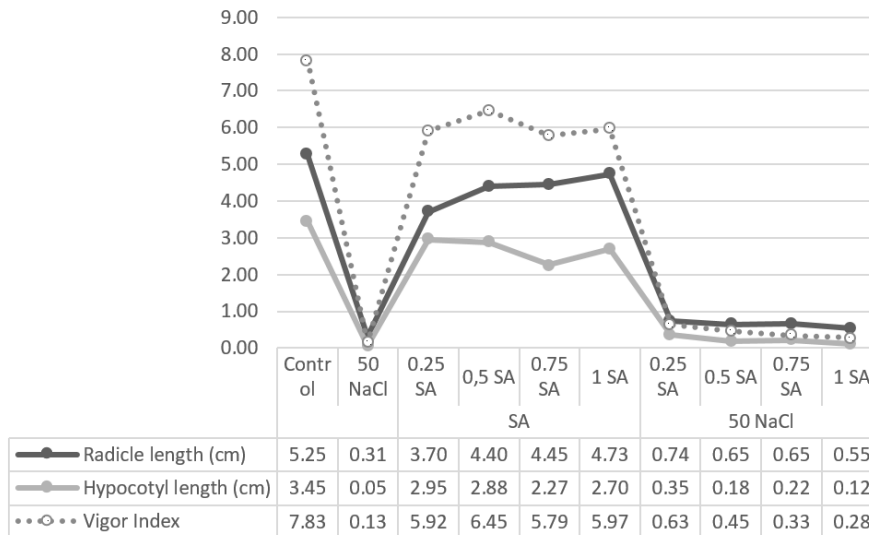


Figure 4. Effect of salt and SA treatments on radicle length, hypocotyl length, and vigor index on 8th days.

Radicle length, hypocotyl length, and vigor index were measured for the control, 50 NaCl concentration, SA doses, and salicylic acid doses under 50 NaCl concentration. The results showed that high salt stress had a negative impact on radicle and hypocotyl lengths, even with different SA doses. The control group had the highest radicle length, hypocotyl length, and vigor index (5.25 cm, 3.45 cm, and 7.8, respectively). Salicylic acid doses appeared to increase radicle and hypocotyl length under 50 NaCl salt stress. Radicle and hypocotyl fresh weight were only measured at different doses of salicylic acid. According to Haileselassie and Gselassie (2012), radicle and hypocotyl lengths are crucial parameters for assessing salt stress. This is because radicles absorb salt from the soil and water and contribute to hypocotyl development. Therefore, measuring radicle and hypocotyl lengths provides initial information on the level of salt exposure experienced by plants. El-Tayeb (2005) and Khodary (2004) found that pre-treating plants with SA increased their weight. Fallahi et al. (2015) reported that an increase in salt content led to a decrease in the fresh weights of rootlets and stems.

5. Conclusion

The study indicated that different combinations of salicylic acid (SA) and salt (NaCl) concentrations on lentil germination had a crucial impact. The results showed that the germination percentage, energy, and rate index values were differently affected by the combination of SA and salt concentrations. The group exposed to the combination of salt and SA had different germination values compared to the control group. However, the germination values were not as low as those observed in the group exposed to 100 NaCl concentration alone. High salt doses hinder nutrient uptake and water absorption, further compromising the overall growth and vigor of the seeds.

It was found that the germination percentage, energy,

and rate index values were higher for the 0.25 SA dose treatment compared to the 1.0 SA dose treatment. This suggests that a low dose of SA had a more positive effect on lentil germination under 50 NaCl concentration. The study also revealed that the effects of SA doses varied depending on the salt concentration. Under 50 NaCl concentration, the germination percentage, energy, and rate index values were higher for all SA doses compared to under 100 NaCl concentration. This indicates that under the lower salt concentration, SA has a more beneficial impact on lentil germination. Overall, increasing doses of SA and salt had a negative influence on lentil germination. However, the combination of SA and salt concentrations, as well as the specific doses used, played a significant role in determining the extent of this negative impact. Higher SA doses lead to reduced radicle growth and shoot development in lentil seedlings. In addition to the negative effect on the germination of lentil seeds, it was worth noting that the increase in SA (salicylic acid) and salt doses can also impact other aspects of seed development.

These findings showed that significance the treatments of SA under salt stress conditions in lentil cultivation to ensure optimal seed germination and subsequent plant growth.

Author Contributions

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

	G.K.T.	B.T.B.	F.B.
C	35	35	30
D	35	35	30
S		100	
DCP	50		50
DAI	50	25	25
L	50	30	20
W	50	30	20
CR	35	35	30
SR	50	30	20
PM	35	35	30
FA	45	10	45

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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BOSCALID + PYRACLOTROBIN RESISTANCE AND EXPRESSION OF SUCCINATE DEHYDROGENASE GENES (*sdhA* AND *sdhB*) IN CHESTNUT BLIGHT FUNGUS *Cryphonectria parasitica*

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Abstract: Sweet chestnut, *Castanea sativa* Mill., is a significant species in Türkiye. The tree has been encountered with numerous diseases. Chestnut blight, caused by *Cryphonectria parasitica* (Murrill) M. E. Barr, is one of the most common diseases having spread to every chestnut-growing region. In this study, the effects of Bellis® (25.2% Boscalid + 12.8% Pyraclostrobin), a fungicide commonly used in plant disease control, were evaluated at different doses (10 ppm and 50 ppm) against the chestnut blight agent *C. parasitica*, which had not been previously studied for its effects *in vitro*. The isolates included two virulent strains (Cp1_2023, Cp2_2023) and two hypovirulent strains (Cp7_2023, Cp9_2023). The effects of Boscalid + Pyraclostrobin on the mycelial growth of the pathogenic fungus were determined for the first time *in vitro*, and these effects were detected at the *sdhA* and *sdhB* genes expression level. The results showed that the mycelial growth and pathogenicity of *C. parasitica* isolates in the fungicide-treated medium were consistent with the gene expression profile. Specifically, the virulent Cp1_2023 isolate exhibited higher mycelial growth, pathogenicity, and gene expression levels compared to the other isolate. In conclusion, it is recommended to conduct more detailed studies before using fungicides and to employ them in controlled environments.


Keywords: Chestnut, *Cryphonectria parasitica*, Fungicide, *Sdh* genes

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1. Introduction

The chestnut tree, *Castanea sativa* Mill., is not only a forest tree comprising 81.232 ha pure forest (OGM, 2021) but with the production of 80.200 tons (FAOSTAT, 2024) is also an important fruit tree as well in Türkiye. Chestnut blight, known as chestnut canker, poses a significant threat to chestnut fruit production. This disease is caused by the Ascomyceteous fungus *Cryphonectria parasitica* (Murrill) M.E. Barr. first documented in Türkiye in 1967 (Delen, 1975), the blight has persisted across all regions of the country ever since (Çeliker and Onoğur, 2001; Erincik et al., 2008; Akıllı et al., 2009; FAO, 2014), affecting chestnut orchards throughout Türkiye.

In the latter part of the twentieth century, the healing of cankers was initially reported in Europe. This phenomenon was attributed to a viral infection of the fungus, specifically identified as *Cryphonectria hypovirus* (CHV), owing to its capability to diminish the virulence of the pathogen (Grente, 1965; Choi and Nuss, 1992; Heiniger and Rigling, 1994; Milgroom and Cortesi, 2004). *Cryphonectria hypovirus* 1 (CHV-1), like other mycoviruses in the *Hypoviridae* family, is a positive

strand-RNA virus that resides in the cytoplasm of its host, reducing the virulence of *C. parasitica*. This characteristic, known as hypovirulence, has been harnessed for the bio-control of chestnut blight in countries where chestnuts are cultivated.

The most effective method in this agent is the implementation of biological control studies. Biological control involves using natural enemies to suppress pathogens and insects. The objective of this approach is based on the transfer of the virus from an infected fungal host to a non-infected one through hyphal fusion (anastomosis), ultimately leading to the overcoming of chestnut disease (MacDonald and Fulbright, 1991; Bisiach et al., 1995).

The most effective approach for controlling chestnut blight appears to be through biological control efforts. Alongside biological control, numerous fungicidal and fungistatic chemicals have been utilized in combatting this fungus. Methyl-2-benzimidazole carbamate, carbendazim, copper oxychloride, benomyl, and azaconazole, along with imazalil, are among the chemicals employed to manage chestnut blight. It has been demonstrated that the efficacy of certain chemicals



is not sustained in long-term therapeutic treatments (Jaynes and Van Alfen, 1977; Anagnostakis, 1982; Canciani et al., 1995; Aksoy and Serdar, 2004; Delen 1980, González-Varela and González, 2007). The feasibility of therapeutic treatments with chemicals does not appear to be a promising option for controlling chestnut blight (Rigling and Prospero, 2018). It has been reported that the application of these fungicides may induce phytotoxic effects and trigger the development of resistance in the fungus (Dias, 2012).

Some fungicide applications in forest areas have been prohibited in many countries. However, alongside these restrictions, some *in vitro* studies have investigated the effects of fungicides on the mycelial growth of the pathogenic fungus. González-Varela and González (2007) explored the impact of six agrochemicals (v-captan, epoxiconazole, azoxystrobin, folpet in combination with cymoxanil and ofurace, carbendazim plus flutriafol, and flusilazole plus carbendazim) on *C. parasitica* in an *in vitro*. Epoxiconazole was identified as the most effective product in *in vitro* studies, inhibiting fungal growth even at the lowest concentrations. Trapiello et al. (2015) demonstrated that epoxiconazole, in particular, is the most effective fungicide in controlling chestnut blight. Cheradil et al. (2022) tested the *in vitro* efficacy of four fungicides (Pictor-dimoxystrobin+boscalid, Amistar Sun-azoxystrobin+difenocanazole, Score 250 EC-difenocanazole, Cupraxat FW-tribasic-copper-sulphate) against *C. parasitica*. They indicated that Score 250 EC and Amistar Sun chemicals, which inhibited fungal mycelial growth, were the most effective fungicides. Azoxystrobin, pyraclostrobin, and boscalid are primary fungal respiration inhibitors widely used in plant diseases control. They have been reported to exhibit both therapeutic and eradicated effects (Rohel et al., 2001; Wong and Wilcox, 2002). The fungicidal activity of boscalid + pyraclostrobin compounds against various diseases is well-documented in numerous studies (Ritchie and Pollard, 2003; Schnabel and Powell, 2003). Pyraclostrobin is categorized as a strobilurin fungicide and belongs to the group of quinone outside inhibitor (QoI) fungicides. QoI fungicides hinder mitochondrial respiration by binding to the Qo site of cytochrome b, thereby interrupting the electron transfer between cytochrome b and cytochrome c1. This disruption leads to a disturbance in the energy cycle of the fungus (Andrade et al., 2022). Boscalid is known as a new fungicide group of succinate dehydrogenase inhibitor (SDHI) (Stammler et al., 2007). Succinate dehydrogenase (SDH), known as respiratory chain complex II, is one of the components of mitochondrial respiration, playing a crucial role in the tricarboxylic acid cycle (TCA cycle) and the electron transport chain (ETC). It is composed of hydrophilic subunits flavoprotein (FP, chain sdhA), iron-sulfur protein (Ip, chain sdhB), and two membrane-anchor proteins (CybL, chain sdhC; CybS, chain sdhD) (Zhang et al., 2019).

SDHI fungicides are crucial for protecting plants from a

variety of phytopathogenic fungi. By attaching particularly to the mitochondrial complex II's ubiquinonebinding site (Q-site), these chemicals prevent fungal respiration (Russel, 2004). Newer active components (such boscalid, penthiopyrad, and fluopyram) exhibit broad-spectrum action against a variety of fungal species, in contrast to the first generation of SDHI fungicides (like carboxin), which was extremely active against fungi. But frequent use of site-specific fungicides, like SDHIs, might cause resistant fungal genotypes to be selected, which could eventually cause a sharp drop in fungicide efficacy (Stammler et al., 2007). As previously mentioned, research on fungicides indicates a reduced sensitivity in various pathogens, particularly observed against compounds like those in the benzimidazole group, phenylamide, EBI, dicarboximide, and dithiocarbamate. The response of pathogens to fungicides is reportedly unaffected by variations in isolate virulence. Another important aspect is that resistance emerges through mutations. Once a fungus gains resistance to a specific fungicide, it can also develop automatic resistance to other fungicides sharing the same mechanism of action. Although effective chemicals against chestnut blight have been identified *in vitro*, their application in forested areas is challenging. Additionally, the resistance that fungi develop over time against these chemicals should be taken into account. However, from a producer's perspective, biological control efforts are a long-term and challenging method of combat, especially in areas such as nurseries and reforestation sites. This highlights the necessity for a swift approach in combating *C. parasitica*, at least in regions where application is more feasible. Delen (1980) found chemical control efforts effective but did not recommend them due to concerns about resistance acquired by virulent isolates and potential adverse effects on hypovirulent isolates.

Previous studies have reported variations in the virulence among *C. parasitica* isolates. The effectiveness of the pathogenicity has been tested by researchers in biological control studies. Therefore, in the efficacy study of Bellis® fungicide, the impact on the mycelial growth of virulent *C. parasitica* with varying virulence and hypovirulent isolates was investigated *in vitro*. Controlling pathogens using chemical methods is highly challenging. However, in contemporary times, especially in plantation areas, the use of pesticides is widespread. To this end, the objective was to determine whether Bellis® fungicide (25.2% Boscalid + 12.8% Pyraclostrobin), commonly used in plant diseases caused by fungi, would affect *C. parasitica* in an *in vitro* setting. The aim was to assess its impact on the mycelial growth of the fungus and determine the expression levels of the fungus's *sdhA* and *sdhB* genes following fungicide applications. Two gene regions have been selected to determine fungicide sensitivity.

2. Materials and Methods

2.1. Collection of Bark Samples

Ten bark samples from the chestnut cankers were collected at Ordu province at Saraycık village in 2023. The samples were taken from the peripheries of the active cankers near the intact tissue by removing the bark, 1.0 cm in diameter, with a cork-borer from the two end points up and below and from the centre (OGM, 2014). The samples were placed between paper towels, kept in cool boxes, and brought to the laboratory, with the relevant information on them.

2.2. Isolation of *Cryphonectria parasitica*

Bark tissues were disinfected in 1% sodium hypochloride for 3 min and dried between paper towels. The bark disks were cut into two pieces transversely, about 2×2 mm were dissected from the adjacent tissue of the intact and diseased portions and they were plated on potato dextrose agar amended methionine and biotin (PDAMB; Difco, Sparks, MD, USA, 40 g, methionin 100 mg, biotin 1 mg, distilled water 1000 mL) medium. The plates were incubated at 25±2 °C. The white growth of the hypovirulent isolates on PDA and was used to distinguish them from the orange growth of the virulent isolates, as reported by Anagnostakis (1977).

2.3. Pathogenicity of *Cryphonectria parasitica* Isolates

Six virulent pure *C. parasitica* isolates namely Cp1_2023, Cp2_2023, Cp3_2023, Cp4_2023, Cp5_2023, Cp6_2023, respectively, were employed. Preliminary pathogenicity testing for virulent isolates was conducted using Golden Delicious apples (Fullbright, 1984). Initially, the apples were surface disinfected via 70% ethanol to cotton swabs. Each apple's surface was then punctured with 0.5 cm holes using a sterile cork borer with a diameter of 5 mm. The mycelial surfaces of seven-day-old *C. parasitica* isolates, cultivated on PDAMB medium, were placed on the apple surfaces with the mycelium facing inward. Sterile agar plugs were employed as controls. Inoculation points were sealed with moist cotton and wrapped with parafilm. The apples were individually bagged and incubated at 23±2 °C. Lesion assessment was conducted by measuring the necrotic area on apples bidirectionally on 15th days, following the method outlined by Fulbright (1984). The study was conducted with six replications, utilizing the Cp1_2023 and Cp2_2023 isolates.

2.4. *In Vitro* Test *Cryphonectria parasitica* Using Fungicide

In vitro studies were conducted to determine the impact of the Bellis® fungicide (25.2% Boscalid + 12.8% Pyraclostrobin) on the mycelial growth of *C. parasitica* isolates. Two virulent *C. parasitica* isolates (Cp1_2023 and Cp2_2023) and two hypovirulent *C. parasitica* isolates (Cp7_2023 and Cp9_2023) were utilized for this purpose. The poisoned food technique (Groover and Moore, 1962; Pundir et al., 2010) was employed to introduce the fungicide into the culture media at two different concentrations: 10 ppm and 50 ppm (Cheradil et al., 2022).

Potato dextrose agar media were prepared, allowed to cool to 60 °C after autoclaving, and then 1000 µL of stock fungicide solutions were added to 20 mL of PDA media. The mixtures were poured into sterile Petri dishes. Subsequently, mycelial plugs from seven-day-old cultures of *C. parasitica* were placed in the center of petri dishes with added fungicide, while similar plugs were placed in fungicide-free petri dishes as controls. All petri dishes were incubated in the dark at 25±2 °C. Fungal colony growth was measured on the third and sixth days of incubation (Cheradil et al., 2022). The percentage of inhibition in *C. parasitica* mycelial growth by the fungicide at different concentrations was determined using the following formula. (Mycelial growth inhibition (%))=(dc-dt/dc) × 100 (%); dc = average diameter of fungal colony in non-poisoned PDA (control), and dt= average diameter of fungal colony in poisoned PDA (Shakeel et al., 2021). The mycelial growth diameter (MGD), expressed in mm, was calculated using the following equation (Owaid et al., 2018). Two perpendicular colony diameters were measured per plate. (MGD (mm)) = (D1 + D2) /2; D1= Colony diameter 1, and D2=Colony diameter 2).

2.5. Bavendamm Test (Phenol Oxidase Test)

This test was conducted to assess whether the application of fungicides had an impact on the presence of mycovirus in hypovirulent isolates. The experiment involved cultivating hypovirulent isolates on treatment agar medium containing tannic acid (Bavendamm's medium), following the method outlined by Rigling et al. (1989). The Bavendamm test was conducted with two replications, and isolates exhibiting a weak color change were considered potential hypovirulent strains. As a virulent control, an EU-26 tester isolate free of CHV-1 was employed.

2.6. RNA Isolation and Complementary (cDNA) Synthesis

For total RNA isolation, virulent *C. parasitica* isolates were cultivated on PDA medium at 25 °C for 7 days. Approximately 50 mg mycelium with conidia were scraped from the agar surface and treated with liquid nitrogen. RNA extraction was according to the manufacturer's guidelines (RNA Extracol; Eurx Ltd, Gdańsk, Poland). The resultant RNA measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). RNA templates were stored at -80 °C till used.

The first strands cDNA synthesis kit (Eurx Ltd, Gdańsk, Poland) was employed to synthesize cDNA from 1 µg total RNA. Reverse transcription was carried out in 20 µl of reaction mixture containing 1 µg total RNA, 4 µL 5× cDNA Buffer, 1 µL mix RT (reverse transcriptase), 1 µL oligo (dT), 1 µL random hexamers, 12 µL nuclease-free water. The thermal cycling conditions of the reaction for cDNA were 10 min at 20 °C, 40 min at 50 °C, 5 min at 85 °C, and finally, 10 min at 4 °C.

2.7. Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The actin was utilized as a housekeeping gene in the qRT-PCR study (Table 1), and the primers were constructed using the Primer3 v4.1.0 tool. qRT-PCR reactions carried out in accordance with manufacturer’s instructions using SYBR™ Green PCR Master Mix in the CFX Connect™ Real-Time PCR Detection System. 12.5 µL of 2 × real-time PCR Mix (SYBR Green I), 0.5 µL of primer, and appropriately diluted cDNA as a template were added to each 25 µL qRT-PCR experiment. The qRT-PCR settings were as follows: 20 seconds at 95 °C, 40 cycles of 30 seconds at 95 °C, 20 seconds at 54 °C, and 10 seconds at 72 °C. For each sample, three technical and biological duplicates were run in each qRT-PCR experiment. The data was analyzed using the 2^{-ΔΔCT} technique, and statistics were computed using one-way ANOVA (Livak and Schmittgen, 2001).

2.8. Statistical Analysis

In order to determine the significance of mean mycelial growth diameter (mm) of the isolates, one-way ANOVA was applied on the percent disease values by using IBM SPSS Statistics 22 package programme and the significance was calculated by Tukey multiple comparison test (P<0.05). Statistical significance was determined by comparing the non-treated control with the treated samples. To compare the mycelial growth diameter (mm) of all isolates, an initial examination of data normality was conducted, and due to the non-normal distribution of the data, Kruskal-Wallis test was applied in some analysis.

3. Results

3.1 Isolation and Their Identification

Ten isolates were identified as *C. parasitica* based on their morphological aspects. Six isolates were identified

as virulent, whereas four isolates were identified as hypovirulent.

3.2 Virulence in Apple

In the study, the pathogenicity of six virulent *C. parasitica* isolates was evaluated on Golden Delicious apples. In the experiment, conducted with six replications, the lesion area on the 10th day for the Cp1_2023 isolate measured 12.1 cm², while for the Cp2_2023 isolate, it was measured as 9.6 cm² (Figure 1). The measurements for other isolates are as follows: Cp3_2023 isolate recorded 9.3 cm², Cp4_2023 isolate measured 8.7 cm², Cp5_2023 isolate measured 5.6 cm², and Cp6_2023 isolate measured 7.2 cm².

3.3 Inhibition of Mycelial Growth of *Cryphonectria parasitica* with Fungicidal Treatment

The mycelial growth of virulent *C. parasitica* isolates subjected to fungicide application at two different concentrations was measured on the third and sixth days. The results are presented in Table 2 and Table 3 (Figure 2). On the 3rd and 6th day measurements, it was determined that the two isolates differed in terms of mycelial growth. Percentages of inhibition of mycelial growth were calculated to assess the effects of fungicide on fungal mycelial development (Table 3). The percentage of inhibition of mycelial growth after the application of fungicide concentrations set at 10 ppm and 50 ppm is presented in Table 4. The mycelial growth of hypovirulent *C. parasitica* isolates was measured on the third and sixth days, and the results are presented in Table 4 and Table 5 (Figure 3). Even at both applied concentrations, the mycelial development of virulent *C. parasitica* continued beyond the sixth day, indicating that the fungus did not completely perish at these doses. In contrast, the growth of hypovirulent *C. parasitica* isolates treated with fungicide was slower, and the applied doses hindered the fungus mycelial growth.

Table 1. Primers sequences used for qRT-PCR experiment

Gene regions	Primers	Primer sequences (5'-3')	Product size (bp)
<i>β-actin</i> (housekeeping)	<i>β-actinF</i>	TGAGCAAGGAGATTACAGCATTGG	150
	<i>β-actinR</i>	CATACTCTGCCTTCGCAATCCAC	
<i>sdhA</i>	<i>SdhAF</i>	GCTAACTCCCTGCTCGATCT	207
	<i>sdhAR</i>	TTCGGTCTGCATGGTCTTCT	
<i>sdhB</i>	<i>sdhBF</i>	AGCATCTTCTCCGGTGTTGA	221
	<i>sdhBR</i>	AGATCGAGCAGGGAGTTAGC	

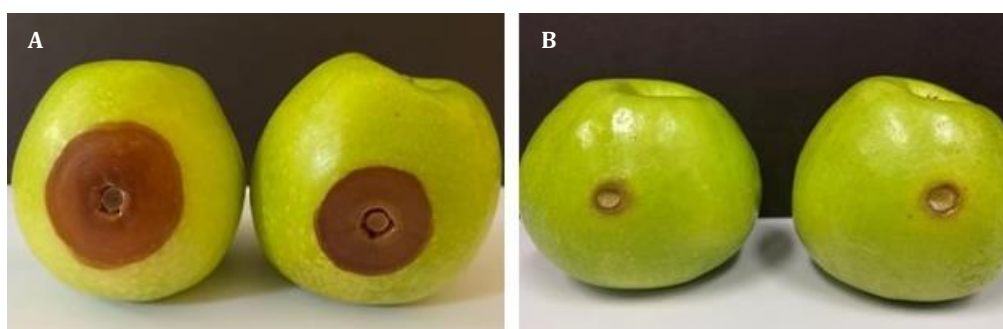


Figure 1. The lesion areas caused by *Cryphonectria parasitica* in Golden Apple test. Apples inoculated with Cp1_2023 and Cp2_2023 (A), control (B).

Table 2. Mean mycelial growth diameter (mm) obtained in third and sixth after treated fungicide

Isolate name	Fungicide concentration					
	Measuring the average size of fungal colony diameter (mm)					
	3 th day			6 th day		
	10 ppm	50 ppm	Control*	10 ppm	50 ppm	Control
Cp1_2023	12.3	9.9	17.4	25.9	23.1	48.1
Cp2_2023	14.1	12.8	26.7	27.3	26.8	58.2

*C= control petri, P<0.05 for 3th measuring; P<0.05 for 6th measuring.

Table 3. Percentages of inhibition of mycelial growth using fungicide on third and sixth days

Isolate Name	Percentages of inhibition of mycelial growth			
	3th day		6th day	
	10 ppm	50 ppm	10 ppm	50 ppm
Cp1_2023	29.3 %	43.1 %	46.1%	51.9%
Cp2_2023	47.2%	52.0%	53.0%	53.9%

Table 4. Mean mycelial growth (mm) obtained in third and sixth after treated fungicide

Isolate name	Fungicide concentration					
	Measuring the average size of fungal colony diameter (mm)					
	3 th day			6 th day		
	10 ppm	50 ppm	Control*	10 ppm	50 ppm	Control
Cp7_2023	10.4	8.6	23.6	20.2	17.0	33.0
Cp9_2023	11.7	10.2	27.9	22.9	20.2	50.4

*C= control petri, P<0.05 for 3th measuring; P<0.05 for 6th measuring.

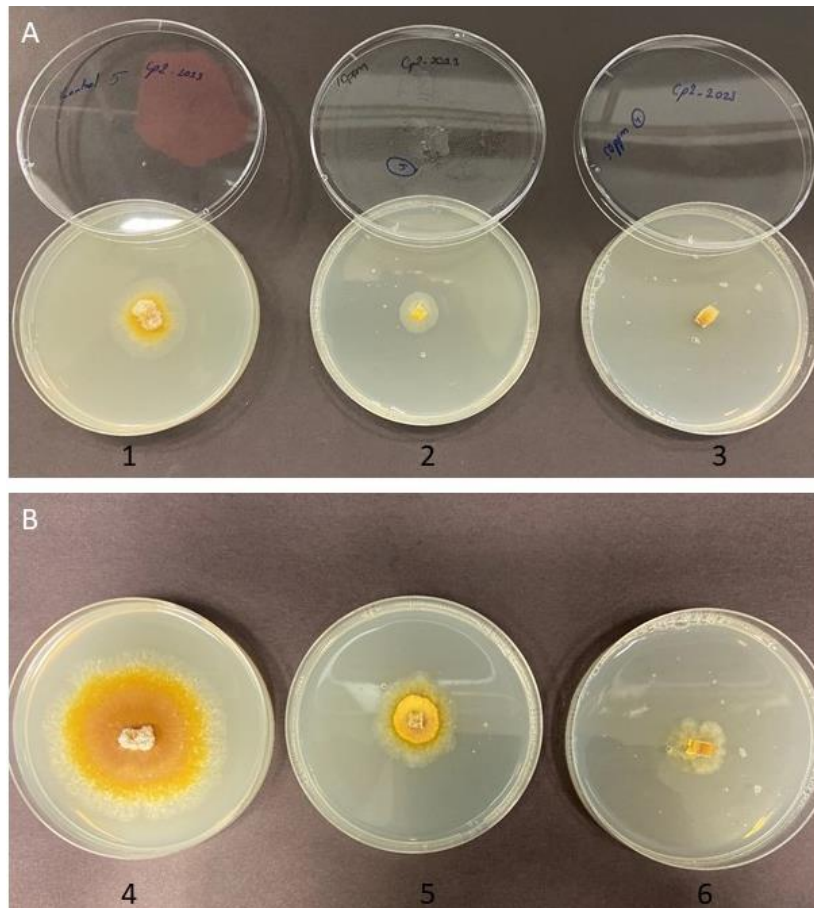


Figure 2. Mycelial growth of *Cryphonectria parasitica* (Cp2_2023) at 3th day on PDA treated with fungicide (A), mycelial growth of control petri (1), mycelial growth at 10 ppm (2), mycelial growth at 50 ppm (3); Mycelial growth of *Cryphonectria parasitica* (Cp2_2023) at 6th day (B), mycelial growth of control petri (4), mycelial growth at 10 ppm (5), mycelial growth at 50 ppm (6).

Table 5. Percentages of inhibition of mycelial growth using fungicide on third and sixth days

Isolate Name	Percentages of inhibition of mycelial growth			
	3 th day		6 th day	
	10 ppm	50 ppm	10 ppm	50 ppm
Cp7_2023	55.9%	63.5%	38.7%	48.5%
Cp9_2023	58%	63.4%	54.5%	59.9%

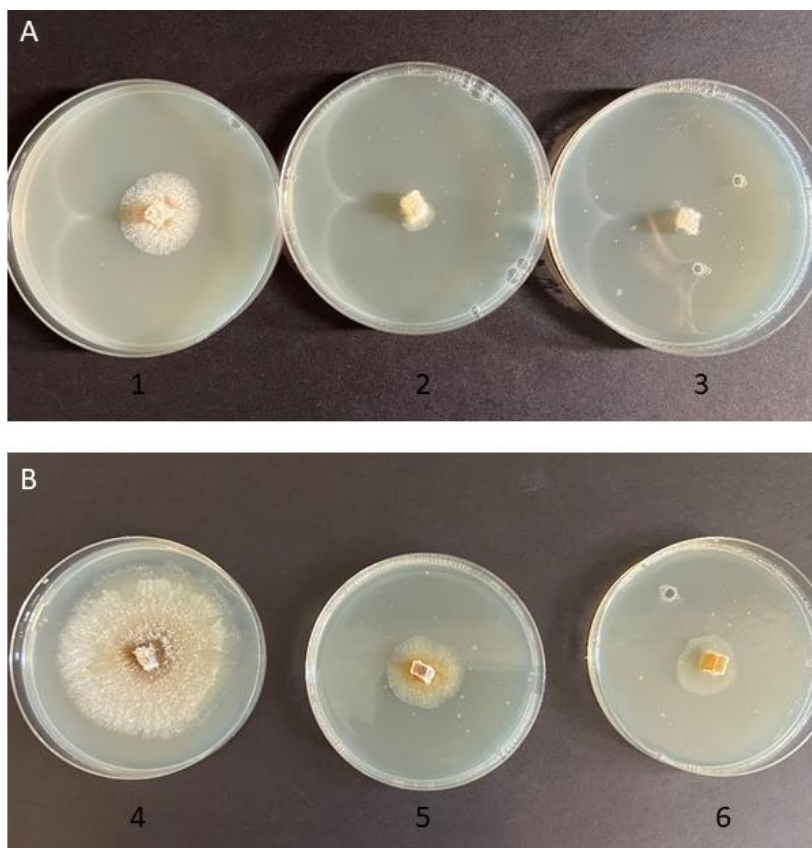


Figure 3. Mycelial growth of *Cryphonectria parasitica* (Cp9_2023) at 3th day on PDA treated with fungicide (A), mycelial growth of control petri (1), mycelial growth at 10 ppm (2), mycelial growth at 50 ppm (3); Mycelial growth of *Cryphonectria parasitica* (Cp9_2023) at 6th day (B), mycelial growth of control petri (4), mycelial growth at 10 ppm (5), mycelial growth at 50 ppm (6).

3.4 Bavendamm Test

Two (Cp7_2023 and Cp9_2023) of all hypovirulent isolates exhibited a weak color change on the Bavendamm test media, whereas the virulent control isolate, EU-26, displayed a dark color change (Figure 4).

3.5 Gene Expression

The molecular resistance of *C. parasitica* isolates to two concentrations of Bellis® was investigated in terms of *sdhA* and *sdhB* gene expression. When comparing the gene expression levels of Cp1_2023 and Cp2_2023 isolates of the highest virulence. It was observed that the expression levels of *sdhA* and *sdhB* genes in the Cp1_2023 isolate were higher than those in the Cp2_2023 isolate (Figure 5). Specifically, the expression levels for Cp1_2023 at 10 ppm and 50 ppm were calculated as 1.16 and 2.51, respectively, while for Cp2_2023, the corresponding expression levels were calculated as 0.72 and 1.55.



Figure 4. Growth of virulent (EU-26=dark colour) and hypovirulent (Cp9_2023=light colour) isolates in Bavendamm test.

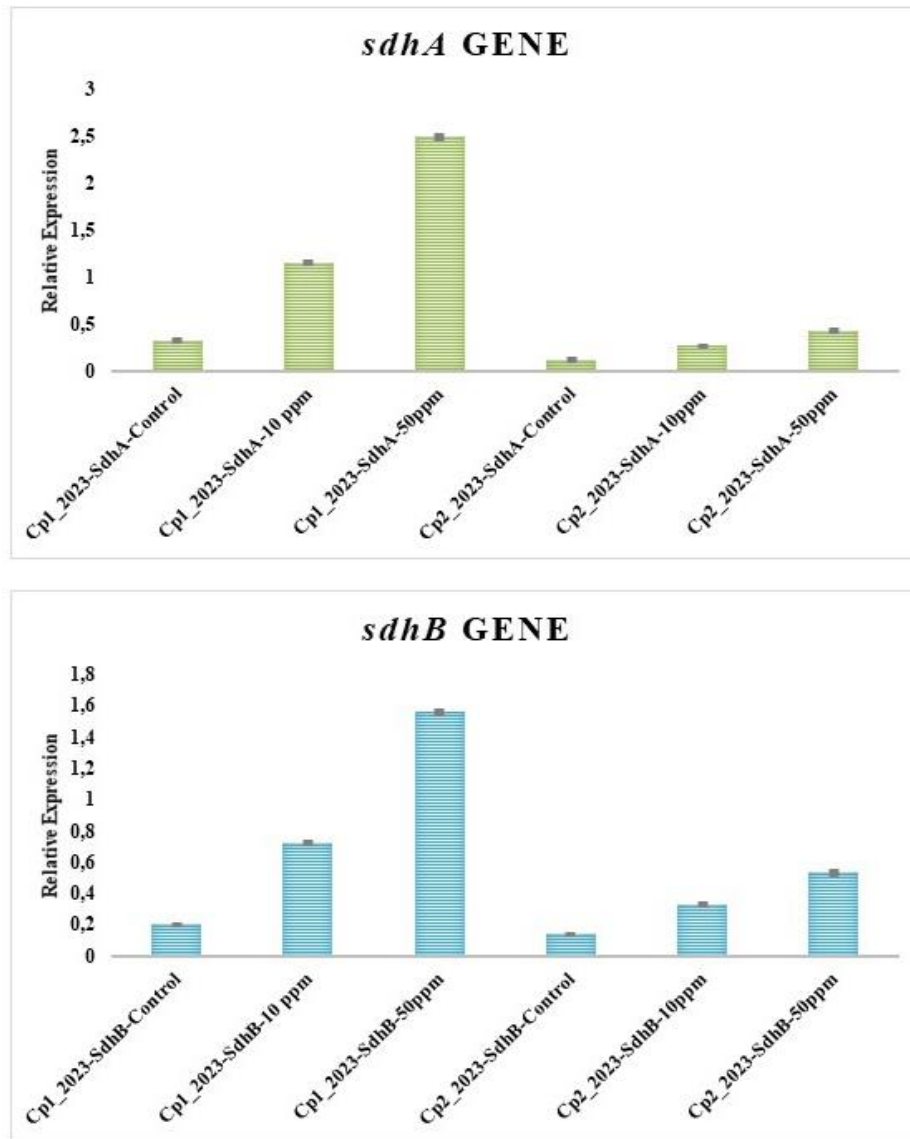


Figure 5. Effect of fungicide application against Cp1_2023 and Cp2_2023 on *sdhA* and *sdhB* genes expression.

4. Discussion

The antifungal effect of the Bellis® fungicide, containing 25.2% Boscalid and 12.8% Pyraclotrobin active ingredients, against two virulent *C. parasitica* and two hypovirulent *C. parasitica* isolates, was determined *in vitro*. The percentage of inhibition caused by the fungicide on the mycelial growth of the fungus was calculated, and it was observed to prevent mycelial growth after colony measurements on the third and sixth days. The applied fungicide concentrations did not completely kill the fungus; however, they exhibited an antifungal effect on the fungus (Table 2, 3). In study conducted by Cheradil et al. (2022), the effects of four fungicides, namely Pictor (dimoxystrobin+boscalid), Amistar Sun (azoxystrobin+difenoconazole), Score 250 EC (difenoconazole), and Cuproxat FW (tribasic-copper-sulphate), on the mycelial growth of *C. parasitica* were investigated. They reported that the most effective fungicides were Score 250 EC and Amistar Sun, indicating their effectiveness on the fungus, even at the lowest

doses, inhibiting the growth of the fungus. González-Varela and González (2007) reported that six agrochemicals (captan, epoxiconazole, azoxystrobin, folpet in combination with cymoxanil and ofurace, carbendazim plus flutriafol, and flusilazole plus carbendazim) exhibited varying degrees of inhibition on mycelial growth of *C. parasitica in vitro*. They noted a considerable variability in the percentage of inhibition across these chemicals. They identified epoxiconazole as the most effective chemical in their findings. However, in our study, the development of virulent *C. parasitica* was monitored after measurements on the sixth day, and it was observed that mycelial growth continued. The doses used were found to initially slow down the growth of the fungus but did not completely eradicate it. Some studies have reported encountering resistance of the fungus to Boscalid + Pyraclotrobin in fungal diseases (Kim and Xiao, 2010; Avenot et al., 2008). In our study as well, resistance of the fungus was encountered and the mycelial growth of the fungus continues after 3th and 6th days measurements.

The pathogenicity of virulent *C. parasitica* isolates test results indicated variations between virulent isolates. Differences in the pathogenicity of the fungus may have affected the rates of fungicide efficacy. The percentage of fungicide inhibition of mycelial growth of the fungus was found to be different between *C. parasitica*. We think that this difference may have been affected by the virulence of the fungus. Prospero and Rigling (2013) noted that none of the chemical alternative approaches have been systematically implemented on a large scale in field settings. González-Varela and González (2007) used chemicals in an *in vivo*, which were thought to have potential effects on fungus control, but these products are prohibited for use against *C. parasitica* in Spain. However, nowadays, nurseries and private garden owners use fungicides (Aiello et al., 2013; Gilardi et al., 2016). Due to the continued use of fungicides, various studies have been conducted to find the most suitable fungicide for controlling *C. parasitica* (González-Varela and González, 2007; Cheradil et al., 2022). Trapiello et al. (2015), in the context of chestnut blight control, evaluated agrochemicals against the pathogen in an *in vivo* environment. Researchers reported that Epoxiconazole was the most effective chemical in disease management. Researchers have suggested that chemicals could be recommended in enclosed spaces or under specific conditions, such as nurseries.

In the biological control studies against chestnut blight, this method is considered the most effective approach (Prospero and Rigling, 2018). In this study, the fungicidal effects were assessed on two hypovirulent isolates. It was determined that the application of fungicide at each dose inhibited the mycelial growth of the fungus. After the application of fungicide, the mycelial growth of hypovirulent isolates exhibited a slower rate compared to virulent isolates. It was observed that, following the sixth-day colony measurement, the hypovirulent isolates did not show any further development. Concluding that fungicide applications disproportionately impact hypovirulent isolates could have detrimental consequences for biological control studies, underscoring the importance of avoiding such misapplications. Additionally, it was observed that the applied fungicide dose did not affect the presence of the virus in hypovirulent isolates, and a Bawendamm test was conducted to confirm the absence of the virus.

Following fungicide application on the Petri dishes, there were variations in colony development among hypovirulent isolates, and a significant difference was identified according to statistical testing. The variation among isolates can be attributed to differences in virus concentration. The hypovirulent *C. parasitica* isolates, which developed in a fungicide-treated were subjected to the Bawendam test. In conclusion, it has been observed that, despite the cessation of mycelial growth in the fungus in a fungicide-treated, it did not lose its hypovirulent characteristic. In other words, as long as the fungicide does not kill *C. parasitica* isolates, the virus can

persist and continue its life within the fungal cells. However, the duration of the virus's persistence can be determined through detailed research. Delen (1980) indicated that the application of carbendazim in combination could provide a long-lasting effect against *C. parasitica* but also mentioned the potential for the fungus to develop resistance. The development of resistance to fungicides by fungi has been reported in several studies (Avenot and Michailides, 2007; Miles et al., 2014).

Fungicide resistance may also be a valuable tool for advancing our understanding of the molecular mechanism of action of a certain class of compounds, even if resistance is a major role in lowering the effectiveness and life of vital, useful fungicides (Steffens et al., 1996). In strawberry fields, kiwifruit, and stored apples, resistance to a fungicide containing the active components boscalid + pyraclostrobin has been reported (Bardas et al., 2010; Kim and Xiao, 2010; Fernández-Ortuño et al., 2012; Fernández-Ortuño et al., 2014).

In our current study, results suggest that the *C. parasitica* isolates exhibit different fungicide phenotypes in response to different SDHI fungicide, Bellis®, concentration treatments and differ in their sensitivity to mycelial development and conidial germination. These results are consistent with the difference in *sdh* genes profile among isolates after fungicide treatment. The data show that the expression level of *sdhA* and *sdhB* genes significantly increased in Cp1_2023 isolate treated with fungicide at 10 ppm and 50 ppm concentration compared to the control. In Cp2_2023 isolate, an increase in the expression level of *sdhA* and *sdhB* genes was observed after fungicide application at two different concentrations compared to the control group. However, the gene profile increase observed in this isolate group was lower than Cp1_2023. In the fungicide experiments the mycelial growth of the two virulent isolates displayed variations. This difference was consistent with the gene expression profile, indicating that the isolate Cp1_2023, with a higher gene expression level, exhibited greater mycelial growth and pathogenicity compared to the other isolate. According to statistical test, the *P*-value for the 3th day was found to be 0.00, and for the 6th day, it was 0.00. This indicates a significant difference between virulent isolates the groups at a 95% confidence level.

Although there is no study on this subject in *C. parasitica*, it has been observed that *sdhA* and *sdhB* genes stand out as a result of boscalid application in *Alternaria solani* Sorauer, resulting in moderate or high-level resistance (Gudmestad et al., 2013). There are two mutations in the *AasdhB* gene sequence of *Alternaria alternata* (Fr.) Keissl. that confer high levels of resistance to boscalid (Avenot et al., 2008), while boscalid resistance in *Didymella bryoniae* (Fuckel) Rehm is the result of mutations in the *Dbsdhb* protein in which histidine is replaced by tyrosine or arginine (Avenot et al., 2012). A similar situation exists in *Botrytis cinerea* Pers. and *Corynespora cassiicola* (Miyamoto et al., 2010; Yin et al., 2011).

5. Conclusion

In plant pathology, reducing the use of chemical fungicides is one of the key objectives. In this study, the effects of the previously untested Bellis® fungicide on the mycelial growth of the chestnut blight agent *C. parasitica* were examined *in vitro*, and the expression levels of the *sdhA* and *sdhB* genes were determined. According to these results, two different doses of the selected fungicide were found to be effective in inhibiting mycelial growth of virulent and hypovirulent isolates. However, it did not completely eliminate virulent *C. parasitica*, and based on the results of the gene expression profile, a resistance mechanism against the fungicide was determined to be present in the fungi. Additionally, for the two selected hypovirulent isolates, fungicide applied at the same doses indicated, according to the Bawendamm test results, that the presence of the virus persisted. It was observed that the development of hypovirulent isolates did not continue compared to virulent isolates. In future studies, a more detailed evaluation of the presence of mycoviruses by examining virus gene regions will provide more comprehensive information. We recommend conducting more detailed studies before the use of fungicides and suggesting for their use in controlled environments.

Author Contributions

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

	E.D.T.	D.Ç.	S.A.Ş.
C	35	35	30
D	30	40	30
S			100
DCP	50	50	
DAI	50	50	
L	60	20	20
W	35	35	30
CR	30	30	40
SR	35	35	30

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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ASSESSING ALUMINUM STRESS RESILIENCE IN COMMON BEAN ROOTS: PHENOTYPIC, HISTOCHEMICAL, AND *PvGST/PvPOD* GENE EXPRESSION ANALYSIS

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Abstract: Common bean (*Phaseolus vulgaris* L.) is grown in various parts of the world. Aluminum (Al) toxicity poses a significant and widespread challenge in marginal areas with unfavorable soil qualities where common bean is grown. In acidic soils, stable forms of Al dissolve into the soil solution and inhibit root growth and function by injuring the root apex with phytotoxic ions. This leads to the development of a smaller root system, adversely affecting crop yield. In this study, the phenotypic evaluation for relative root elongation of 10 common bean genotypes/cultivars under Al stress (50 μ M), the impact of Al toxicity using different histochemical dyes (Evan's blue and Schiff's reagent) and the expression levels of *PvGST* (Glutathione S-transferases) and *PvPOD* (peroxidase) genes in the root tissues of the most resistant/sensitive common beans under Al stress (50 μ M) and control conditions (0 μ M) were investigated. The maximum relative root elongation value (71.9%) was found in Önceler-98 cultivar, while the lowest value (14.1%) was obtained from Blksr-19 genotype. Histochemical applications used in the study supported phenotypic results. The cracks at the root tip and high blue color intensity were detected in Schiff's reagent and Evan's blue dyes in the Blksr-19, respectively. The expression levels of *PvGST* and *PvPOD* genes in the root tissue of the Blksr-19 (Al-sensitive) were highly upregulated at 24 h of Al stress treatment. The results revealed that these genes might be involved in the common bean root tissue's defense mechanism against Al stress for the first time. The findings herein will help plant breeders develop common bean cultivars tolerant to Al toxicity.

Keywords: Aluminum stress, Breeding, Gene expression

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1. Introduction

Common bean (*Phaseolus vulgaris* L.) is among the most significant edible legume grains that can be consumed directly by humans. The seeds of common bean include protein, vitamins and minerals (Baloch and Nadeem, 2022; Yeken, 2023). The Food and Agriculture Organization (FAO) reported that on almost 35.9 million hectares, more than 27.7 million tons of common beans were produced in 2021 (FAO, 2023). This plant is produced on marginal land in different parts of the world when the potential yield is limited by adverse soil qualities (Eticha et al., 2010; Yang et al., 2011). Al toxicity is one of the most limiting factors in plant growth and development, particularly in acidic soils with a pH below 5 (Tóth et al., 2021). Toxic levels of soluble Al limit roots' development and function by damaging the root apex (Yang et al., 2011). These processes also impact other plant parts, resulting in a significant reduction in yield (Ambachew et al., 2023). Al toxicity can lead to poor root development, root browning, stunted plant growth, and reduced nutrient and water uptake efficiency by the roots (Bartoli et al., 2017). Al can bind to the cell wall, causing it to become rigid (Delhaize et al., 1993). The

integrity of the cell membrane is evaluated using Evan's blue staining. In the staining process, living cells exclude the dye due to the semipermeable nature of cell membranes, but damaged cells are unable to eliminate the dye and so are stained blue (Ikegawa et al., 2000). The intensity of Evans blue's absorbance increases proportionally to the amount of cell membrane damage. On the other hand, Al can alter the lipid peroxidation in the plasma membrane and the calcium ion homeostasis, inhibit nutrients and water uptake, and reduces the photosynthetic rate (Tóth et al., 2021). The use of Schiff's reagent method allows for the identification of lipid peroxidation on the root surface. This technique was successfully applied in pea roots to identify aldehyde functions that originate from the peroxidation of membrane lipids and are attached to the membrane protein under Al stress (Yamamoto et al., 2001). Plants can improve their ability to remove ROS by increasing the activity of antioxidants, such as glutathione S-transferase and peroxidase, when exposed to oxidative stress (Mhamdi et al., 2010; Nanda et al., 2010). Glutathione S-transferases (GSTs) play significant roles in detoxifying xenobiotics and toxic lipid peroxides,



regulating signal transduction, defense against heavy metals and ozone damage, glucosinolate biosynthesis and metabolism in plants (Abdul Kayum et al., 2018). Additionally, they regulate plant growth and development (Jiang et al., 2010). On the other hand, peroxidase (POD) plays a crucial role during stress conditions in plants, removing hydrogen peroxide generated (Tóth et al., 2021). There are many studies on Al stress in common bean (Rangel et al., 2007; Eticha et al., 2010; Rangel et al., 2010; Yang et al., 2011; Butare et al., 2012; Bartoli et al., 2017; dos Santos Neto et al., 2020; Ambachew and Blair, 2021; Tóth et al., 2021; Ambachew et al., 2023). However, to the best of our knowledge, there is no study in the literature on the expression levels of *PvGST* and *PvPOD* genes under Al stress in common bean.

The improvement of new common bean cultivars with resistance to Al stress is one of the main goals of breeding programs (Butare et al., 2012; Ambachew and Blair, 2021). The existence of genetic variation in common bean in response to Al toxicity has led plant breeders to discover genotypes better suited to Al stress and to improve cultivars with better agronomic features (dos Santos Neto et al., 2020). In soils contaminated with Al, tolerant cultivars provide a sustainable and cost-effective solution that can increase yield gains. For this purpose, this study aimed to (1) assess the phenotypic variation for relative root elongation of common bean genotypes/cultivars under Al stress, (2) determine the impact of Al toxicity using different histochemical dyes, and (3) investigate the expression levels of *PvGST* and *PvPOD* genes in the root tissues of the most resistant/sensitive common beans under Al stress and non-stress conditions. Findings herein could help to develop modern cultivars with Al-toxicity resistance in common bean breeding programs.

2. Materials and Methods

2.1. Plant materials

The eight common bean genotypes (Blksr-14, Blksr-19, Brs-4, Brs-23, Brs-24, Dzc-2, Dzc-3, and Ylv-14) collected different provinces of Türkiye (Tübitak Project ID: 115R042) and two commercial cultivars (Bulduk and Önceler-98) were used as plant materials in this study.

2.2. Plant Growth, Al treatment, phenotypic evaluation and histochemical staining

Al exposure to common bean seedlings was performed as described earlier with some modifications (Kariya et al., 2017). The experiment was conducted in three replicates. Briefly, seeds of common bean genotypes/cultivars were surface-sterilized using domestic bleach (5% sodium hypochloride), and sown in the sterile peat soil. The sowing trays were watered to moistened peat. The growing conditions were 25°C and 16-h light [at ~150 µmol photons m⁻² s⁻¹] and 8-h dark photoperiod. Four days after germination, seedlings were uprooted carefully and thoroughly washed without damaging the roots under running tap water. Al treatment media

contained 500 µM CaCl₂ supplied with AlCl₃ at 50 µM and pH adjusted with 1 N HCl at 4.5 for 24 h. At the same time, control (without Al) media just contained 500 µM CaCl₂ and pH adjusted with 1 N HCl at 4.5 for 24 h. Magenta boxes (H 100 mm × W 60 mm × D 60 mm) were covered with black PVC tape to protect the roots from light except for the lid side which was left open. Seedlings were clamped in between slits of sponge and left floating over the water surface. Aeration was provided continuously by an air pump over 24 h during the Al treatment.

Root lengths of seedlings were measured using a ruler before and after the Al exposure. Relative root elongation (RRE) was measured using the following formula (equation 1).

$$RRE = \frac{\text{root elongation with Al}}{\text{root elongation without Al}} \times 100 \quad (1)$$

After phenotypic evaluation, the impact of Al toxicity was detected using Evan's blue for plasma-membrane integrity and aldehyde detection using Schiff's reagent essentially as described earlier by Yamamoto et al. (2001). Briefly, roots were washed with distilled water thoroughly and stained with Schiff's reagent for 20 min. After staining, the roots were rinsed with a sulfite solution. To detect integrity of plasma-membrane, roots were stained with Evan's blue solution (0.025% [w/v] for 10 min. After staining, roots were washed extensively to remove excess of the dye. After Schiff's reagent and Evan's blue staining, root sections of 10 mm were observed under a light microscope Leica DM1000 LED (Leica, Weztlar, Germany).

2.3. Plant Growth and Al Treatment for Gene Expression Analysis

The most Al tolerant (cultivar; Önceler-98) and sensitive (genotype; Blksr-19) common beans were selected for gene expression analysis results of the relative root elongation and histochemical staining. The genotype and cultivar were grown again as mentioned above and treated with Al similarly. After treatment, the root samples were collected at 24 hours for both the treated and control groups. The collected samples were maintained at -80°C for RNA isolation.

2.4. RNA extraction, DNase treatment and cDNA synthesis

According to the manufacturer's instructions, total RNA was extracted from 150 mg of all root samples using NucleoZOL reagent (MACHEREYNAGEL GmbH, Dueren, Germany). Then, Thermo Fischer Scientific RNase-Free DNase was used to conduct DNase digestion on the resulting RNA. Following the manufacturer's protocols, using the Thermo Fisher Scientific RevertAid First Strand cDNA Synthesis Kit, complementary DNA (cDNA) was generated from 2 µg of total RNA. The resulting cDNA samples were diluted, and then for quantification studies stored at -20 °C. Using a DS-11 FX+ series spectrophotometer (Denovix Inc., Wilmington, DE, USA), nucleic acid measurements was performed.

2.5. Primers for Quantitative Real-Time PCR (qRT-PCR)

The primers of *PvGST* and *PvPOD* genes previously described by Oliveria et al. (2015) in *P. vulgaris* were used in the study. The β -*TUB* was previously used as the

reference gene for the qRT-PCR analysis in common bean under Al stress (Eticha et al., 2010). Thus, it was involved in this study as the reference gene. The sequences of the primers used in the study are given in Table 1.

Table 1. Sequences of primers utilized for qRT-PCR investigation

Primers	Forward/Reverse	Sequences	References
<i>PvGST</i> (Glutathione S-transferase)	F	AGCTCTTCAAGGACACTGAGCCAA	Oliveira et al. (2015)
	R	AAAGGCTGTGGATGCTGCACTAGA	
<i>PvPOD</i> (Peroxidase)	F	TCCTTTTCAGCACTTTCCT	Eticha et al. (2010)
	R	AGAAAGCAGTGTCTTGTGG	
β - <i>TUB</i> (Beta-tubulin)	F	CCGTTGTGGAGCCTTACAAT	(2010)
	R	GCTTGAGGGTCTGAAACAA	

2.6. qRT-PCR analysis

Using CFX Connect Real-Time PCR System (Bio-Rad, Hercules, CA, United States), the quantitative Real-Time PCR analysis was employed. The reaction mix includes 1 μ L of cDNA, 12.5 μ L of RealQ Plus 2x Master Mix Green without ROX (Ampliqon, Odense, Denmark), 10 μ L of RNase-free ddH₂O and 0.75 μ L each of primers (10 μ M). The qRT-PCR was employed as one cycle at 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 61°C for 45 s, and 72°C for 20 s. Afterward, using the CFX Maestro Software, Ct values were calculated. The accuracy of each amplicon was verified by performing melting curve analyses after the final PCR cycle. The samples were incrementally heated from 65 to 95°C during the analysis. To ensure accuracy, each sample was used as three technical duplicates. As a reference gene, β -*TUB* was used in this study (Eticha et al., 2010).

2.7. Statistical Analyses

According to the relative quantitative method ($2^{-\Delta\Delta Ct}$), the relative expression profiles were determined in root tissue subjected to control and Al treatments (Livak and Schmittgen, 2001). Analysis of variance (ANOVA) was done using the expression data. Using SAS statistical

software, the mean values were compared according to Least significant difference (LSD) test at a significance level ($P < 0.05$). Graphics were constructed in GraphPad Prism version 6.04.

3. Results and Discussion

Many biotic and abiotic stress factors have adversely affected common bean production (Şen et al., 2020; Palacioğlu et al., 2021; Tóth et al., 2021; Çelik et al., 2023). When the common bean is exposed to stress, environmental and genetic factors influence the function and structure of the roots (Rangel et al., 2010). They enable them to sense and respond to environmental challenges. Therefore, it is important to protect roots from stresses such as Al toxicity, which can inhibit root function and growth. Understanding the genetic variation of local genotypes for Al stress is one of the most crucial tasks of a breeding program (Ambachew and Blair, 2021). In this study, root lengths of seedlings were measured using ruler before and after the Al exposure, and the relative root elongation of common bean genotypes/cultivars were determined (Figure 1).

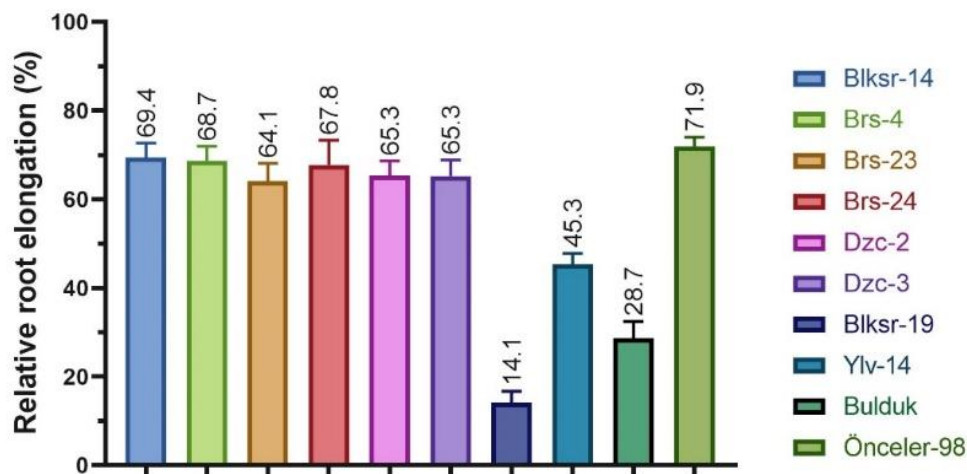


Figure 1. The relative root elongation values of common bean genotypes/cultivars under Al stress.

Blksr-14, Dzc-2, Dzc-3, Brs-4, Brs-23, Brs-24 and Önceler-98 revealed high relative root elongation values. On the other hand, Blksr-19, Ylv-14 and Bulduk had low relative root elongation values of 14.1%, 45.3% and %28.7, respectively. The highest relative root elongation was obtained in the Önceler-98 cultivar (71.9%), while the lowest relative root elongation was determined in Blksr-19. It was reported that the root is the most sensitive plant organ, and the first symptom of Al toxicity is decreased root growth (Delhaize et al., 1993; Tóth et al., 2021). Rangel et al. (2007) examined the short- and medium-term effects of Al treatment (20 μ M) on root growth and Al accumulation in Al-resistant and Al-sensitive common bean genotypes. They reported that root elongation in both genotypes was significantly inhibited within the initial 3-4 hours of Al treatment. Ambachew and Blair (2021) assessed 227 common bean genotypes to explore their tolerance to Al toxicity (50 μ M) and to determine candidate genes linked to Al tolerance. It was observed that the number of root forks, number of links, root surface area, number of root tips,

total root length and root volume decreased under Al toxicity treatment. Very recently, Ambachew et al. (2023) investigated the relationship between root characteristics and genetic variation among 262 common bean genotypes under different Al treatments. They found that the 50 μ M Al treatment was sufficient to elucidate genotypic differences in the studied root traits. Under Al-toxicity treatment, a decrease in all root traits compared to the control group was observed. The histochemical procedure has an advantage over the biochemical procedure. This procedure shows the localization of the Al-enhanced peroxidation of lipids in situ on the root surface with high sensitivity. The roots of common bean genotypes and cultivars were dyed with Schiff's reagent and Evan's Blue to determine the impact of Al toxicity. Positive results were detected in Al-treated roots when compared to controls (Figure 2). As a result of staining the roots with Schiff's reagent dye, the cracks at the root tip of the Blksr-19 genotype, which has the lowest relative root elongation, especially under Al stress, were seen in Figure 2.

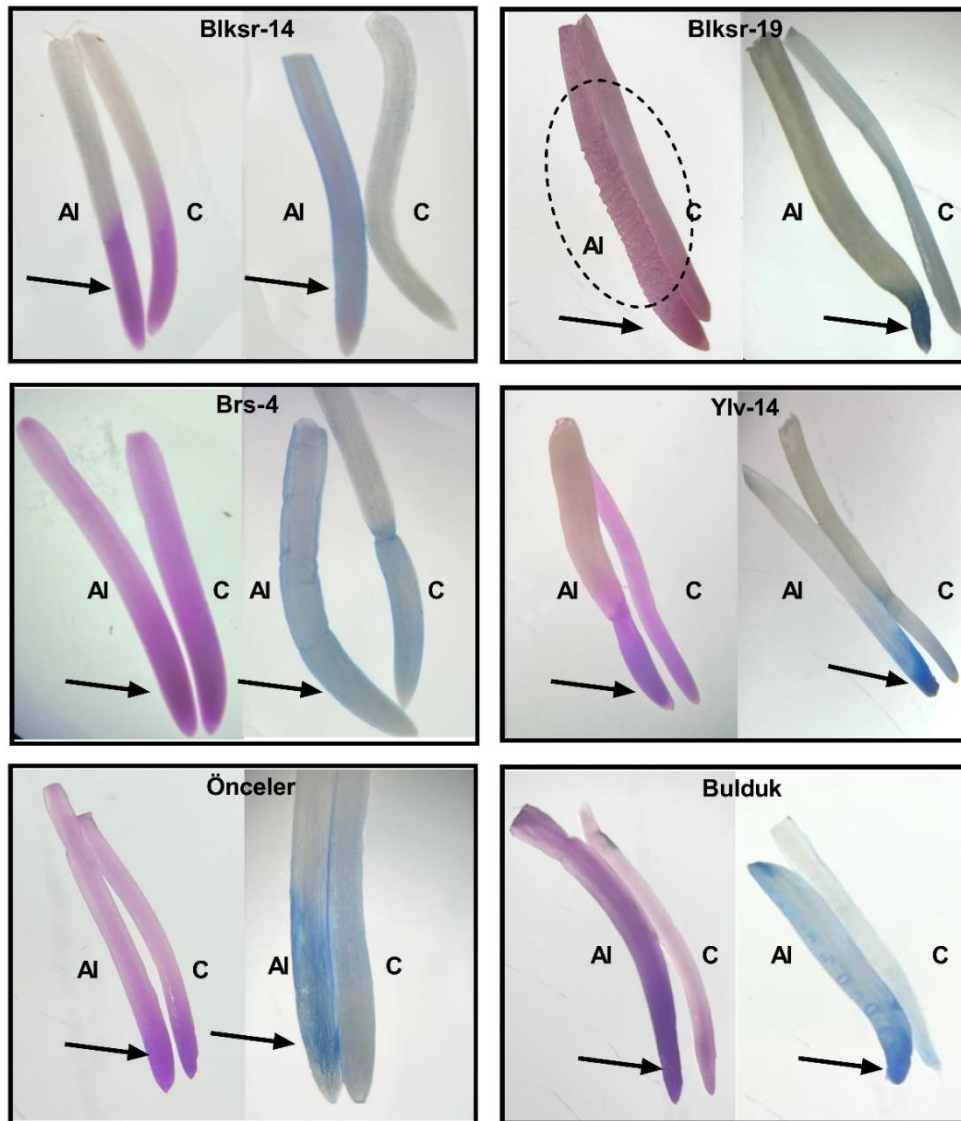


Figure 2. Histochemical detection of the Al effect in common bean roots. The roots were stained with Schiff's reagent (pink color) and Evan's blue (Blue color). Al= Al treated roots, C= control roots.

Yamamoto et al. (2001) investigated the response of pea roots to Al stress using histochemical and biochemical techniques. They reported that the cracks in the pea roots under Al stress are caused by differential cell expansion created by root elongation inhibition. As a result of dyeing the roots with low relative root elongation under Al stress with Evan's blue, it was seen that the color intensity is quite high at the tips of the roots (Blksr-19, Ylv-14 and Bulduk) when compared to controls. On the other hand, there was low color intensity at the tips of the roots in Önceler-98, Brs-4 and Blksr-14 having high root elongation. Al sensitivity is concentrated at the tip of the root, particularly in the transition zone located 1-2 mm behind the root (Rangel et al., 2007; Butare et al., 2012). This leads to changes in the cell wall and plasma membranes, which affect the mechanical characteristics of the cell wall. These changes play a significant role in inhibiting root elongation caused by Al (Yang et al., 2011). Our phenotypic evaluation results were supported by the histochemical analyses.

The expression levels of the *PvGST* and *PvPOD* genes were determined in root tissues of Önceler-98 and Blksr-19 at different time points (0 and 24 h). As seen in Figure 3, the Al stress findings showed that both genes were upregulated in the Blksr-19 (Al-sensitive) root tissue. This genotype had higher expression levels than the tolerant cultivar (Önceler-98) for both genes. The expression levels of the *PvGST* gene in Önceler-98 showed no statistical differences compared to Al stress treatment at 24 h. On the other hand, Blksr-19 revealed a 2.49-fold increase at 24 h compared to the control. When comparing Al-treated plants to the control, significant differences in gene expression for the *PvPOD* gene were detected. The transcript level of the *PvPOD* gene was significantly decreased at 24 h in Önceler-98, while this level was significantly increased 3.76-fold at 24 h in Blksr-19. Dmitriev et al. (2016) reported that exposure to

Al leads to the production of ROS and lipid peroxidation in plants. Plants facing oxidative stress, improve their ability to scavenge ROS by improving the enzymatic activity of antioxidants such as glutathione S-transferase, as well as peroxidase, superoxide dismutase and catalase (Mhamdi et al., 2010; Nanda et al., 2010). It has been observed that there is an enhancement in GST expression under Al stress in both sensitive and resistant maize lines (Cançado et al., 2005), *Arabidopsis* (Ezaki et al., 2004), and pea roots (Panda and Matsumoto, 2010). This indicates the participation of GST in the response of plants to Al stress. On the other hand, differential transcript level of *PvPOD* gene under Al treatment was detected in common bean genotype/cultivar. In previous study, Eticha et al. (2010) reported that peroxidases may assist in the plant's resistance to Al by detoxifying ROS, which are produced as a result of oxidative stress induced by Al. Tóth et al. (2021) observed differential POD activity under Al treatment in common bean. They found that POD activity was significantly higher in 12 cultivars and lower in others. The *PvPOD* gene was expressed differentially during the *Sclerotinia sclerotiorum* infection and BCMV infection in common bean (Oliveira et al., 2015; Yeken et al., 2024). Al tolerance in common bean is a complex process that involves the coordination of different genes (Ambachew and Blair, 2021). The effect of Al on plant growth and development varies based on factors such as concentration, species, genotype, cultivar, and duration of exposure (Kopittke et al., 2016). We suppose that *PvGST* and *PvPOD* genes might be involved in the defense mechanism against Al stress in the root tissue of common bean. However, further research on this subject, including different Al doses and different time intervals, and a better understanding of the molecular mechanisms are required.

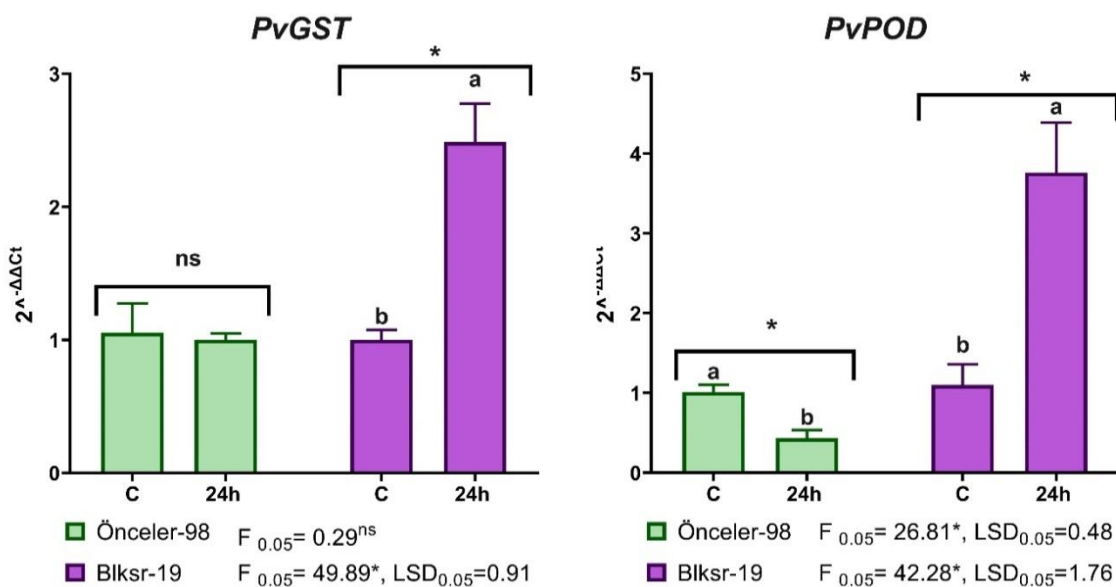


Figure 3. The differences in expression levels of *PvGST* and *PvPOD* genes in roots subjected to Al stress. * significant ($P < 0.05$), ns: non-significant, C= control.

4. Conclusion

This study evaluated the variation of relative root elongation under Al stress and the impact of Al toxicity using different histochemical dyes in common bean genotypes/cultivars. Moreover, the expression levels of *PvGST* and *PvPOD* genes against Al stress in the root tissue of the common bean were investigated for the first time. The obtained results in this study will enhance the knowledge of the literature on the *PvGST* and *PvPOD* genes in response to the Al stress in common bean. The use of the genetic resources discovered herein will hopefully allow the production of common bean cultivars resistant to Al toxicity. These sources might be used in Al tolerance breeding projects in the near future.

Author Contributions

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

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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COMPARISON OF NONLINEAR MODELS TO DESCRIBE THE GROWTH OF TUJ AND ROMANOV X TUJ (F1) LAMBS

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Abstract: This study aimed to identify the most suitable model for explaining weight changes in purebred Tuj (n=35) and Romanov x Tuj (RoxTuj) (F1) (n=25) lambs using non-linear models. Single-born lambs of both breeds and genders were included in the evaluation. Five different non-linear growth models were compared: Brody, Gompertz, Logistic, Richards, and Weibull. The best model for describing growth was chosen based on four criteria: coefficient of determination (R^2), mean square error (MSE), Akaike information criterion (AIC), and Bayesian information criterion (BIC). Models with the highest R^2 and the lowest MSE, AIC, and BIC values were considered the best fit for the data. It was observed that the Brody model had the highest R^2 and lowest MSE, AIC and BIC values for Tuj and RoxTuj (F1) female and male lambs. The Gompertz, Logistic, and Richards models exhibited similar predictive performance. In contrast, the Weibull model produced significantly different results compared to the other models when predicting weight changes. Therefore, the Brody model was identified as the most effective model for explaining growth patterns in both Tuj and RoxTuj (F1) lambs.

Keywords: Growth curves, Non-parametric models, Tuj, Romanov

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1. Introduction

In the context of animals, growth refers to the dynamic changes in weight and volume that occur during specific developmental stages based on age. These changes are influenced by a multitude of environmental factors acting upon the genetic potential, resulting in a complex and intricate process (Yakupoğlu, 1999). Growth curves, also known as age-growth curves, depict the trajectory of growth over a defined time period. They provide insights into the inherent capacity of animals to grow and develop, as well as the interplay between this capacity and environmental conditions throughout their lifespan (Efe, 1990).

The primary purpose of growth curves is to predict an individual's growth at later ages, enabling the selection of animals with favourable growth traits at an early stage (Efe, 1990; Tekel, 1998). Interpretation of changes in values across different age points and data summarization with fewer parameters is key objectives. Summarization involves estimating growth curve parameters through statistical modeling (Akbaş, 1995; Bilgin et al., 2004; Esenbuğa et al., 2000; Kopuzlu et al., 2014).

While the shape of growth curves may vary based on species, breed, sex, and environmental conditions, linear models can describe growth until adult weight in sheep. However, since growth rates exhibit a sigmoidal pattern

in later stages, linear increase is not sufficient. Consequently, non-linear models have been developed to capture the temporal changes (Akbaş, 1995; Esenbuğa et al., 2000; Kopuzlu et al., 2014).

The use of growth curves in animal husbandry originated with Brody's estimation of various growth characteristics using growth models and gained prominence through the work of Richards. Commonly employed growth curve models include Gompertz, Logistic, Brody, and Richards, which facilitate the prediction of growth traits in animal husbandry (Bilgin et al., 2004).

The objective of this study is to identify the most effective model for predicting the growth of female and male lambs from the Tuj and RoxTuj (F1) crossbreeds, utilizing age-body weight data and employing certain nonlinear models.

2. Materials and Methods

The animal material for this study comprises 35 pure Tuj lambs and 25 Romanov x Tuj (RoxTuj) (F1) crossbred female and male lambs (single-born), which were reared at the Sheep Farm of Atatürk University Food and Livestock Application and Research Center. Within the first 24 hours of birth, each lamb's weight was meticulously measured using scales accurate to 20 grams, and they were individually identified with plastic earrings. At an average age of 75 days, weaning was



carried out, and the lambs were then transferred to pasture. The same care and feeding practices were maintained for all lambs on the farm.

Following birth, lambs were weighed every 15 days to track their live weight changes. The study utilized body weight data collected at 15-day intervals from birth up to 24 weeks of age to analyze growth curves. Various nonlinear models (Table 1), including Brody, Gompertz, Logistic, Richards, and Weibull, were employed to describe growth over time. By evaluating coefficients of determination (R^2), mean square error (MSE), Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC), the most suitable growth model for Tuj and RoxTuj (F1) crossbred lambs were identified. The coefficient of determination (R^2) serves as a measure of how much variation in the data set can be explained by

the generated growth curve model, ranging between 0 and 1. In the calculation of AIC values, the formula “ $AIC=n*\ln(SSE/n)+2k$ ” is used, while for BIC values, the formula “ $BIC=n*\ln(SSE/n)+k*\ln(n)$ ” is employed. Here, n represents the number of observations, k denotes the number of model parameters, and SSE represents the sum of squared errors.

From a biological perspective, the parameters derived from different functions have specific interpretations. Y_t represents the observed weight at age t . The parameter A signifies the weight at maturity, which is the weight limit as age (t) approaches infinity. B corresponds to the initial weight, and k denotes the growth rate. These parameters were estimated using the Levenberg-Marquardt iteration method via the NLIN procedure in the SPSS program.

Table 1. Growth curve models

Growth Model	
Brody	$Y_t = A[1 - B * \exp(-k * t)]$
Gompertz	$Y_t = A * \exp[-B * \exp(-k * t)]$
Logistic	$Y_t = A [1 + B * \exp(-k * t)]^{-1}$
Richards	$Y_t = A[1 - B * \exp(-k * t)]^m$
Weibull	$Y_t = A - B * \exp(-k * t^m)$

3. Results and Discussion

The growth curve parameters for Tuj and RoxTuj (F1) lambs, as estimated by nonlinear models, are displayed in Table 2. When Table 2 ve 3 is examined, the A parameter in Tuj female and male lambs is highest with the Richard model and lowest with the Weibull model; In RoxTuj (F1) lambs, the highest was determined by the Brody model and the lowest was determined by the Logistic model. The B parameter was determined to be highest with the Weibull model and lowest with the Brody and Richard model for both genotypes in males and females. The k parameter was determined as the highest by the Logistic models and the lowest by the Richard and Brody models. The m parameter was determined as the highest in males

and females by the Richard model, and the lowest by the Weibull model.

In this study, the parameter values estimated from all models except the Richards model were found in Akbaş et al. (1999) Kıvrıkcık and Dağlıç breeds; In Esenbuga et al. (2000) İvesi, Morkaraman and Tuj breeds; Köyceğiz (2003) in İvesi and Morkaraman male and female lambs; Bilgin et al. (2004) in Awasi and Morkaraman breeds; Aytekin and Zülkadir (2013) in Malya sheep; It was found to be lower than the values reported by Kopuzlu et al. (2014) in Hemsin breed male and female lambs. The values reported by Yıldızbaş (2016) for the A parameter in Romanov lambs were observed to be higher than the values obtained from all models in this study.

Table 2. The estimated parameter values, and their standard errors for Tuj and RoxTuj (F1) male lambs with nonlinear growth models

Models		A	B	k	m
Brody	Female	45.73±2.29	0.95±0.007	0.040±0.004	-
	Male	48.73±3.13	0.94±0.008	0.04±0.004	-
Gompertz	Female	35.15±2.85	2.15±0.076	0.093±0.007	-
	Male	37.78±4.32	2.17±0.072	0.087±0.006	-
Lojistik	Female	33.44±2.28	5.02±0.29	0.125±0.005	-
	Male	34.37±1.22	5.31±0.47	0.137±0.006	-
Richard	Female	108.82±12.23	0.95±0.008	0.028±0.005	1.97±0.71
	Male	99.23±14.25	0.94±0.008	0.030±0.005	1.91±0.71
Weibull	Female	29.02±11.28	26.49±2.02	0.12±0.009	1.35±0.12
	Male	31.96±11.07	28.35±1.95	0.08±0.009	1.65±0.13

Table 3. The estimated parameter values, and their standard errors for RoxTuj (F1) male lambs with nonlinear growth models

Models		A	B	k	m
Brody	Female	135.27±20.19	0.99±0.007	0.006±0.006	-
	Male	152.73±25.25	0.99±0.008	0.006±0.008	-
Gompertz	Female	27.22±2.76	2.23±0.06	0.06±0.007	-
	Male	31.08±3.22	2.20±0.07	0.06±0.003	-
Lojistik	Female	23.34±2.08	5.89±0.19	0.25±0.004	-
	Male	24.28±2.12	6.03±0.27	0.37±0.006	-
Richard	Female	71.13±11.54	0.94±0.008	0.03±0.004	1.95±0.56
	Male	85.27±16.36	0.94±0.008	0.03±0.004	2.20±0.71
Weibull	Female	30.12±10.58	23.56±1.85	0.09±0.01	1.54±0.34
	Male	36.27±11.07	29.62±1.72	0.10±0.01	1.73±0.61

In Table 4, the Weibull model exhibited the highest MSE values for both female and male lambs of the Tuj and RoxTuj (F1) breeds, while the Brody model had the lowest MSE values. Regarding R², the Brody model yielded the highest values for both males and females, whereas the Weibull model had the lowest R² values. Additionally, AIC and BIC values were highest in male and female lambs with the Weibull model, while the lowest values were detected in the Brody model.

In both genotypes, the Brody model demonstrated the best fit with the highest R², lowest MSE, AIC, and BIC values for both male and female lambs. Conversely, the Weibull model exhibited the poorest fit. The deviation in predictions by the Weibull model is clearly observed in Figures 1 and 2. Furthermore, upon analyzing the figures, it is evident that the Logistic model provides close predictions to the observed values in Tuj lambs, while in

RoxTuj (F1) crossbreds; it tends to overestimate the observed values.

Similar to our study, Lambe et al., (2006), Malhado et al., (2009), Daskiran et al. (2010), Özdemir and Dellal (2009) determined the Brody, Logistic and Gompertz models as the best models. Unlike our study, Mohammadi et al., (2019) reported that the Logistic model was the model that showed the worst fit. Bilgin et al. (2004), Kopuzlu et al., (2014) and Nimase et al., (2018) reported that the Brody model can be recommended because it has fewer parameters than other models and is easy to interpret.

As a conclusion, it was observed that all five models describe growth well except for weibull. However, the Brody model was the model that best described the growth as it had the highest R² value in both Tuj and RoxTuj (F1) male lambs.

Table 4. Goodness of fit criteria (MSE, R², AIC ve BIC) results for models değerleri

Models		Tuj				RoxTuj (F1)			
		MSE	R ²	AIC	BIC	MSE	R ²	AIC	BIC
Brody	Female	2.74	99.4	13.09	15.96	3.23	99.7	12.96	15.28
	Male	3.51	99.7	18.24	21.33	3.91	99.8	17.32	19.64
Gompertz	Female	3.89	97.7	15.24	17.56	4.23	99.3	16.98	19.3
	Male	4.02	97.5	21.75	24.07	4.96	99.5	20.13	22.45
Lojistik	Female	3.81	96.1	16.21	18.53	4.85	99.0	18.51	20.83
	Male	3.77	96.9	19.99	22.89	4.65	99.1	19.95	22.27
Richard	Female	3.96	96.3	16.57	19.06	3.99	96.2	17.82	20.91
	Male	4.51	96.9	24.76	27.08	4.23	97.5	22.14	25.23
Weibull	Female	8.44	90.5	16.82	19.82	9.28	89.2	19.82	22.91
	Male	12.27	91.2	25.34	28.34	13.44	90.3	21.95	25.04

MSE= mean square error; R²= coefficients of determination; AIC= akaike information criterion; BIC= bayesian information criterion; RoxTuj: Romanov x Tuj crossbreed.

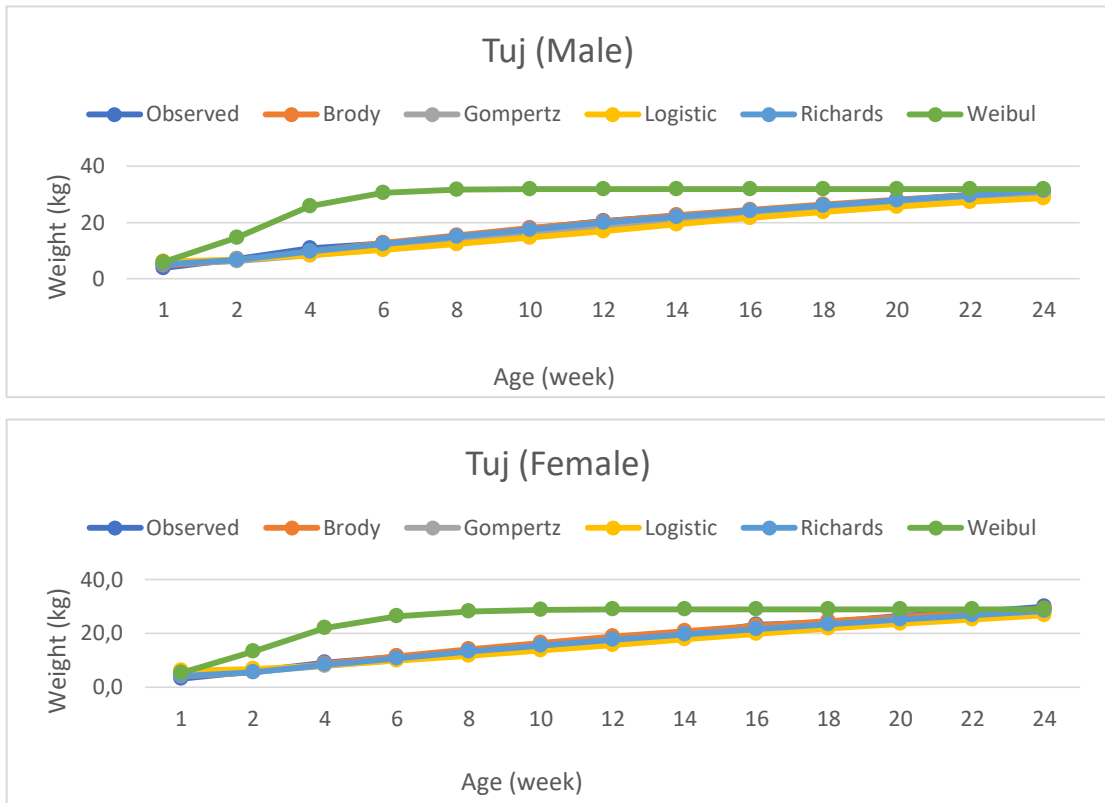


Figure 1. Growth curves of Tuj female and male lambs observed and estimated by different nonparametric models

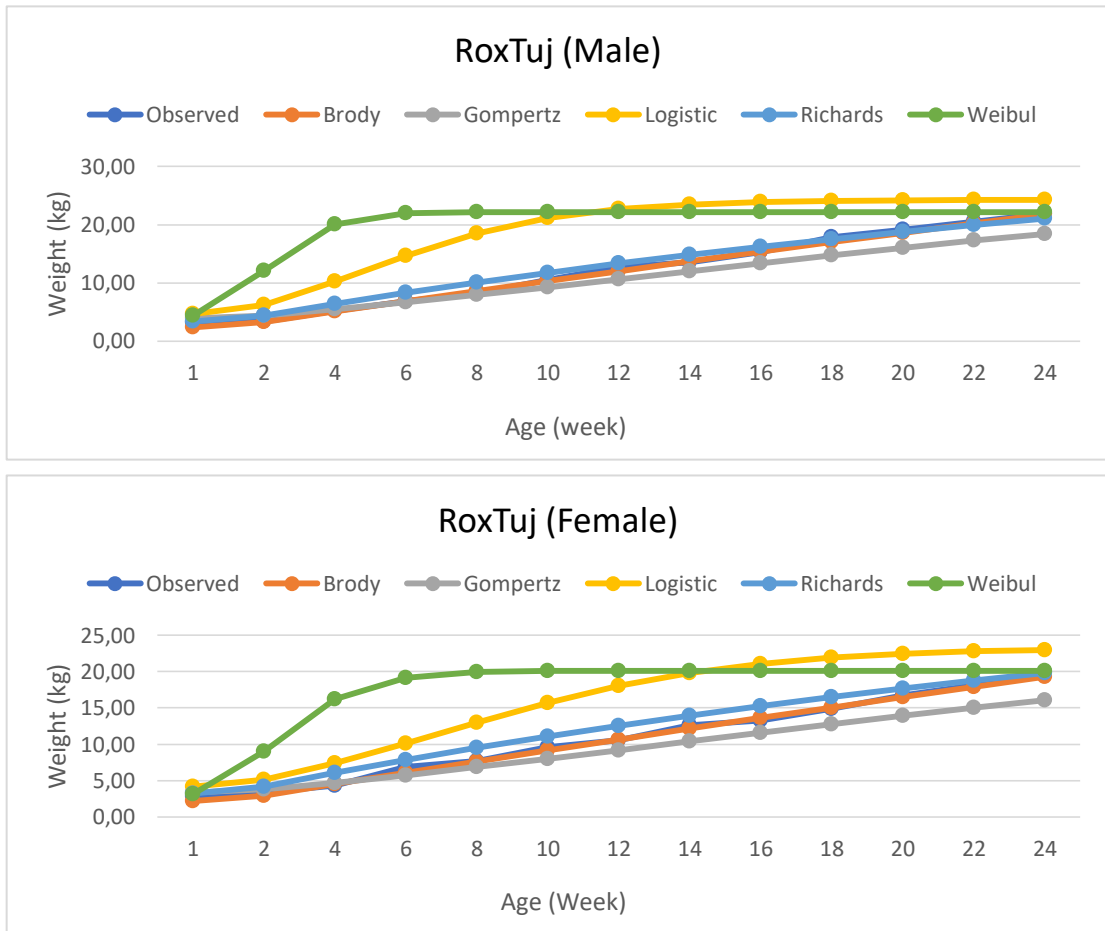


Figure 2. Growth curves of RoxTuj (F1) female and male crossbred lambs

Author Contributions

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

	Ü.D.	N.E.
C		100
D	25	75
S		100
DCP	50	50
DAI	25	75
L	50	50
W	50	50
CR	50	50
SR	75	25
PM	25	75

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The study was approved by The Local Animal Care and Ethics Committee of Atatürk University, Erzurum, Türkiye (approval date: May 29, 2018, protocol code: 2018/64).

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EFFECTS OF DIFFERENT SALT STRESS AND TEMPERATURE APPLICATIONS ON GERMINATION IN MUNG BEAN (*Vigna Radiata* (L.) R. WILCZEK) GENOTYPES

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Abstract: Abiotic stress factors are among the primary elements hindering plant growth and development. Initial growth and development in plants are significantly affected by temperature and salinity. The study aimed to investigate the growth and development parameters of two different mung bean genotypes under different salt concentrations and temperatures. Four different salt doses (0, 5, 10, and 15 EC) and three different temperatures (15, 20, and 30°C) were used in the study. Germination percentage, shoot and root lengths, shoot fresh and dry weights, and ion leakage parameters were examined in the study. As a result of the study, the highest germination rate, shoot and root lengths, and fresh and dry weights were determined at 30°C under control, 5 EC salt applications, while the lowest was recorded at 15°C under 15 EC salt applications. The lowest ion leakage was determined in the control application at 15°C, while the highest was observed at 30°C under 15 EC applications. Increasing temperature positively affected growth parameters. It was determined that salt stress could be tolerated up to a certain level with high temperatures. This study conducted on mung bean is indicative of developing varieties tolerant to temperature and salt stress, which are important issues today.

Keywords: Abiotic stress, Temperature, Mung bean, Germination

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1. Introduction

Seed germination is a crucial agricultural characteristic with profound effects on plant growth and productivity (Demirkaya et al., 2017). Biotic factors such as seed structure and environmental factors significantly influence the germination and growth process. Additionally, there exists a range of abiotic factors that substantially affect seed germination and development, including drought, nutrient deficiencies or excesses, salinity, extreme temperatures, terrestrial and atmospheric pollution, and radiation (Dadaşoğlu and Ekinci, 2013; Çakmakçı and Dallar, 2019; Demirkaya et al., 2020). Abiotic stress factors significantly limit agricultural production on a global scale (Kopecká et al., 2023). Among abiotic stressors, salt stress negatively affects plant growth and development (Okumus et al., 2023). High salt concentrations hinder water uptake during the initial stages of seed development, slow down embryo growth and development, and prolong the germination process (Munns and Gilliam, 2015). High salt concentrations reduce the water potential inside seeds, leading to osmotic stress, which hinders seed embryo hydration and complicates germination (Shabala and Pottosin, 2014).

Salinity directly affects plants through osmotic and ion stress, while its indirect effects result from structural damage in plants and the synthesis of toxic compounds due to these stress factors. Effects of salt stress include the synthesis of AOT (accumulation of organic osmolytes and toxic ions) compounds, metabolic toxicity, inhibition of photosynthesis, prevention of K⁺ uptake, and cell death, which disrupt DNA, protein, chlorophyll, and membrane function (Botella et al., 2005; Hong et al., 2009; Çakmakçı and Dallar, 2019). The germination characteristics of seeds can vary significantly depending on the plant species and environmental conditions, especially under different temperature regimes. Different temperature levels can have notable effects on germination rate, percentage, and subsequent seedling development (Kaya et al., 2006). Temperature stress can substantially reduce seed germination percentage, germination time, and seedling vigor in many plants (Lamichaney et al., 2021). High temperatures lead to cellular dehydration, causing a reduction in cell size and ultimately a decrease in growth (Arun-Chinnappa et al., 2017).

In this study, the effect of different salt stress and temperature applications on germination in mung bean



(*Vigna radiata* (L.) R. Wilczek) was investigated. Mung bean is an annual plant belonging to the legume family commonly used in world agriculture (Ram et al., 2019). Mung bean is rich in protein (22-28%), fiber, and minerals, and it is also abundant in magnesium, iron, B vitamins, and folate (Singh et al., 2017). Besides its high nutritional content, mung bean is a highly digestible legume (Benlioğlu and Özkan, 2020). Mung bean typically grows erect and can reach heights ranging from 30 to 90 cm, with flowers of various colors such as white and yellow (Huyen et al., 2014). Cultivation of mung bean in temperate regions is characterized by its short vegetative period and broad adaptability (Hanumantha Rao et al., 2016). Due to its low production cost and rich nutrient content, mung bean can be cultivated as an alternative crop in areas where protein sources are limited (Benlioğlu and Özkan, 2020). It is crucial to cultivate high-nutrient plant species under the influence of abiotic stress factors. Salinity and temperature are significant factors affecting early seedling development. The study aimed to investigate the effects of salinity and temperature on seed germination in mung bean.

2. Material and Methods

In this study, the tolerance to temperature and salinity was examined. The experiment was three-factorial and set up according to a split-split plot design in randomized complete blocks. As plant material, two different mung bean genotypes obtained from Argentina (Genotype 1) and Antalya (Genotype-2) were used. The study involved 3 different temperatures, 2 mung bean genotypes, and 3 different salt concentrations, conducted with 3 replications NaCl (Merck, Germany) was used for salt stress in the study. The salt levels were adjusted to control (0 EC), 5 EC, 10 EC, and 15 EC. The study was conducted at controlled conditions of 15°C, 20°C, and 30 °C in incubator.

The seeds to be used in the study were sterilized with 1% sodium hypochlorite for 5 minutes followed by rinsing with distilled water three times. The seeds were sown between 3 filter papers in groups of 25 and sealed with a ziplock bag to prevent moisture loss. 7 mL of solution was added to each filter paper. Seeds were considered germinated when the root (≥ 2 mm) emerged, and germinated seeds were counted for 14 days. Germination percentage (number of germinated seeds/25 x 100) was calculated at the end of the 14th day, and shoots and root length, fresh weight, dry weight, and ion leakage data were examined in randomly selected 10 seedlings.

Ion leakage was measured according to the method described by Aydın (2018). After washing with distilled water, the fresh shoots (0.5 g) were kept in 10 ml of distilled water at room temperature for 24 hours to measure the EC of the solution (O.D1). Then, after being autoclaved at 121°C for 20 minutes and cooled, the EC was measured again (O.D2), and ion leakage in leaf tissues was calculated using the following equation (Aydın, 2018).

$$\% \text{ Ion leakage} = (O.D1 / O.D2) \times 100$$

2.1. Statistical Analysis

The statistical analysis was conducted using the factorial experimental design with four replications in a randomized complete block design. The data obtained from the study were analyzed using the "JMP 13.2.0" software according to the factorial experimental design in randomized complete blocks. The treatment means were compared using Tukey's Honestly Significant Difference (HSD) test (Snedecor and Cochran, 1967). Also, PCA and correlation analyses were carried out JMP 13.2.0 software.

3. Results and Discussion

Upon examination of the features considered in the experiment, statistically significant differences between the applications were found. Additionally, the temperature*salt interaction was found to be statistically significant at the 5% level. In the genotype originating from Argentina, Genotype-1, the highest germination rate was determined to be 100% at 30°C under control conditions and with a 5 EC salt application, while the lowest rate was 23% at 15°C with a 15 EC salt application. However, a significant increase in germination rate of cowpea seeds was observed under high-temperature conditions. Specifically, the germination rate determined at 30°C with a 15 EC salt application (76%) was higher than that obtained under control conditions at 15°C (69%). It was determined that under high temperatures, salt stress could be tolerated up to a certain level.

Similar results were also recorded for shoot length and root length. The lowest values for shoot length and root length were determined in the applications at 15°C. The highest shoot length (13.08 cm) was determined at 30°C in the control group, followed by a 10.53 cm shoot length in the 5 EC salt applications at 30°C, with the control group at 20°C having a lower shoot length (9.06 cm). Root length, on the other hand, was found to be highest at 30°C and 20°C with values exceeding 10 cm, followed by the 5 EC salt applications at 30°C and 20°C. It is evident that shoot and root formation performance is significantly lower under low-temperature conditions.

When examining fresh and dry weights, the highest fresh weight was determined as 5492 mg in the control application at 30°C and 5250 mg in the control application at 20°C, while the lowest was determined as 19.50 mg in the 15 EC salt applications at 15°C. In terms of dry weight, it was determined as 430.53 mg in the control application at 30°C and 413.58 mg in the control application at 20°C, while the lowest was determined as 2.05 mg in the 15 EC salt applications at 15°C. Ion leakage was evaluated, with the highest percentage of 58.93% observed in the 15 EC application at 30°C, and the lowest percentage of 34.25% observed in the control application at 15°C (Table 1).

Table 1. Germination Rate (%), Shoot and Root Length (cm), Fresh and Dry Weights (mg), and Ion Leakage (%) of Genotype-1 at Different Temperature and Salt Concentrations

Temperature	Treatment	Germination Rate (%)	Shoot Length (cm)	Root Length (cm)	Fresh Weight (mg)	Dry Weight (mg)	Ion Leakage (%)
15°C	Control	69.00±1.17f	0.82±0.11h	0.77±0.19de	193.75±56.4fg	25.10±11.2ef	34.25±0.87i
	5 EC	51.00±1.17h	0.56±0.11hi	0.62±0.19de	127.25±56.4fg	20.60±11.2ef	41.03±0.87fgh
	10 EC	45.00±1.17i	0.29±0.11i	0.38±0.19de	51.75±56.4g	6.05±11.2f	46.62±0.87de
	15 EC	23.00±1.17j	0.16±0.11i	0.15±0.19e	19.50±56.4g	2.05±11.2f	48.43±0.87cd
Mean		47.00	0.46	0.48	98.06	13.45	45.58
20°C	Control	88.00±1.17b	9.06±0.11c	10.43±0.19a	5250.00±56.4ab	413.58±11.2a	38.66±0.87gh
	5 EC	82.00±1.17cd	7.25±0.11d	7.70±0.19b	4680.00±56.4c	384.70±11.2a	42.74±0.87efg
	10 EC	79.00±1.17de	3.23±0.11f	4.50±0.19c	3735.00±56.4d	326.13±11.2b	50.91±0.87bcd
	15 EC	58.00±1.17g	1.88±0.11g	1.05±0.19de	513.25±56.4fg	73.00±11.2de	55.12±0.87ab
Mean		76.75	5.36	5.92	3544.56	299.35	46.86
30°C	Control	100.00±1.17a	13.08±0.11a	10.75±0.19a	5492.50±56.4a	430.53±11.2a	37.54±0.87hi
	5 EC	100.00±1.17a	10.53±0.11b	7.90±0.19b	4800.00±56.4bc	409.50±11.2a	43.99±0.87ef
	10 EC	87.00±1.17bc	4.49±0.11e	4.31±0.19c	2345.00±56.4e	166.75±11.2c	52.47±0.87bc
	15 EC	76.00±1.17e	2.28±0.11g	1.24±0.19d	610.00±56.4f	83.63±11.2d	58.93±0.87a
Mean		90.75	7.60	6.05	3311.88	272.60	48.23
Temperature x Salt		**	**	**	**	**	*
*P<0.05; **P<0.01							

In the 2nd genotype originating from Antalya, the highest germination rate was determined to be 87% under the control condition at 30°C, while the lowest was 25% under the application of 15 EC salt at 15°C. Significant increases in germination rate at high temperatures were observed, like those originating from Argentina. When shoot and root lengths were examined, the highest shoot length was determined to be 12.07 cm under the control condition at 30°C and 8.93 cm under 5 EC application, whereas the lowest was 0.14 cm under the application of 15 EC salt at 15°C. Regarding root length, the highest was determined to be 10.30 cm under the control condition at

30°C and 9.79 cm at 20°C under the control condition. When considering fresh and dry weights, the highest fresh weight was 5010 mg under the control condition at 30°C and 4474 mg at 20°C, while the lowest was 18 mg under the application of 15 EC salt at 15°C. In terms of dry weight, it was 479.25 mg under the control condition at 30°C and 420 mg at 20°C under the control condition, with the lowest being 1.83 mg under the application of 15 EC salt at 15°C. Ion leakage was evaluated, with the highest being 53.84% under the application of 15 EC at 30°C, and the lowest being 30.53% under the control condition at 15°C (Table 2).

Table 2. Germination rate (%), shoot and root length (cm), fresh and dry weights (mg), and ion leakage (%) of genotype-2 at different temperature and salt concentrations

Temperature	Treatment	Germination Rate (%)	Shoot Length (cm)	Root Length (cm)	Fresh Weight (mg)	Dry Weight (mg)	Ion Leakage (%)
15°C	Control	65.00±1.28ef	0.64±0.15f	0.70±0.20d	173.00±47.77e	17.50±3.16fg	30.53±0.96h
	5 EC	57.00±1.28g	0.52±0.15f	0.54±0.20d	105.25±47.77e	11.57±3.16g	37.64±0.96fg
	10 EC	39.00±1.28i	0.26±0.15f	0.30±0.20d	48.00±47.77e	5.00±3.16g	41.62±0.96ef
	15 EC	25.00±1.28j	0.14±0.15f	0.13±0.20d	18.00±47.77e	1.83±3.16g	44.57±0.96de
Mean		46.5	0.39	0.42	86.06	8.98	38.60
20°C	Control	79.00±1.28bc	8.66±0.15b	9.79±0.20a	4474.25±47.77b	420.00±3.16b	34.12±0.96gh
	5 EC	73.00±1.28cd	6.85±0.15c	7.26±0.20b	3879.50±47.77c	372.50±3.16c	42.73±0.96de
	10 EC	59.00±1.28fg	2.77±0.15d	4.13±0.20c	3187.50±47.77d	307.50±3.16d	47.36±0.96cd
	15 EC	50.00±1.28h	1.65±0.15e	1.01±0.20	408.75±47.77e	42.87±3.16f	52.25±0.96ab
Mean		65.25	4.98	5.54	2987.50	285.72	44.11
30°C	Control	87.00±1.28a	12.07±0.15a	10.30±0.20a	5010.00±47.77a	479.25±3.16a	37.47±0.96fg
	5 EC	81.00±1.28ab	8.93±0.15b	7.40±0.20b	4617.50±47.77ab	430.50±3.16b	41.46±0.96ef
	10 EC	68.00±1.28de	3.52±0.15d	3.85±0.20c	2797.50±47.77d	215.25±3.16e	48.05±0.96bc
	15 EC	58.00±1.28g	1.80±0.15e	1.14±0.20d	413.25±47.77e	47.48±3.16f	53.84±0.96a
Mean		73.50	6.58	5.67	3209.56	293.12	42.21
Temperature x Salt		*	**	**	**	**	*
*P<0.05; **P<0.01							

In the study, the effect of 4 different salt doses (0, 5, 10, and 15 EC) and 3 different temperatures (15, 20, and 30°C) on germination parameters was supported by PCA analyses. According to the calculated PCA analysis, the graphs explain the applications by 93% (Component 1 %77.9; Component 2 %15.3). Upon examination of the obtained findings, a linear relationship between temperature and mung bean germination is evident. With increasing temperature, there is an increased tendency

for root and shoot formation (Figure 1). With increasing temperature, the application of low doses of salt is positioned at the same place as the control. Looking at the ion leakage graph, it can be observed that salt applications are positioned in the opposite direction to germination parameters. As the salt application in the environment increases, so does the ion leakage (Figure 1).

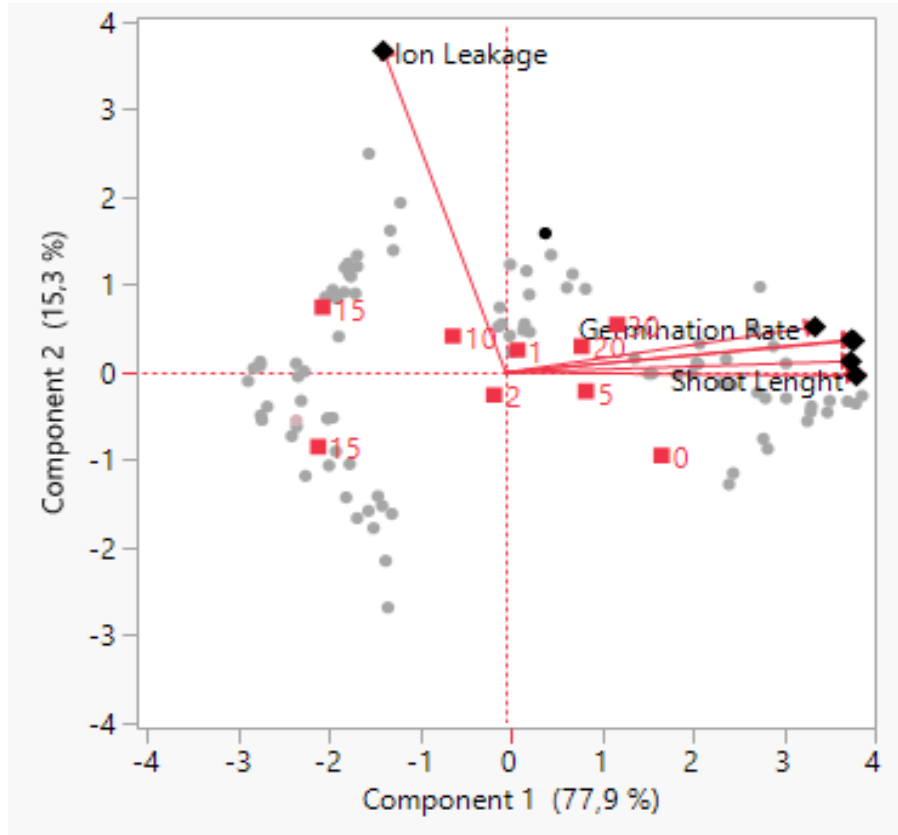


Figure 1. Principal Component Analysis (PCA) between germination parameters of mung bean with different salt and temperature treatments.

The PCA graph, which evaluates the interaction of variety, salt, and temperature treatments on the germination parameters of mung bean seeds, also supports the findings obtained (Figure 2). While germination rates vary according to variety, germination parameters are positively affected under conditions where low salt and high temperature treatments are applied together. Under high temperature conditions, the application of low salt doses has yielded results similar to the control.

Salt and temperature stress significantly reduces the growth and development of plants during their initial growth stages. Salt and temperature stress can directly lead to plant death and simultaneously significantly reduce plant growth, seed germination, root and shoot length (Yılmaz et al., 2023). When both genotypes were examined, an increase in germination rates was observed with increasing temperatures. In parallel, plant growth and development were negatively affected by increasing salt doses. In other studies, in beans (*Phaseolus vulgaris*

L.) (Kibar et al., 2020), they found that increasing salt doses (0, 50, 100, and 200 mM) resulted in a decrease in germination rate from 88.16% to 31.11% and significant decreases in other parameters such as shoot and root length, as well as fresh and dry weights. In maize (*Zea mays* L.) (Çakmakçı and Dallar, 2019), they observed a decrease in germination rate from 81.27% to 45.87% with increasing temperatures and concluded that increasing temperature and salt application negatively affected growth and development. In beans (*Phaseolus vulgaris* L.) (Yılmaz et al., 2023), the effect of 18 local genotypes and a 200 mM salt concentration on growth and development was investigated, and it was found that the salty environment reduced germination by 38%. Salt stress negatively affects shoot and root lengths (Saha et al., 2010). Root length ensures more efficient utilization of soil minerals and water by the plant; therefore, root length is an important parameter during initial growth and development (Rabie, 2005). Significant decreases in shoot and root lengths were observed at high salt doses

in both genotypes. Similar decreases have been observed in other studies (Çakmakçı and Dallar, 2019). Most mung bean genotypes exhibit tolerance to moderate salt levels (9-18 mhos cm) (Singh and Singh, 2011). In the obtained results, resistance was observed up to 15 EC salt at 20 and 30°C. This finding is consistent with similar studies (Benlioğlu and Özkan, 2020).

Both fresh and dry weights were similarly negatively affected by low temperature and high salt density. In other studies, it has been noted that increasing salt densities not only affect shoot and root lengths but also lead to decreases in fresh and dry weights (Misra and Dwivedi, 2004).

Salinity causes yield losses in more than 50% of the world's arid and semi-arid regions (Naeem et al., 2020). The high osmotic effect, ion toxicity, oxidative stress, and nutrient deficiencies in these areas adversely affect plant

growth (Naeem et al., 2020). High salt concentrations lead to membrane breakdown, increasing ion leakage (Kalisz et al., 2023). The highest ion leakage values were observed in G1 and G2 under 15 EC application at 30°C, while the lowest was observed in G1 and G2 under 10°C control application. A lower ion leakage value indicates higher tolerance to salt stress (Okumuş et al., 2023). In a study on red clover, ion leakage ranged from 31.74% to 68.36% with increasing salt doses (Okumuş, 2022). Kurt et al., in their study on soybean (*Glycine max*) in 2023, reported that ion leakage in leaf tissues increased with increasing salt doses.

In other studies, it has been found that ion leakage increases with increasing salt concentrations, and the reason for this is reported to be the parallel increase in ion leakage with salt doses, resulting from damage to cell membrane integrity and stability (Oral et al., 2020).

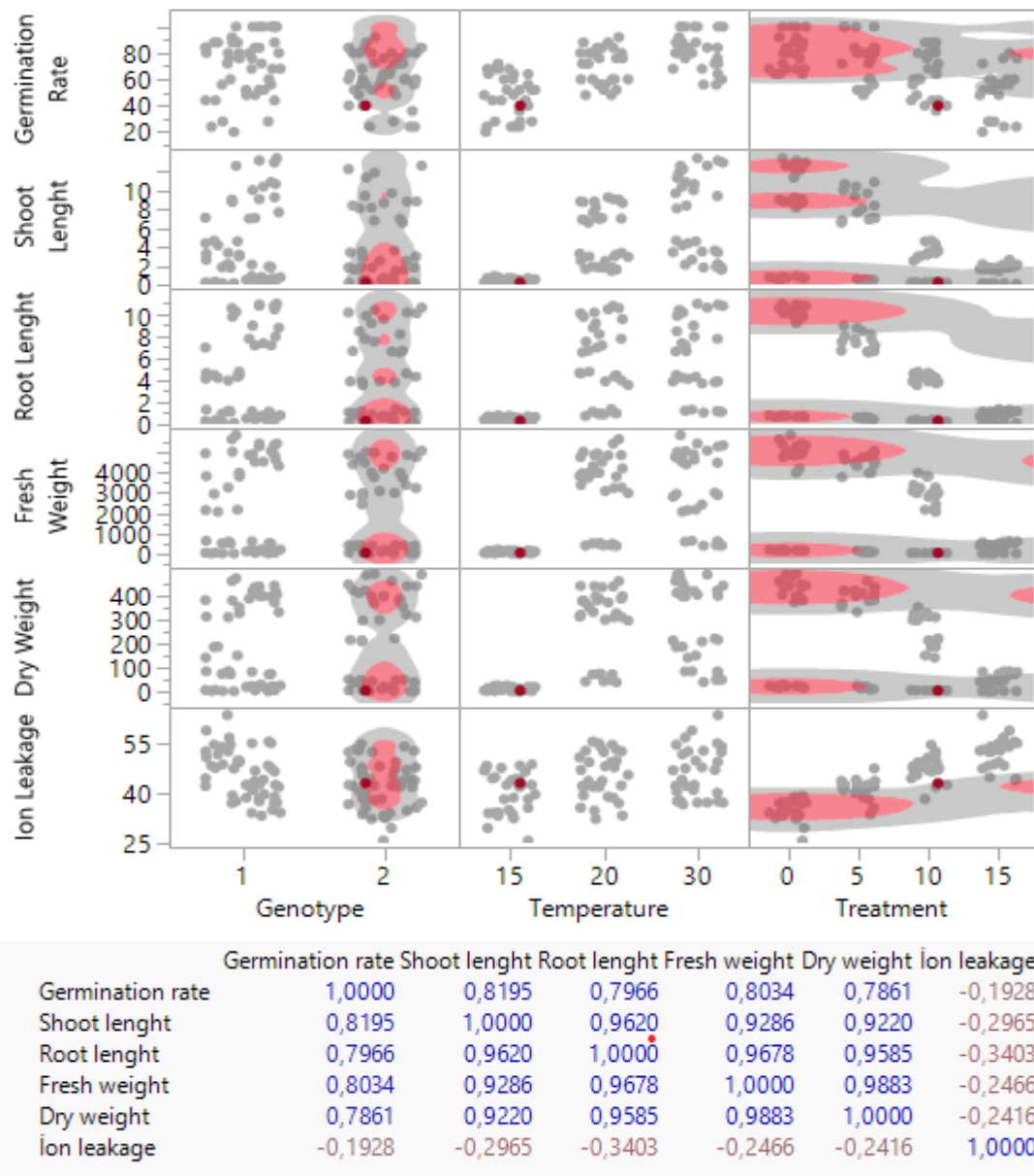


Figure 2. Scatterplot matrix and correlation of the germination parameters of mung bean with different salt and temperature treatments.

4. Conclusion

Salinity and high temperature are significant abiotic stress factors that adversely affect plant growth and productivity, limiting agricultural production. Bean species are among the most sensitive vegetables to salinity. In this study, the effects of different salt and temperature treatments on germination parameters in mung beans were investigated. The research revealed that salinity greatly inhibited germination in mung bean seeds. As salt concentration increased, a significant decrease in germination rate was observed. With increasing salt stress, there was an elevation in ion leakage, accompanied by ion toxicity. It is believed that this results in the blockage of water passage in seeds and limits seed germination as the salt concentration in the environment increases.

Similar results were obtained with the two genotypes used in the study. Additionally, it was observed that the damage caused by salt stress decreased with increasing temperature. This study, designed with two different genotypes, aimed to determine the germination characteristics of mung beans. However, it is a fundamental study and provides guidance for drought and salinity-resistant variety breeding, in line with sustainable development goals, responsible production and consumption, climate action, and addressing global climate issues.

Author Contributions

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

	O.O.	A.D.S.
C	50	50
D	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50

C=Concept, D= design, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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GLOBAL RESEARCH ON BLUETONGUE: A BIBLIOMETRIC ANALYSIS

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Abstract: Bluetongue (BT) is a viral disease characterized by swelling and cyanosis of the tongue, capable of infecting both domestic and wild animal species. Bluetongue virus (BTV) can cause an outbreak in any region of the world where favorable temperature and vector presence conditions exist. For this reason, BTV studies remain an active field in the literature and the research network of this field continues to develop. This paper aims to identify the most influential research elements and collaborations in the BTV field, with a discussion of results based on existing literature. To discover the current literature on BTV, the Web of Science database was employed. The dataset consisted of 1315 articles. The “Bibliometrix” R package and the VOSviewer program were utilized for data analysis. The number of BT studies rose dramatically between 2005 and 2016 with the appearance of BTV in Europe. The USA and England were determined as the most influential countries. The *Veterinary Microbiology* and *Medical and Veterinary Entomology* journals were identified as the most prominent journals of the field. It has been determined that serological studies have been a focal point and collaborations have been strengthened in the field of BTV. Conversely, financing sources and collaboration levels regarding BTV studies were found to be inadequate in African and Asian countries. This paper is expected to provide information to researchers interested in BTV studies about the intellectual structure of the field.


Keywords: Bluetongue, Culicoides, Surveillance, Serology, Veterinary bibliometric

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1. Introduction

Bluetongue (BT) is a non-infectious, arthropod-borne viral disease of ruminants. The causative agent of the disease is known to be the Bluetongue virus (BTV), which belongs to the Orbivirus genus of the Reoviridae family (Mertens et al., 2005; Ranjan et al., 2015). BTV is recognized as a potentially devastating disease of ruminants by the OIE (World Organization for Animal Health) and FAO (Food and Agriculture Organization) (Rao et al., 2017). BT was first emerged in South Africa. In the following years, it was seen in Europe, America, the Middle East, and Asian countries (Monaco et al., 2006). BTV causes economic losses due to high morbidity, mortality, abortion, fetal abnormalities, and weight loss in ruminants. Beyond impeding the progress of countries in the domain of animal husbandry, it also adversely impacts the national economies by incurring supplementary expenses for disease management (Gethmann et al., 2020).

BTV is transmitted to the host through specific species of mosquitoes belonging to the genus *Culicoides* (Batten et al., 2008). BTV outbreaks exhibit seasonality, typically emerging during the summer and autumn seasons. These occurrences can emerge in any part of the world characterized by tropical, subtropical, and temperate

climatic zones, with the presence of vectors and suitable hosts. BTV has the potential to impact both domestic and foreign ruminants. However, clinical manifestations are typically evident in sheep whereas, cattle and other domestic ruminants mostly exhibit subclinical symptoms. Typically, clinical symptoms of BTV infection include fever, edema of the ears, oral ulcers, cyanosis of the tongue, and muscle necrosis, often resulting in fatal outcomes (Savini et al., 2008; Schulz et al., 2012).

Bibliometrics is a quantitative analysis and visualization of research outputs derived from databases within a specific research area (Yu et al., 2020). Bibliometric analysis employs a set of metrics to assess and evaluate researchers, institutions, journals, countries, and research topics. Bibliometric studies aim to provide researchers with a priori information through a map of the relevant field (Wang et al., 2020).

A literature survey showed that bibliometric analysis has become a prevalent tool for uncovering the intellectual structure of the field of animal diseases in recent years. For example, Kai et al. utilized bibliometric analysis to determine research topics for Brucellosis disease. Their findings revealed that studies mostly focused on cattle as an intermediate host and epidemiological studies on brucellosis were estimated to increase in the coming years (Kai et al., 2023). Elisha et al. examined research



funding and collaborations in African countries on the Anthrax outbreak. They reported strong cooperation in the African continent between Kenya, Nigeria, South Africa, and South Africa identified as the country providing the most financing (Elisha et al., 2024).

This study explores the current trends, cooperations, and contributions within the field of BTV. Through this examination, the study aims to present researchers with a general framework of the field and general knowledge of the related literature. The most influential researchers, institutions, journals, countries, as well frequently used keywords were identified and discussed based on the quantitative metrics.

2. Material and Method

2.1. Research Methods

In general, bibliometric analysis is composed of 2 steps, performance analysis and visualization. Performance analysis enables meticulous measures of research elements according to certain metrics. On the other hand, the visualization step includes visual presentations of results obtained through performance analysis to facilitate a clear understanding and interpretation of the findings (Zupic and Čater, 2015). Accordingly, changes in research trends over time and gaps within a given field can be revealed; additionally, popular topics and the

most influential research items (researchers, journals, countries, etc.) can be identified (Aria and Cuccurullo, 2017). In the performance analysis, various indicators, including the *h*-index, *g*-index, *m*-index, citation count, publication count, Journal Impact Factor (JIF), and JIF Quartile, were employed. Furthermore, bibliometric laws such as Lotka’s and Bradford’s Laws were utilized.

2.2. Data Sources and Statistical Analysis

Clarivate Analytics' database Web of Science (WoS) was used to explore the existing literature on Bluetongue disease in veterinary medicine. WoS and Scopus, as the largest databases, are known for publishing studies that adhere to rigorous publication ethics (Merigó and Yang, 2017). A search conducted on 3 January 2024 using the terms “bluetongue” OR “bluetongue virus” OR “BTV” resulted in 2351 studies in the field of veterinary sciences. After limiting the search to document type (research article) and language (English), 1640 studies were listed. Upon content review, 325 articles related to other animal diseases or those concentrating on *Culicoides* mosquitoes, the vector of the Bluetongue virus without directly addressing the BT virus were excluded from the initial pool of 2351 studies. Consequently, the bibliographic dataset consisted of 1315 articles complying with the search parameters (Figure 1).

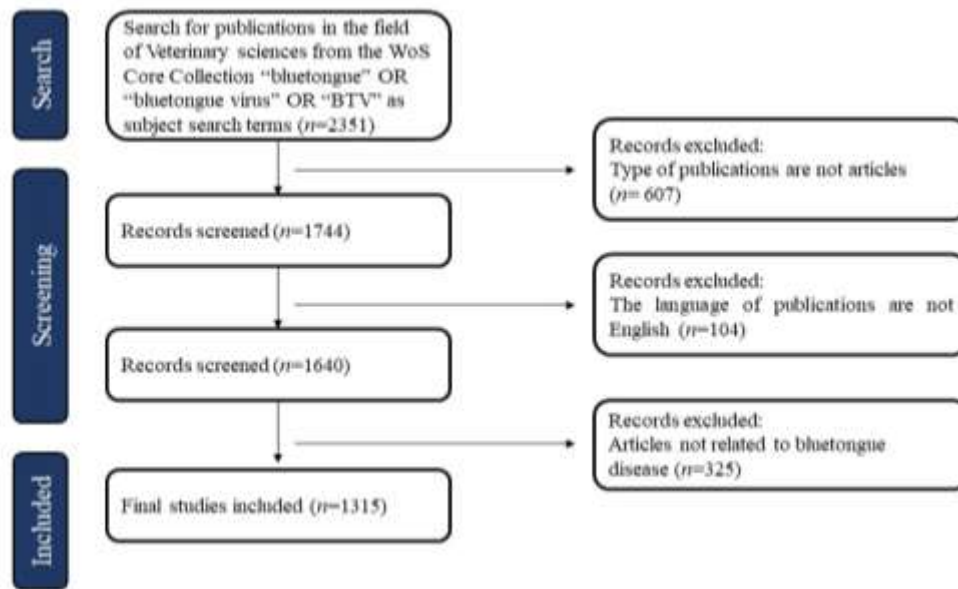


Figure 1. The publication selection process for bibliometric analysis.

Many statistical programs are used for bibliometric analysis. In this study, R “Bibliometrix” package was preferred for bibliometrics analysis. Furthermore, for visualization of the findings, VOSviewer, a commonly used program in bibliometric analyses, was employed (Aria and Cuccurullo, 2017; Van Eck and Waltman, 2017). The performance analyses of academicians, journals, institutions, and countries were conducted using the “Bibliometrix” package. Co-authorship and keyword network analyses were performed on the VOSviewer program.

3. Results

3.1. General Publication Trends

A general examination of the bibliometric data revealed that articles from 65 different journals published between 1980 – 2023 were included in the dataset. Over this 43-year duration, a total of 1315 articles meeting the search criteria were published. The cumulative count of authors amounted to 4189 and the number of single-authored articles was 69. The citation count per article was 20.62 and the total citation count was 17782. The

bibliographic data comprised 1706 keywords plus and 2182 author keywords. Author collaboration statistics revealed that the average number of articles per author was 0.31, authors per article were 3.18, co-authors per article were 5.42, the international co-authorship rate was 25.93%, and the collaboration index was 3.32. The number of authors contributed to these studies was 4189 and author names were used 7127 times. The author footprint index was found to be 0.75. The majority of the publications were (94.75%) multi-authored (Table 1).

Table 1. Main statistics on Bluetongue studies

Description	Results
Main Information About Data	
Timespan	1980:2023
Sources (journals, books, etc.)	116
Documents	1315
Annual growth rate (%)	2.31
Document average age	15.8
Average citations per doc	20.62
References	17782
Document Types	
Article	1246
Article; early access	5
Article; proceedings paper	37
Document Contents	
Keywords Plus (ID)	1706
Author's Keywords (DE)	2182
Authors	
Authors	4189
Author appearances	7127
Authors of single-authored documents	49
Authors of multi-authored documents	4140
Authors Collaboration	
Single-authored documents	69
Multi-authored documents	1246
Authors per document	3.18
Documents per author	0.31
Co-Authors per document	5.42
Collaboration index	3.32
Author footprint index	0.75
International co-authorships (%)	25.93

The examination of the publication trend of BTV studies revealed a significant increase in the number of articles between 1980 – 2023. The annual growth rate was 2.1%. However, this growth did not follow a linear trajectory. Until 2005, the number of articles was steady with an annual average of about 20 but rose dramatically between 2005 – 2016 maintaining a continuous upward trend. From 2016 onward, the number of articles showed a decline with an annual average of 30 until the beginning of 2024 (Figure 2).

Figure 3 visualizes the relationships between the top 20 most influential “sources (SO)”, “authors (AU)”, and “keywords (DE)”. Accordingly, *Transboundary and*

Emerging Diseases and *Veterinary Microbiology* were identified as the most influential journals in the field. Philip Scott Mellor and Stephan Zientara emerged as two of the most influential authors. The most frequently preferred keywords were “bluetongue”, “bluetongue virus”, and “culicoides”.

3.2. Most Influential Journals

Utilizing Bradford's law, 5 journals were determined as primary sources in the field. These journals were *Veterinary Microbiology*, *Transboundary and Emerging Diseases*, *Preventive Veterinary Medicine*, *Medical and Veterinary Entomology*, and *Journal of Medical Entomology*. An overall examination of the *h*-index, *g*-index, the number of articles, and the number of citations indicated that the most influential journals were *Veterinary Microbiology* and *Medical and Veterinary Entomology*, respectively. The aims and scope of *Veterinary Microbiology*, the most influential journal in the field, were comprehensively examined and it was found that the journal mostly includes innovative studies on viral and bacteriological diseases in domestic and farm animals. The examination of BTV articles in the field indicated that studies mostly focused on serological and molecular BTV research. On the other hand, the *Medical and Veterinary Entomology* journal focused on important research on insects, ticks, and other arthropods in the veterinary field. The review of BT virus studies in this journal indicated that studies were on culicoides, the vector of BTV, and the diseases caused by it. The international collaboration of the journals mostly exceeded 20% (Table 2).

3.3. Analysis of Prolific Authors

The metrics indicating the scientific productivity of the top ten most influential authors in the field of BTV are listed in Table 3. Philip Scott Mellor (England) and Nigel James Maclachlan (USA) held the top two positions based on *h*-index and *g*-index scores, total publication count, and citation count. The author with the highest *m*-index score was Simon T. Carpenter (England). The international collaboration rate among the top ten most influential authors in the field of BTV was found to be high (average 49.86%). The only author with an international collaboration rate below 40% was Bennie Irve Osburn. Lotka's law estimates that 60% of authors contribute to the field with one, 15% with two, and 7% with three articles (Sudhier, 2013). It was determined that in the BTV field, 75.3% of the authors contributed to the field with one, 13% with two, and 4.8% with three articles. Therefore, the distribution did not comply with Lotka's law. It was observed that the number of single-authored articles was higher than expected, while the number of three-authored articles was lower. The primary reason for this result can be attributed to the popularity of the field between 2008 and 2016, with the number of studies during this period accounting for 41.5% of the total studies in the literature. Furthermore, according to Lotka's law, authors with more than 5 articles can be considered core authors in the BTV field.

To determine the collaborations among the most influential authors in the field, the co-authorship interactions between the authors with at least 5 articles on BTV were visualized as shown in Figure 4. According to the analysis of the authors with the highest co-

authorships, a total of 131 authors combined under 12 groups were identified, accounting for 508 interactions. The top three authors with the highest co-authorship interactions were Giovanni Savini (86), Stephan Zientara (82), and Corinne Sailleau (73), respectively.

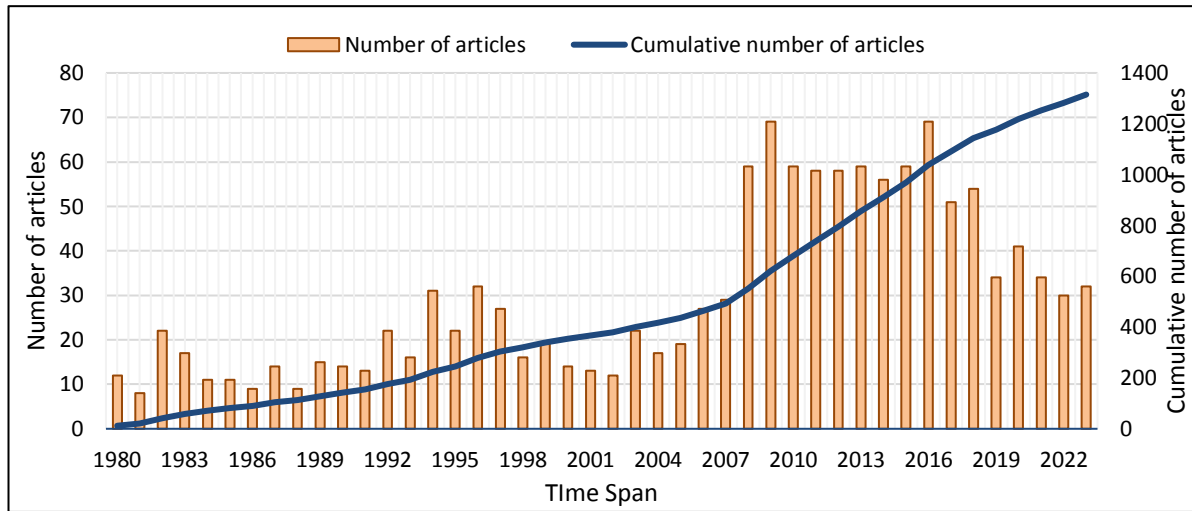


Figure 2. Annual scientific production on Bluetongue in Veterinary Medicine.

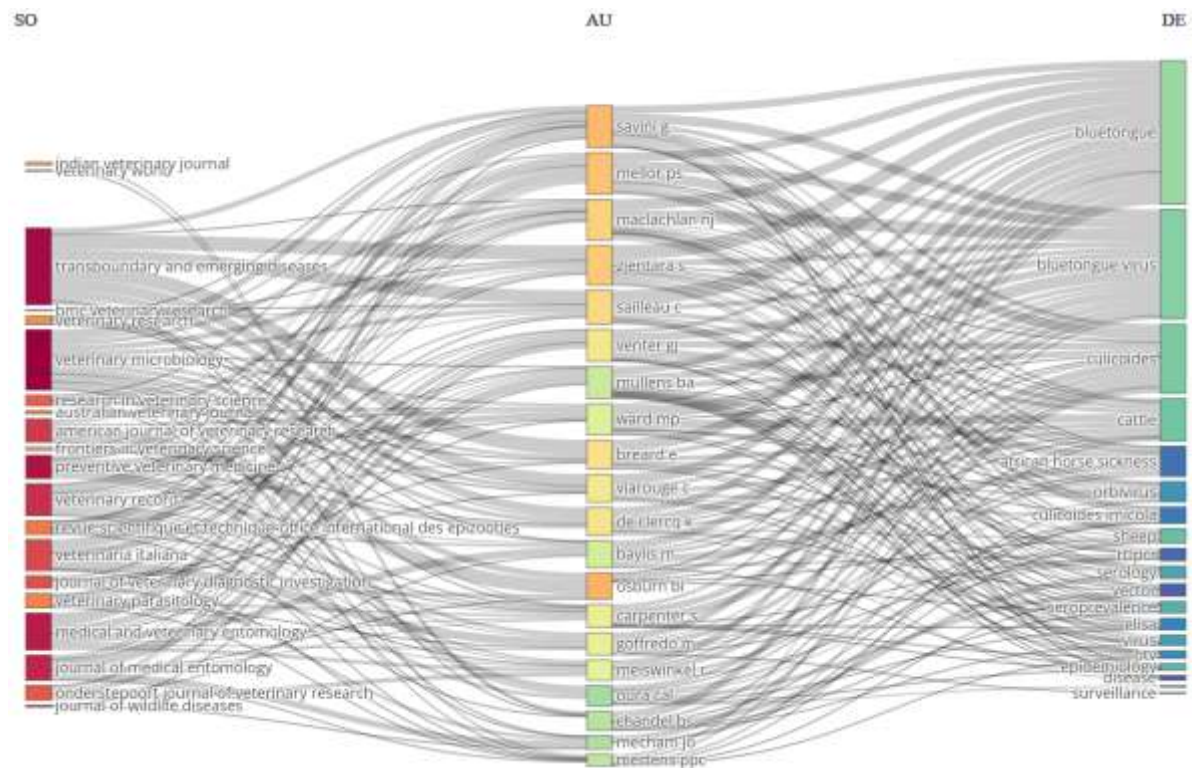


Figure 3. Bluetongue three area graph, Sources (left), Authors (middle), and Keywords (right).

Table 2. The *h*-index, *g*-index, *m*-index, and other scientific indices of the journals (top 10)

Source	h index	g index	m index	TC	NP	CI	IC (%)	JIF	JIF Quartile	Country
Veterinary Microbiology	33	50	0.75	3599	137	26.27	26.37	3.3	Q1	Netherlands
Medical and Veterinary Entomology	28	43	0.80	2120	71	29.86	44.48	1.9	Q2	England
Preventive Veterinary Medicine	28	46	0.70	2441	81	30.14	40.89	2.6	Q1	Netherlands
Veterinary Record	27	47	0.61	2358	62	38.03	4.22	2.2	Q2	England
Transboundary and Emerging Diseases	24	37	1.50	1808	93	19.44	43.54	4.3	Q1	Germany
American Journal of Veterinary Research	23	34	0.52	1417	58	24.43	10.14	1	Q3	USA
Journal of Medical Entomology	23	35	0.59	1417	66	21.47	29.81	2.1	Q2	USA
Journal of Wildlife Diseases	21	29	0.58	1075	53	20.28	29.85	1.3	Q2	USA
Veterinary Parasitology	18	24	0.64	760	24	31.67	32.74	2.6	Q1	Netherlands
Journal of Veterinary Diagnostic Investigation	16	25	0.50	697	35	19.91	23.13	1.5	Q2	USA

NP= number of publications, TC= total citations, CI= citation impact, IC= international collaborations, JIF= journal impact factor.

Table 3. The *h*-index, *g*-index, *m*-index, and other scientific indices of the authors (top 10)

Author	h index	g index	m index	TC	NP	CI	IC (%)	Country
Mellor, Philip Scott	27	39	0.675	2252	39	57.74	60	England
Maclachlan, Nigel James	24	36	0.585	1692	36	47.00	61.5	USA
Zientara, Stephan	19	30	0.864	929	37	25.11	54.8	France
Meiswinkel, Rudy	18	20	0.600	1251	20	62.55	44.4	Italy
Osburn, Bennie Irve	18	28	0.409	901	43	20.95	10	USA
Carpenter, Simon T.	16	20	0.842	1053	20	52.65	62.9	England
Sailleau, Corinne	16	23	0.727	591	33	17.91	44.7	France
Savini, Giovanni	16	30	0.842	987	40	24.68	52.3	Italy
Venter, Gert Johannes	16	22	0.485	883	22	40.14	54.9	South Africa
Baylis, Matthew	15	19	0.577	1009	19	53.11	53.1	England

NP= number of publications, TC= total citations, CI= citation impact, IC= international collaborations.

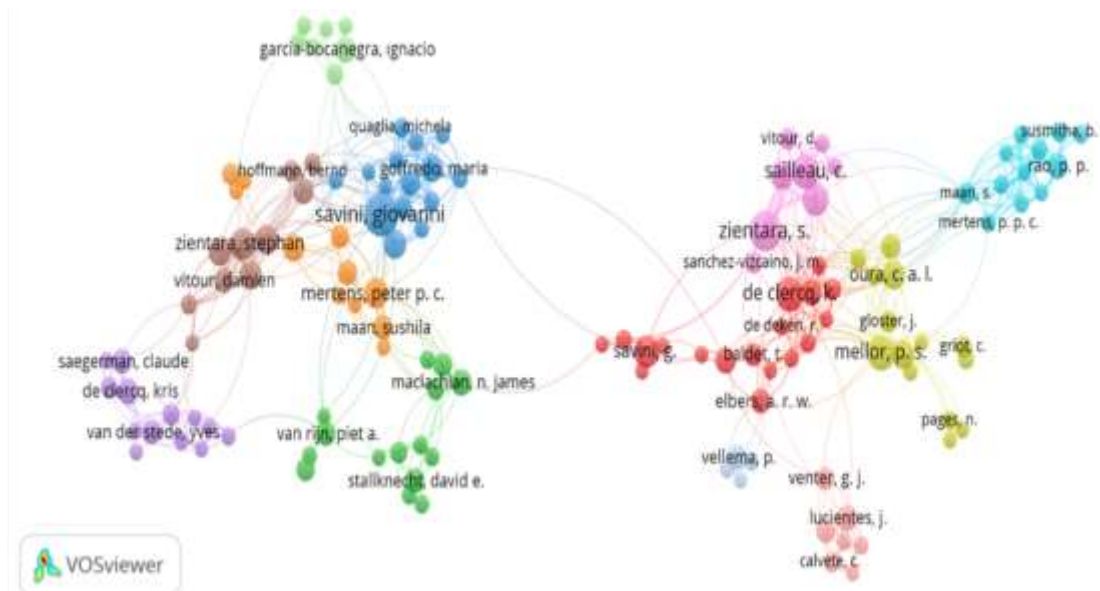


Figure 4. Co-authorship network.

3.4. Contribution of Institutions

In total, 1173 institutions associated with a total of 1315 articles on BTV were determined. The article count per institution was almost 1. This figure indicates the high cooperation in the field. The productivity of institutions on BTV studies was examined and UC Davis (USA), Institute of Animal Health (Japan), and University of Georgia (USA) were identified as the institutions with the highest number of articles. Furthermore, these institutions were also identified as the top three institutions according to *h-index* scores. Regarding international collaborations among institutions, the top three were the University of Liege (70.04), Pirbright Institute (69.30), and Onderstepoort Veterinary Institute (66.56), respectively (Table 4).

Table 4. The *h-index* and other scientific indices of Affiliations (top 10)

Affiliation	Articles	IC (%)	Country
University of California Davis	164	42.10	USA
Institute of Animal Health	135	27.21	Japan
University of Georgia	71	32.26	USA
University of Pretoria	63	51.44	South Africa
Indian Veterinary Research Institute	55	26.66	India
Pirbright Institute	50	69.30	England
Onderstepoort Veterinary Institute	43	66.56	South Africa
University of Florida	42	37.28	USA
University of Liege	41	70.04	Belgium
University of California Riverside	35	42.37	USA

IC: international collaboration.

3.5. Contribution of Countries

Table 5 presents some metrics including article and citation counts of countries on BTV studies. Countries with the highest article counts were the USA and England. These 2 countries accounted for 28.9% of the global BTV literature. Furthermore, the Netherlands was identified as the country with the highest category-specific citation index (CNCI). Multiple country publication (MCP) counts of the countries were examined and Belgium (0.462) was identified as the country with the highest MCP (0.462), despite it did not achieve an MCP ratio of 50%. Moreover, although the USA had the highest publication count, it exhibited the lowest MCP ratio (0.134).

The collaboration network of the countries was examined and it was found that 49 countries grouped under 16 clusters. However, only 4 clusters could build nodes within them. The dimensions of the nodes within the network represent the publication frequency of countries in the selected field. Countries within the same cluster demonstrated collaborations on research publications. According to the collaboration network, the highest collaborations were observed in the clusters involving the USA, England, and France. Considering the number of nodes and total node powers, the USA, England, France, and Italy were identified as the most influential countries in the collaboration network (Figure 5).

Table 5. Publication count and collaboration metrics of the countries (top 10)

Country	TC	NP	CI	Frequency	SCP	MCP	MCP Ratio	Links	TLS
USA	6141	268	22.91	0.204	232	36	0.134	48	134
England	3775	112	33.71	0.085	77	35	0.313	46	209
India	799	105	7.61	0.080	95	10	0.095	5	12
Australia	1283	72	17.82	0.055	59	13	0.181	17	41
France	1572	71	22.14	0.054	43	28	0.394	24	143
Spain	1494	68	21.97	0.052	52	16	0.235	21	74
Italy	1894	65	29.14	0.049	43	22	0.338	28	111
Belgium	1443	52	27.75	0.040	28	24	0.462	12	84
South Africa	1371	51	26.88	0.039	40	11	0.216	24	84
Netherlands	1781	42	42.40	0.032	28	14	0.333	13	81

NP= number of publications, TC= total citations, CI= citation impact, SCP= single country publication, MCP= multiple country publication, TLS= total link strength.

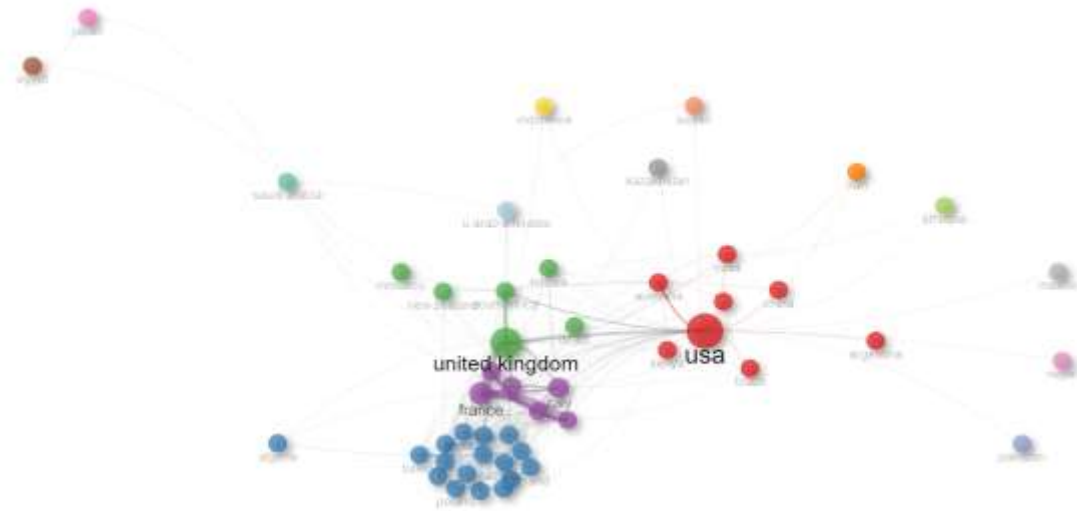


Figure 5. Map of cooperation between countries.

3.6. Keyword Analysis

Keyword analysis is important to assess the evaluation of the studies and research topics in a given field. The keyword network showing the most frequently used author keywords (used at least 5 times) is illustrated in Figure 6. The evolution of keywords used in the field indicates the changing significance of concepts in BTV studies over the years and reveals the current research topics. Accordingly, the keyword network indicated that the terms “bluetongue”, “bluetongue virus”, and “culicoides” were frequently used together. Furthermore, the terms “vector borne diseases”, “surveillance”, “wildlife”, and “serotype 4” were often used in recent studies. The examination of the keyword network also revealed that studies on vector-borne diseases and the seasonality of the disease were mostly focused on cattle species. Moreover, a higher concentration of studies on the BTV serotype 8 was found in South Africa; while the

number of studies examining the effects of BT on abortion in camels was higher in Saudi Arabia. Additionally, since epizootic hemorrhagic disease and BTV belong to the Reoviridae family, serological and experimental infection studies using both agents were frequently conducted.

3.7. Co-citation Analysis

Co-citation analysis reveals how frequently two reports are cited together by other studies. A minimum co-citation count of 20 was set for BTV studies and a total of 218 journals were listed. The top 3 journals with the highest citations were *Veterinary Record* (1647 citations, JIF 2023 = 2.2, Q2), *Veterinary Microbiology* (1515 citations, JIF 2023 = 3.3, Q1), and *American Journal of Veterinary Research* (1259 citations, JIF 2023 =1, Q3). In general, these journals exhibited high Journal Impact Factor (JIF) values and were considered prestigious and influential within the field (Figure 6).

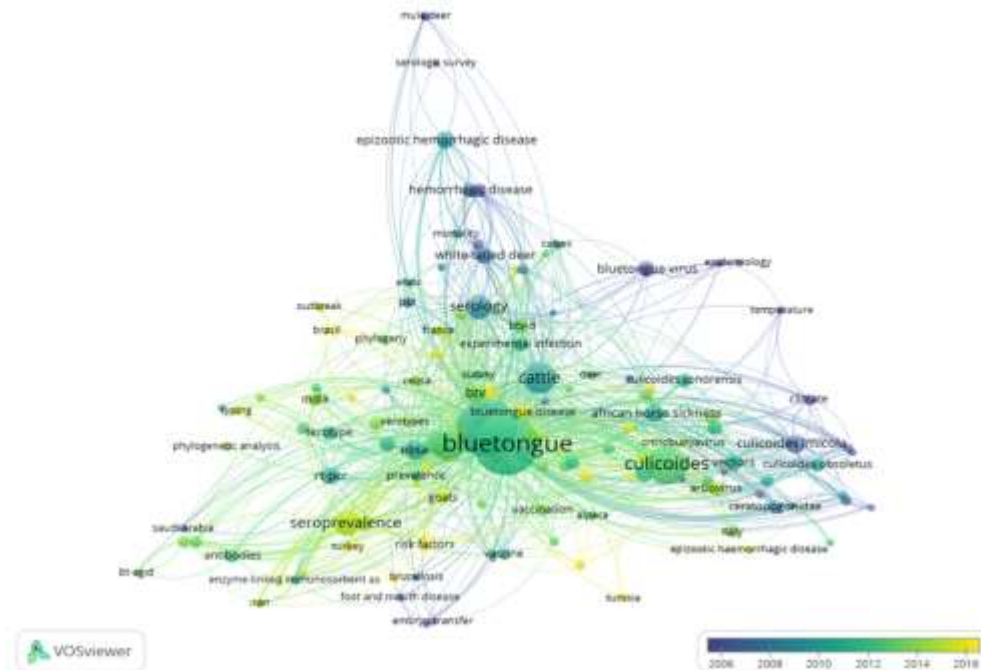


Figure 6. Network analysis of authors' keywords.

Co-cited references were examined and accordingly, 136 references were determined among 17758 references with a minimum co-citation count of 20. The study exploring the transfer of BTV to the host by *Culicoides*, titled "*Culicoides Biting Midges: Their Role as Arbovirus Vectors*", authored by Philip Scott Mello emerged as the highest co-cited research (Year: 2000, co-citations:176). The study with the second highest co-citation count was "*Climate change and the recent emergence of bluetongue in Europe*" by Bethan V. Purse. In this study, Purse examined the epidemiology of BTV in Europe (Year: 2005, co-citations:146). It was found that the top 10 articles with the highest co-citation counts were about the prevalence of BTV among different animal species and regions.

4. Discussion

BTV was initially confined to the South African region but has spread globally over time. BTV causes economic losses worldwide primarily due to the impact of regional *Culicoides* vectors (Subhadra et al., 2023). Therefore, BTV remains an attractive topic among researchers. Due to the ongoing relevance of the topic, the intellectual development of BTV studies was examined using the data obtained from the WoS database. The annual growth rate of studies in this field was found to be %2.31. The volume of scientific studies across all disciplines has exhibited an average annual growth rate of 3% over the past 30 years (Bornmann and Mutz, 2015). Accordingly, it can be argued that BTV studies exhibited a similar growth with all literature. Regarding publication trends on BTV, a dramatic rise was observed between 2008 and 2018 with a relatively stable pattern in other periods. Starting in 2006, BTV cases (BTV-8) rapidly spread in Europe. During this period, 50,000 new cases were reported in Europe, particularly in North Europe, and new BTV strains were identified. Furthermore, vaccine development studies have also started during this period (Niedbalski and Fitzner, 2018). Due to vaccine development studies with a focus on serological studies and research on *Culicoides* vectors, the number of BTV-related studies has displayed a dramatic rise in European countries. The growing research in this field has revealed that the virus tends to propagate in various serotypes among domestic animals (Mayo et al., 2020). Due to the risk of the emergence of new serotypes of BTV, future funding should be directed toward economically disadvantaged countries, particularly in Africa, to facilitate efforts in combating the disease (MacLachlan and Osburn, 2006). Financial support to projects on BTV plays a crucial role in the collective effort to combat the virus by facilitating rapid global sharing of information. For instance, BTV-GLUE is a project carried out in collaboration with MRC-University of Glasgow Centre for Virus Research, Nottingham University, The Pirbright Institute, and PALE-Blu (Pathogen Livestock Environment interaction involving bluetongue) (Singer et al., 2019). For the development of genomic studies on

BTV, institutions from Asian and African countries should also be included in institutional collaboration projects. Considering the obtained keyword network, the increase in virus-vector and seroprevalence studies was found to be remarkable due to the role of the vector in the spread of BTV. Furthermore, RT-PCR and ELISA were identified as current diagnosis tools.

The spread of BTV in Europe has prompted some authors to develop a higher interest in this field and specialize accordingly. Two of the most influential authors were Philip Scott Mellor and Nigel James Maclachlan. These authors also developed collaboration networks with their high participation in the field. The only author from South Africa, the homeland of the disease, is Gert Johannes Venter. Furthermore, the top ten most influential journals in the field of BTV mostly implement the open-access publication method. It is important to note that authors from South Africa and other underdeveloped or developing countries, lacking the opportunity to cover open access fees, often prefer journals with lower citation indices. Journals focusing on BTV research were mostly animal diseases and parasitology journals. Despite being the first country in article count, the USA displayed a relatively lower MCP value for these articles. Additionally, since the disease is affected by seasonality due to *Culicoides* vectors, it is observed in only certain regions of the USA (Rivera et al., 2021).

The findings indicated that researchers from the USA mostly established collaborations with China, Brazil, and Argentina. Among European countries, a strong collaboration, particularly England-based, was observed. Additionally, England was the country with which South Africa established the largest number of collaborations.

To prevent new BTV-related outbreaks, developed countries should enhance serological studies with African countries and establish a more extensive collaboration network. As BTV occurs in different serotypes regionally, cooperation networks were mostly observed between countries from the same continent (Maclachlan and Mayo, 2013). In general, Asian and African countries exhibited lower article counts and collaborations.

5. Conclusion

BTV is a viral disease occurring globally in various serotypes, primarily transmitted by *Culicoides* vectors, causing substantial economic losses in ruminants. Accordingly, the development of BTV studies, increase/decline trends over time, as well as the most influential authors, countries, institutions, and journals were examined over a 43-year period using the WoS database. A total of 1315 scientific papers were statistically reported using mapping methods.

A comprehensive examination revealed a shift in publication trends towards the USA and the European continent, with the emergence of BTV in the USA and Europe after 2000. Insufficient collaborations in African

and Asian countries have prevented the establishment of adequate research accumulation in these regions. For this reason, research centers should be established with the support of developed countries and institutions, involving underdeveloped (especially African) countries. Therefore, by fostering collaboration, BTV's new serotypes can be identified earlier, and efforts for vaccine development and prevention practices can be carried out through global platforms.

This study, by offering a comprehensive overview of the field, can serve as a guide for researchers in their future studies on the BT virus. Through this paper, researchers can access various useful information including details about other researchers interested in BTV and serotypes of the disease in their regions. This study presents an objective structure of the field quantitatively but has some limitations. The WoS database offers an extensive collection of academic journals and is a commonly utilized resource in bibliometric studies. The main limitations of this study are that studies outside the WoS database are not included, and only English studies are incorporated into the analysis.

Author Contributions

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

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

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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ENVIRONMENTAL IMPACT ON ECONOMICALLY SIGNIFICANT TRAITS IN CENTRAL ANATOLIAN MERINO SHEEP

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Abstract: This research delves into the pre-weaning growth and fleece characteristics of the Central Anatolian Merino sheep breed, focusing on Türkiye's National Community-Based Small Ruminant Breeding Program. The study encompasses Central Anatolian Merino lambs born between 2016 and 2021 across 22 farms in Ankara province, amassing a dataset of around 35,344 observations. Economically important traits such as birth weight (BW), weaning weight (WW), average daily weight gain (ADWG), Kleiber ratio at weaning (KR), fibre diameter, and fibre length (measured in approximately 4,809 observations) were scrutinized. Rigorous statistical analyses, including outlier identification, normality assessment, and the development of linear mixed models, were employed to unravel the impact of environmental factors on these traits. Significant findings emerged, indicating that birth weight, weaning weight, and the Kleiber ratio were substantially influenced by variables such as sex, birth type, birth season, birth year, and flock size. Moreover, average daily weight gain exhibited noteworthy variations attributed to gender, birth type, birth season, birth year, flock size, and other environmental factors, underscoring their collective impact on growth. Fleece traits displayed considerable diversity influenced by gender, birth type, birth season, birth year, and flock size. This study sheds light on the intricate interplay between environmental factors and pre-weaning growth traits within the Central Anatolian Merino sheep breed. Beyond its scientific contributions, this research provides valuable insights aimed at bolstering productivity. The adaptability of the Central Anatolian Merino breed to arid climates and challenging pasture conditions positions it as a key player in Türkiye's broader agricultural development.


Keywords: Pre-weaning growth traits, Kleiber ratio, Fleece characteristics, Environmental factors, Central Anatolian Merino Sheep


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1. Introduction

Sheep, one of the earliest domesticated species, have played a significant role throughout history as a source of animal protein need, as well as for their skin and wool used in clothing and shelter (Demiryürek et al., 2017). Sheep offer several advantages over cattle breeding due to their ability to adapt to challenging climatic conditions and utilize low-quality pastures. Furthermore, sheep breeding is a vital component of rural economies, family-based farm livelihood, and traditional lifestyles, contributing to food security with its consistent output (Alhamada et al., 2017).

Türkiye holds a prominent position in sheep farming, boasting approximately 41 million sheep, with 90.8% being domestic sheep and 9.2% crossbred Merino sheep (TUIK, 2023). Crossbreeding studies initiated in the 1930s aimed to enhance the wool quality and meat productivity of indigenous breeds. As a result of these efforts, many genotypes such as Karacabey Merino,

Central Anatolian Merino, Malya, and Ramlıç have been developed up to the present day (Atav et al., 2022; Bağkesen and Koçak, 2018; Unlusoy and Ertugrul, 2016; Koyuncu and Uzun, 2009; Yılmaz et al., 2012). The Central Anatolian Merino sheep breed's population is rapidly expanding due to its exceptional meat production and wool quality, making it a favourite among breeders (Ingham et al., 2007).

Pre-weaning growth traits are influenced by a combination of genetic and environmental variables. Genetic factors, such as the lamb's and its parents' genetic potential, play a crucial role in influencing the development rate and efficiency of lambs during the pre-weaning stage. Breeding strategies that select animals with desirable development traits may contribute to increased pre-weaning growth rates in future generations (Balasundaram et al., 2023). Therefore, countries implement breeding programs as a critical step towards ensuring food security, health, economy, and a



sustainable future for the growing population and future generations. It is essential for food security policies and practices to consider the needs and welfare of future generations (Gul et al., 2023).

In Türkiye, breeding programs focus on various native and Merino crossbreed sheep breeds, selecting breeding stock based on birth weight, weaning weight, milk yield, average daily gain, feed efficiency, and wool quality characteristics. These phenotypic traits, essential in the sheep breeding programs, play a critical role in the selection of animals for the next generation (Atav and Buğdayci, 2022; Eskandarinasab et al., 2010; Gül et al., 2019; Keskin et al., 2017; Mahala et al., 2020). Since the initiation of the Central Anatolian Merino sheep community-based breeding initiative in Ankara in 2016, there has been significant success. This initiative is part of the Ministry of Agriculture and Forestry's "National Sheep and Goat Breeding Project under Farmers Conditions," conducted in collaboration with several universities, research institutions, sheep and goat breeder groups, and individual breeders. Consequently, the objective of this study was to characterize the distributions of birth weight, weaning weight, average daily gain, Kleiber ratio, wool diameter, and wool length in Central Anatolian Merino sheep raised around Ankara, as well as to estimate the effects of certain environmental factors on these traits.

2. Materials and Methods

2.1. Animals and Phenotype

This study focuses on Central Anatolian Merino lambs born in 22 herds participating in the National Community-Based Small Ruminant Breeding Program in Ankara province, Türkiye, spanning from 2016 to 2021. The geographical coordinates of the area are 39°41' N; and 33°01' E, known for its challenging pasture conditions and characterized by a continental climate featuring cold, snowy winters and hot, dry summers. The

region experiences an average annual rainfall of 389 mm, a temperature of 11.7 °C, and an altitude of 938 m. Despite its considerably poor pasturelands, sheep utilize grazing areas from April to November. During lambing season and adverse winter conditions, sheep are provided an average of 0.8 kg/day of concentrate feed in enclosed shelters. After an approximate 90-day nurturing period, lambs are weaned by their dams. Key traits, including birth weight (BW), weaning weight (WW), average daily weight gain (ADWG), and Kleiber ratio at weaning (KR), were recorded, resulting in approximately 35,344 observations. Additionally, wool diameter and wool length were measured in roughly 4,809 observations. Regularly recorded data include birth and weaning dates, sex, birth type (singlets/twins), dam age (6 levels), years (2016 to 2021), herd size (3 levels), and season (3 levels). Weaning weight represents interpolated weights of animals at 90 days, which is the average weaning day. ADWG was calculated using linear statistics based on BW and WW. The Kleiber ratio at weaning (KR) was determined as ADWG divided by $WW^{0.75}$. A detailed data structure, along with sample size after eliminating outliers, is presented in Table 1. The analysis of fibre diameter and staple length required collecting approximately 50 g of fleece from the caudal region of the scapula at ages 0-6, 12-24, 24-36, and 36 months. These samples were numbered and presented to the laboratory. Fibre analysis was conducted using the OFDA 2000 (BSC Electronics, Ardross, Australia), an optical fibre diameter analyzer installed in the Ankara Sheep and Goat Breeders Association Laboratory. During analysis, a portion of the fibre was aligned and straightened before being inserted into the device. Fibers were then automatically optically measured in micrometres (µm) for diameter and millimetres (mm) for length. Samples from four different age groups were analyzed, and descriptive statistical characteristics are presented in Table 2.

Table 1. Descriptive statistics of pre-weaning growth traits

Trait	BW (kg)	WW (kg)	ADWG (g)	KR
Number of observations	35344	35344	35344	35344
Mean	4.06	26.94	252.95	21.17
Standard error	0.00	0.04	0.38	0.01
Minimum	1.40	11.66	71.74	10.90
Maximum	7.97	45.95	483.16	27.53
Coefficient of Variation	19.17	24.80	28.09	11.21

BW= birth weight, WW= weaning weight, ADWG= average daily weight gain, KR= kleiber ratio.

Table 2. Descriptive statistics of wool traits

Trait	Diameter(µ)	Length(mm)
Number of observations	4,809	4,809
Mean	24.65	51.30
Standard error	0.03	0.27
Minimum	17.70	15.00
Maximum	29.90	115.00
Coefficient of Variation	9.80	37.02

2.2. Statistical Analysis

Outliers in the observations, identified as values exceeding the mean by 3 standard deviations, were carefully examined. The normality of responses was assessed using the Shapiro-Wilk test, and visual inspection of variance homogeneity was performed through a plot derived from the residual vs. fitted values of the responses.

2.2.1. Environmental variables and model development

Initially, the impact of environmental variables on growth performance (sex, birth type, season, dam age, herd size, and birth year) and wool quality attributes (diameter and length, considering age and years) was investigated to formulate final linear mixed models. Data management and statistical analyses were conducted using the "lme4," "lmerTest," and various additional basic packages within the R statistical environment (R Core Team, 2020).

2.2.2. Linear mixed models

After fitting the final models for the characteristics, linear mixed models were employed to evaluate the influence of environmental variables. These models provided the least square means of the components. Differences between groups for crucial variables were then assessed using Duncan's Test.

2.2.3. Final linear mixed model descriptions

Model for pre-weaning growth traits (BW, WW, ADWG, KR):

$$Y_{ijklmp} = \mu + s_i + t_j + p_k + y_l + d_m + h_p + Z_{1h} + Z_{2p} + e_{ijklmp}$$

μ : Intercept

s_i : Fixed effects of sex

t_j : Fixed effects of birth type (2 levels)

p_k : Fixed effects of period (3 levels)

y_l : Fixed effects of birth year (6 levels)

d_m : Fixed effects of dam age (6 levels)

h_p : Fixed effects of herd size (3 levels)

Z_{1h} : Random herd effects

Z_{2p} : Maternal permanent environmental effects

e_{ijklmp} : Residual error of observations in the model

Model for wool quality traits (WD and WL):

$$Y_{ij} = \mu + y_i + a_j + Z_{1h} + Z_{2p} + e_{ij}$$

μ : Intercept

y_i : Fixed effects of sample take a year (3 levels)

a_j : Fixed effects of age (4 levels)

Z_{1h} : Random herd effects

Z_{2p} : Maternal permanent environmental effects

e_{ij} : Residual error of observations in the model

These models were designed to comprehensively capture the intricate relationships between the dependent variables and the specified fixed and random effects.

3. Results and Discussion

3.1. Birth Weight

Upon scrutinizing the influence of fixed factors, we observed that all factors affecting birth weight were statistically significant, as illustrated in Table 3. Analyzing the results derived from least squares means,

male lambs exhibited a birth weight of 4.06 ± 0.01 kg, while female lambs showed 3.88 ± 0.01 kg. Furthermore, our study revealed a significant disparity in birth weight between male and female groups. In comparison with the findings of Sezenler et al. (2013), our study reported higher birth weights for both male and female lambs, whereas studies by Aktaş et al. (2015, 2016) and Ceyhan et al. (2009) indicated lower weights. Considering our results alongside those of other studies, a consistent trend emerged, indicating that male lambs generally have higher birth weights than females (Ceyhan et al., 2009; Aktaş et al., 2015, 2016; Ceyhan et al., 2009; Koyuncu and Uzun, 2009; Sezenler et al., 2013). Variations in birth weight among studies may be attributed to the effects of pregnancy care and nutrition (Sen et al., 2016; Koyuncu et al., 2017).

In our study, the birth weight of single and twin lambs was reported as 3.99 ± 0.01 kg and 3.95 ± 0.01 kg, respectively (Table 3). The difference in birth weights between single and twin lambs was found to be significant ($P < 0.001$). Our study indicated a minimal difference, favouring single-born lambs by 0.06 grams, representing the lowest disparity among the studies compared to the literature (Aktaş et al., 2016). The smaller gap in birth weight between single and twin lambs in our study may suggest that ewes with twin pregnancies adequately meet the nutritional needs of the lambs during the gestation period.

When exploring the impact of dam age on birth weight, the least squares means revealed birth weights of 3.96 ± 0.01 , 3.99 ± 0.01 , 3.96 ± 0.01 , 3.97 ± 0.01 , 3.95 ± 0.01 , and 3.97 ± 0.01 kg for ewes aged 2, 3, 4, 5, 6, and above 7 years, respectively. A significant difference in birth weights among age groups was observed ($P < 0.05$). Ewes aged 3 years had the highest birth weight, while those aged 6 years had the lowest. In comparison with the study by Aktaş et al. (2015), our results showed higher birth weights across all age groups. While several studies found the impact of ewe age on birth weight to be insignificant (Ceyhan et al., 2009; Koyuncu and Uzun, 2009), our study, in line with Aktaş et al. (2015), suggests its significance.

Analyzing the impact of the birth year on birth weight in our study, the weights for the years 2016 to 2021 were 3.87 ± 0.01 , 3.93 ± 0.01 , 4.01 ± 0.01 , 4.02 ± 0.01 , 3.91 ± 0.01 , and 4.05 ± 0.01 kg, respectively. The highest birth weight was observed in 2021, with a significant difference in birth weights over the years ($P < 0.001$). Similar to other studies (Aktaş et al., 2016; Ceyhan et al., 2009; Sezenler et al., 2013), our results suggest variations in birth weights across years, with factors such as care, nutrition, climate, and herd management playing a role.

In our study, when considering farms based on flock size, birth weights for flock sizes of 0-250, 250-500, and 500 and above were 3.86 ± 0.01 , 4.00 ± 0.01 , and 4.03 ± 0.01 kg, respectively. The highest birth weights were observed in farms with 500 and above in flock size, while the smallest birth weights were in farms with 0-250 in flock size. The

effect of flock size on birth weight was significant ($P<0.001$). Examining the impact of flock size, it can be inferred that larger farms provide better pregnancy care and nutrition, resulting in heavier lambs at birth.

3.2. Weaning Weights

The weaning weights for males and females, as detailed in Table 3, were 27.92 ± 0.06 kg and 26.71 ± 0.07 kg, respectively. The difference between male and female groups was statistically significant in our study ($P<0.001$). Our results differed from those of Ceyhan et al. (2009), who reported lower weaning weights for both male and female lambs, while Koyuncu and Uzun (2009) obtained higher values. Considering the results, it can be inferred that variations in genotype and environment

contribute to differences in weaning weights.

For single-born and twin-born lambs in our study, weaning weights were reported as 27.59 ± 0.06 kg and 27.04 ± 0.07 kg, respectively. The difference between single and twin lambs was significant ($P<0.001$). Similar to Sezenler et al. (2013), our study reported higher weaning weights for both male and female lambs compared to their findings, while Özcan et al. (2002) found lower weaning weights for Turkish Merino lambs on the 90th day. As observed in other studies, single-born lambs tend to have higher weaning weights than twin-born lambs, influenced by differences in pasture conditions, genotype, and nutrition.

Table 3. Least square mean of pre-weaning traits and Kleiber ratio

Fixed Effects	BW (kg)			WW (kg)			ADWG (g)			KR		
	n	LSM ± SE	P-value	n	LSM ± SE	P-value	n	LSM ± SE	P-value	n	LSM ± SE	P-value
Sex			***			***			***			***
Male	19121	4.06 ± 0.01^a		19121	27.92 ± 0.06^a		19121	263.77 ± 0.68^a		19121	21.53 ± 0.02^a	
Female	16223	3.88 ± 0.01^b		16223	26.71 ± 0.07^b		16223	252.68 ± 0.71^b		16223	21.30 ± 0.02^b	
Birth type			***			***			***			***
Single	17657	3.99 ± 0.01^a		17657	27.59 ± 0.06^a		17657	260.92 ± 0.69^a		17657	21.48 ± 0.02^a	
Twin	17687	3.95 ± 0.01^b		17687	27.04 ± 0.07^b		17687	255.53 ± 0.71^b		17687	21.35 ± 0.02^b	
Dam Age (Year)			*			***			***			***
2	7999	3.96 ± 0.01^a		7999	26.94 ± 0.09^a		7999	254.31 ± 0.91^a		7999	21.31 ± 0.03^a	
3	6564	3.99 ± 0.01^b		6564	27.11 ± 0.09^b		6564	255.50 ± 0.96^a		6564	21.29 ± 0.04^a	
4	5832	3.96 ± 0.01^c		5832	27.88 ± 0.10^c		5832	264.33 ± 1.02^b		5832	21.62 ± 0.03^c	
5	4945	3.97 ± 0.01^d		4945	27.34 ± 0.10^d		4945	258.49 ± 1.09^b		4945	21.41 ± 0.04^b	
6	4085	3.95 ± 0.01^e		4085	27.08 ± 0.11^e		4085	256.20 ± 1.16^b		4085	21.35 ± 0.04^b	
7 and upon	5919	3.97 ± 0.01^f		5919	27.55 ± 0.10^f		5919	260.51 ± 1.02^c		5919	21.49 ± 0.03^d	
Birth year			***			***			***			***
2016	4623	3.87 ± 0.01^a		4623	26.75 ± 0.11^a		4623	253.10 ± 1.13^b		4623	21.31 ± 0.04^b	
2017	5833	3.93 ± 0.01^b		5833	26.30 ± 0.10^b		5833	247.66 ± 1.06^b		5833	21.12 ± 0.04^a	
2018	6604	4.01 ± 0.01^c		6604	27.32 ± 0.09^c		6604	258.64 ± 1.01^c		6604	21.48 ± 0.03^c	
2019	6550	4.02 ± 0.01^c		6550	28.26 ± 0.09^d		6550	268.08 ± 0.95^d		6550	21.72 ± 0.03^d	
2020	6327	3.91 ± 0.01^b		6327	27.92 ± 0.09^d		6327	264.00 ± 0.95^d		6327	21.52 ± 0.03^c	
2021	5407	4.05 ± 0.01^d		5407	27.35 ± 0.10^e		5407	257.85 ± 1.04^c		5407	21.33 ± 0.03^b	
Herd Size			***			***			***			***
<250	4607	3.86 ± 0.01^a		4607	26.95 ± 0.10^a		4607	255.55 ± 1.14^b		4607	21.38 ± 0.04^b	
250-500	18868	4.00 ± 0.01^b		18868	25.99 ± 0.06^b		18868	243.56 ± 0.68^b		18868	20.92 ± 0.02^a	
>500	11869	4.03 ± 0.01^b		11869	29.00 ± 0.06^c		11869	275.56 ± 0.68^c		11869	21.94 ± 0.02^c	
Season			***			***			***			***
Winter	24311	4.01 ± 0.01^c		24311	26.78 ± 0.05^a		24311	251.10 ± 0.50^a		24311	21.10 ± 0.02^a	
Spring	7919	3.87 ± 0.01^b		7919	27.69 ± 0.08^b		7919	262.80 ± 0.84^b		7919	21.56 ± 0.03^b	
Autumn	3114	3.96 ± 0.01^b		3114	27.48 ± 0.13^b		3114	260.76 ± 1.34^c		3114	21.58 ± 0.04^c	
Intercept	35344	4.00 ± 0.02		35344	28.55 ± 0.17		35344	271.78 ± 1.82		35344	21.92 ± 0.06	

Examining the impact of dam age on weaning weight, the least squares means indicated weaning weights of 26.94 ± 0.09 , 27.11 ± 0.19 , 27.88 ± 0.10 , 27.34 ± 0.10 , 27.08 ± 0.11 , and 27.55 ± 0.10 kg for ewes aged 2, 3, 4, 5, 6, and above 7 years, respectively. Similar to birth weight, the highest weaning weight was observed in lambs born to 4-year-old ewes. Our results, when compared with Ceyhan et al. (2009), indicated higher weaning weights for all age groups, except for lambs born to 2-year-old ewes. In contrast, Koyuncu and Uzun (2009) reported

lower weaning weights for Karacabey and Merino lambs. The significant differences among age groups highlight the impact of ewe age on weaning weight ($P<0.001$). The least-square mean averages of weaning weights according to birth years are presented in Table 3. Accordingly, the weaning weights for the years 2016 to 2021 were 26.75 ± 0.11 , 26.30 ± 0.10 , 27.32 ± 0.09 , 28.26 ± 0.09 , 27.92 ± 0.09 , and 27.35 ± 0.10 kg, respectively. The lowest weaning weight was observed in 2017, while the highest was in 2019. Significant differences in

weaning weights among birth years were observed ($P<0.001$). Consistent with other studies (Aktaş et al., 2016; Ceyhan et al., 2009; Sezenler et al., 2013), our results indicate variations in weaning weights across years.

In our study, the weaning weights for flock sizes of 0-250, 250-500, and 500 and above were determined as 26.95 ± 0.10 , 25.99 ± 0.06 , and 29.00 ± 0.06 kg, respectively. Notably, farms with 500 or more sheep exhibited the highest weaning weights. These findings imply that larger farms might employ more professional care and nutrition practices compared to smaller and medium-sized counterparts. The impact of flock size on weaning weight was significant in our study ($P<0.001$).

According to the least squares means, weaning weights for Winter, Spring, and Autumn were 26.78 ± 0.05 , 27.69 ± 0.08 , and 27.48 ± 0.13 kg, respectively. A significant difference in weaning weights among seasonal groups was observed ($P<0.001$). Yilmaz et al. (2007) reported higher weaning weights for lambs born in Winter in their study on Norduz lambs. In a study on Ivesi lambs investigating development based on birth month, Gül et al. (2020) found that lambs born in October had higher weaning weights compared to other months. Genotypic and environmental factors contribute to these variations.

3.3. Average Daily Weight Gain

As indicated by the least squares means in Table 3, males demonstrated a higher average daily weight gain (ADWG) compared to females, and this gender difference was significant in our study ($P<0.001$). Consistent with other studies, our findings suggest that, depending on genotypic and environmental factors, males tend to experience a higher daily weight gain (Behrem, 2021b). When evaluated based on birth type, a significant difference was found between single-born and twin-born lambs in our study ($P<0.001$). Single-born lambs exhibited a higher ADWG compared to twin-born lambs. Khan et al. (2020) also reported similar results, indicating that single-born lambs tend to have a higher ADWG than twin-born lambs. The observed differences can be attributed to variations in care, nutrition, and genotype.

The highest ADWG, based on the least squares means, was observed in lambs born to 4-year-old ewes. A significant difference in ADWG among ewe age groups was evident ($P<0.001$). In line with Li et al. (2022), who conducted a study on Alpine Merino, our results highlight the importance of ewe age groups in determining ADWG. The impact of ewe age on growth characteristics varies depending on genotypic and environmental factors.

Analyzing ADWG's increase over the years, the highest ADWG was observed in 2019, while the lowest was in 2017. The effect of years on ADWG was deemed significant ($P<0.001$). Considering the impact of climate-dependent pasture efficiency and the increasing costs of feed over the years, the changes in ADWG between years can be explained. When examined based on flock size, the highest ADWG was identified in large-scale farms with a

capacity of 500 or more. The difference among farms was significant ($P<0.001$). Similar to birth weight and weaning weight, the variation in ADWG is influenced by environmental factors. Seasonal analysis of ADWG reveals that ADWG in Spring and Autumn is higher than in Winter. The difference in ADWG among seasons is statistically significant ($P<0.001$). Yilmaz et al. (2007) reported that Norduz lambs born in Winter had higher ADWG compared to those born in Spring.

3.4. Kleiber Ratio

Kleiber Ratio (KR) in our study was determined as 21.53 ± 0.02 for male lambs and 21.30 ± 0.02 for females, with a significant difference between the two groups ($P<0.001$). In line with studies conducted by Mahala et al. (2020) and Sofla et al. (2011), gender appeared to have a notable impact, with males exhibiting higher ratios than females. However, Behrem (2021a) reported the opposite trend in a study on the Akkaraman breed, where females displayed higher ratios. These variations could be attributed to genotypic and environmental factors.

According to birth type, KR for single-born and twin-born lambs was 21.48 ± 0.02 and 21.35 ± 0.02 , respectively, as seen in Table 3. It was observed that single-born lambs had slightly higher ratios than twins, and the difference between the groups was significant ($P<0.01$). Comparing our results with Sofla et al. (2011), the ratios for single-born lambs were similar but lower for twins in our study. Regarding KR results based on dam age, the highest ratio was observed in lambs born to 4-year-old ewes, while the lowest was in those born to 2-year-old ewes. The difference among dam age groups was significant ($P<0.001$). Variations in feeding conditions due to the age of the ewes could account for the observed differences among groups.

Based on the least squares means, the highest KR and weaning weight in birth years were similarly found in 2019 in our study. The impact of birth year groups on the KR ratio was significant ($P<0.001$). Regarding flock size, the highest KR ratio was observed in farms with 500 or more sheep. The difference in KR ratios among flock size groups was significant ($P<0.001$). Analyzing KR based on seasons revealed that lambs born in Autumn had higher ratios compared to other seasons, and the difference among seasonal groups was significant in our study ($P<0.001$).

3.5. Fleece Traits

This study investigated the impact of age on the fleece diameter (μ) and length (mm) of Central Anatolian Merino sheep. The least-square means (LS) for sheep across different age groups and years are presented in Table 4. Notably, lambs exhibited the finest fleece, with the diameter progressively increasing from lamb to 36 months and older, reaching 22.49μ , 25.28μ , 25.42μ , and 25.44μ , respectively. The differences among age groups were statistically significant ($P<0.001$), emphasizing the age-dependent nature of fleece characteristics, particularly in lambs.

Table 4. Least square mean of wool quality traits.

Fixed Effects	Diameter(μ)			Length(mm)		
	n	LSM \pm SE	P-value	n	LSM \pm SE	P-value
Year			NS			**
2019	1668	24.54 \pm 0.05		1668	52.03 \pm 0.27 ^b	
2020	1707	24.70 \pm 0.05		1707	50.25 \pm 0.27 ^a	
2021	1434	24.74 \pm 0.05		1434	52.22 \pm 0.29 ^b	
Age			***			***
0-6 Month	1216	22.49 \pm 0.06 ^a		1216	25.45 \pm 0.32 ^a	
12-24 month	1168	25.28 \pm 0.06 ^b		1168	65.42 \pm 0.32 ^d	
24-36 month	1177	25.42 \pm 0.06 ^b		1177	58.06 \pm 0.32 ^c	
36 and upon month	1248	25.44 \pm 0.06 ^b		1248	57.06 \pm 0.31 ^b	
Intercept	4809	25.52 \pm 0.07		4809	57.78 \pm 0.40	

Comparing these findings across years, the differences between age groups were found to be insignificant, except for lambs. The fleece diameter in Merino sheep typically falls within the range of 18 to 23, meeting the fine standards required by the textile industry. Several studies on the same breed reported fleece diameters of 24.1 μ to 25.9 μ , 28.67 μ , 23.5 μ to 20.6 μ , 20.6 μ to 26.4 μ , and 22.88 μ (Atav et al., 2020; Behrem et al., 2022; Sönmez, 1959), showcasing variations that could be attributed to factors such as genetics and nutrition. A noteworthy observation from this study and others is that fleece quality tends to be age-dependent, peaking in quality up to 3 to 4 years and subsequently declining. The physical condition of the sheep, especially the metabolic changes in primary and secondary follicles as they mature, plays a pivotal role in determining fleece quality. These changes can result in variances in fleece quantity and quality, leading to a decline in fleece quality with advancing age (Behrem and Gül, 2022). Beyond fineness, fleece length is another crucial factor in the textile industry. Results from this study revealed significant differences in fibre length among age groups ($P < 0.001$). Specifically, the 12-24 age groups displayed the longest fibres (65.42 mm), while lambs had the shortest fibres (25.45 mm). The discrepancy in lambs' fiber structure is attributed to the collection of samples at the age of 0-6 months. Yearlings, on the other hand, exhibited longer fibres due to the first shearing occurring at the age of 15-18 months. Comparative studies showed similar results of 28.1 mm, 64.6 mm, and 83.6 mm for lambs, yearlings, and 2.5-year-olds, respectively (Behrem and Gül, 2022). It's noteworthy that the findings in this study deviate from the 9-12 cm length values reported in a Central Anatolian Merino study and the 7.9 cm fibre length observed in a study on Anatolian Merinos (Sertkaya and ÖZTÜRK, 2022). This variability is influenced by factors such as shear number, genetics, and nutrition. Ideally, fleece for the textile industry should have a length of less than 150 mm (Scobie et al., 2015). The observed significant differences when comparing age groups by years emphasize the importance of considering these factors in understanding fleece characteristics in Central Anatolian Merino sheep ($P < 0.01$).

4. Conclusions

The significant influence of environmental factors on pre-weaning growth traits and fleece characteristics highlights their pivotal role in shaping the economic attributes of the Central Anatolian Merino sheep breed. Harnessing and optimizing these environmental factors have the potential to yield positive impacts on productivity concerning these traits. This study underscores the crucial need to consider and prioritize environmental factors when integrating the Central Anatolian Merino breed into selection programs. The findings from this research provide invaluable insights for improving productivity and ensuring long-term sustainability, particularly in the realm of growth characteristics specific to the Central Anatolian Merino breed. The resilience of Central Anatolian Merino to arid climates and challenging pasture conditions underscores its significance. Increased acknowledgement and further exploration of its genetic attributes, especially regarding growth, wool quality traits, and adaptation characteristics, are poised to make substantial contributions to Türkiye's overall development. This research lays the foundation for a more comprehensive understanding of the breed's potential, paving the way for informed decisions in breeding and husbandry practices, ultimately contributing to the breed's continued success and its role in Türkiye's agricultural advancement.

Author Contributions

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

	Y.A.	S.B.	M.K	S.T.D.	Ç.Y.
C	20	20	20	20	20
D	100				
S		50	50		
DCP		20		40	40
DAI	25	25	25	20	05
L	20	20	20	20	20
W	20	20	20	20	20
CR	20	20	20	20	20
SR	20	20	20	20	20
PM	20	20	20	20	20
FA	20	20	20	20	20

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The measurements of the study were conducted under the supervision of the Animal Experiments Local Ethics Committee of the International Center for Livestock Research and Training (approval date: May 15, 2023, protocol code: 2023/05).

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