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NOVEL PHYTOGENICS' IMPACT ON WEANED PIGS' GROWTH PERFORMANCE, HEAMATOLOGY AND SERUM BIOCHEMICAL INDICATORS

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Abstract: This experiment was carried out to investigate a novel phytogenics' (PCCPR) an acronym for (peppermint, celery, coriander, parsley and rosemary leaf meal mixture) impact on weaned pigs' performance, heamato-biochemical indicators. A total of forty cross bred weaned male pigs (Large white × Landrace) with an initial body weight of 7.33 ± 0.38 weaned at 28 days of age were individually housed in an open sided pen were randomly divided into four treatment group of six pigs each. Basal diet were adequate in all nutrients (NRC, 2012). The animals were fed as follows; basal diet with antibiotics (neomycin – 1.5 g/kg) in treatment one, treatment two, three and four were fed basal diet supplemented with PCCPR at 5 g, 10 g and 15 g/kg respectively. The experiment lasted for two months and all necessary management practices were observed. Average daily weight gain and average daily feed intake were similar in diet three and four compared to the other groups ($P<0.05$). Dietary supplementation of PCCPR resulted in a numerical increase in pack cell volume, haemoglobin and red blood cell, white blood cell and its differentials (monocytes, eosinophils, basophils and leucocytes) and a remarkable improvement in mean corpuscular volume mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations. All the serum biochemical parameters were significantly affected by the treatments ($P<0.05$). However, all values were within the normal physiological range for healthy pigs. It was concluded that the use of PCCPR up to 15 g/kg could boost swine productivity at weaned stage without causing any negative impact on the health status of animals.

Keywords: Phytogenics, Phytochemicals, Haematology, Food safety, Swine, Management

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1. Introduction

Interest in pig nutrition and the widespread use of antibiotic-free feeding systems has been sparked by public outrage over possible risks associated with antibiotic resistance to the health of humans. As a result, feed additives that can be employed as in-feed antibiotic substitutes in pig feeding regimens have been developed (Caroline, 2019). Due to their wide variety of efficacies and their impacts on sustainability and safety, phytogenics are one of the most important substitutes for antibiotics because they have an exciting potential in animal feeding (Jan et al., 2016). Phytogenic feed additions include a variety of plants, including herbs, spices, and essential oils obtained from plants (Luis, 2012; Singh et al., 2022).

Due to the substantial amount of phytochemicals in them, they are well known for having good benefits on animals in the form of flavoring, antioxidant, anti-inflammatory, antiviral, antifungal, and antibacterial qualities (Manu, 2006; Alagbe et al., 2022a). The variances in efficiency between phytogenic feed additives are explained by the chemical makeup of these additives, which highlights some variations due to their ingredients and other influencing factors like climate, location, harvest stage, and storage conditions (Jan et al., 2016). Additionally, several natural plant bioactives can have a growth-promoting effect by favorably affecting the morphology and physiology of the gastrointestinal tract and most likely by either stimulating or inhibiting a specific metabolic pathway (Sandra, 2020).

The effects of plant chemicals (phytochemicals) directly on gut tissues and digestive enzyme secretions or indirectly through stimulatory or inhibitory effects on microbial populations, immune modulation, anti-inflammatory and antioxidant effects have been observed may affect nutrient digestion and absorption (Chris, 2010). The release of brush border enzymes and pancreatic enzymes can both be favourably influenced by mixtures of various phytogenic feed additives (Manu, 2018). Trypsin, an enzyme involved in breaking down proteins, increased by 13% when phytogenics (spices and herbs) were added to the diet of monogastric animals in vivo. Amylase, which breaks down starch, sucrase, and maltase, which breaks down the disaccharide maltose, also showed increases in their respective carbohydrate-digesting capacities (Manu, 2006).

With all of these advantages of phytogenic feed additives eliminate the risks that conventional antibiotics offer to animal health (antimicrobial resistance), and harmful residues in animal products. To avoid toxicity and improve food safety, it is necessary to investigate the usage of additional medicinal plants with pharmacological qualities and perhaps conduct study on their tolerable levels. This experiment was designed to examine a novel phytogenics' impact on weaned pigs' growth performance, heamatology and serum biochemical indicators.

2. Materials and methods

2.1. Location of the Test

The research was conducted at livestock department of Sumitra Research Institute in Gujarat, India, which is situated at 23° 13' N and 72° 41' E and has a 1600 km coastline (Bose Ashish, 1991). The study was carried out between January to March, 2022.

2.2. Gathering and Preparation of Phytogenic Feed Additive

Leaves of Rosemary (*Rosmarius officinalis*), peppermint (*Mentha piperita*), celery (*Apium graveolens*), coriander (*Coriandrum sativum*) and parsley (*Petroselinum crispum*) were harvested from Sumitra Research Institute gardens in Gujarat. The leaves were identified and authenticated by a certified taxonomist, washed with running tap water separately and the placed in different plastic sieve for the water in each leaf to drain for 10 minutes. Afterwards, they were shade dried separately under room temperature for 16 days until each leaf attain a constant weight. Each leaf is powdered using an electric blender (Kenwood 500 Watts commercial high speed blender, Japan) and stored in a labeled zip lock bags stored in the refrigerator (Haier Thermocool, Model: HRF-95EX, Nigeria) at a temperature of 4 degrees Celsius. 200 grams of each powdered leaf sample was transferred into another electric blender and agitated for 10 minutes to allow a homogenous mixture to form a new phytogenic feed additive (PCCPR) an abbreviation for peppermint, celery, coriander, parsley and rosemary leaf. This was thereafter taken to the laboratory for additional testing. The following standard laboratory techniques were used to determine the phyto-constituents in PCCPR.

2.3. Chemicals and Reagents for Analysis

Aluminium chloride, Sodium hydroxide, Sodium carbonate solution, concentrated sulfuric acid, Potassium hexacyanoferrate solution, acetic acid solution, ethanol, vanillin solution, sodium nitrate.

2.4. Equipment Used During Analysis

YSTE – UV5600 UV/VIS Spectrophotometer which uses imported flanged deuterium lamp with low stray light and a rigid die-cast aluminum base as its optical mount. It is capable of setting up various standard curves. It has the following technical specifications; wave length range (190 – 1100 nm), band width (2nm), wavelength accuracy (± 0.8 nm), wavelength repeatability (0.3nm), wavelength setting: auto, photometric accuracy (± 0.3 % T), photometric repeatability (± 0.02 A/h), stray light (≥ 0.05 % T), detector (silicon photodiode).

2.5. Estimation of Flavonoids

Using catechin as a reference, the total flavonoid content was calculated using the aluminium chloride technique. After adding 0.1 mL of aluminum chloride and 0.2 mL of 5% sodium nitrite, 0.5 grams of PCCPR was added. 2 mL of 1 M sodium hydroxide was added to the reaction mixture after the mixture had been incubated for 6 minutes at room temperature. Immediately, 10 mL of distilled water were added to the final volume. Using a spectrophotometer, the reaction mixture's absorbance at 410 nm was evaluated in comparison to a blank.

2.6. Total Phenolic Content

The Folin-reagent Ciocalteu's was used to determine the total phenolic contents in PCCPR. In the method, 0.5 grams of PCCPR and 0.4 mL of 1:10 v/v diluted FCR were combined. 4 mL of sodium carbonate solution was added after 5 minutes. The tubes were filled to their final content with 10 mL of distilled water and left to stand at room temperature for 90 minutes. A spectrophotometer was used to test the sample's absorbance at 850 nm in comparison to the blank. The phenolic content of the oil was expressed as milligrams of catechol per dry gram of dry weight, and a calibration curve was created using catechol solution as the standard with the standard graph given by and the total phenolic content of the sample was reported as milligrams of catechol per dry gram of dry weight (Otles and Yalcin, 2012).

2.7. Estimation of Saponins

Using a vanillin and concentrated sulfuric acid colorimetric technique, saponin was quantified. 0.4 milliliters of 77% sulfuric acid, 0.5 milliliters of freshly made vanillin solution, and 0.2 milliliters of PCCPR were combined. The mixture was allowed to cool to room temperature before being heated in a water bath for 15 minutes at 60 degrees centigrade. A spectrophotometer was used to detect the absorbance at 545 nm.

2.8. Estimation of Total Steroid Content

In accordance with reports by Madhu *et al*. (2016), 0.5 grams of PCCPR was added to a 10 mL volumetric flask. Potassium hexacyanoferrate solution (0.5% w/v, 0.5% w/v, 2 mL) was added after that. Prior to getting diluted with distilled water to the right concentration, the substance was heated for 20 minutes at 40-50 degrees centigrade in a steam bath with regular shaking. The absorbance was calculated at 380 nm and compared to a reagent blank.

2.8. Alkaloids Complex

Using the gravimetric technique, the alkaloids content of PCCPR was determined (Adeniyi *et al*., 2009). Alkaloids were precipitated by mixing 20 mL of acetic acid solution in ethanol (10% w/v) with 0.5 grams of PCCPR and placing the mixture on a water bath. The alkaloids were then precipitated by adding drops of extremely concentrated ammonium hydroxide. After the precipitate reached a constant weight, it was transferred to

desiccators and reweighed.

2.9. Total Tannins Estimation

The Folin-Ciocalteau technique was employed to determine the total tannin concentration (Biswas et al., 2020). 0.5 mL of metaphosphoric acid, 1.5 mL of 90 % ethanol, 1.5 mL of 1.0 mol/mL Na_2CO_3, and 1.5 mL Folin-Ciocalteau were added to 1.0 grams of PCCPR to dilute it (100 mL). The combination was thoroughly blended and then let to cool for 20 minutes at room temperature. Then, using a spectrophotometer, the absorbance of the standard curve and PCCPR were compared to a blank at 880 nm.

2.10. Mineral Composition of PCCPR (novel phytogenics)

Composition of calcium, phosphorus, potassium, magnesium, manganese, zinc, iron, sodium, copper, chromium, nickel and boron were analyzed using trace series atomic absorption spectrometer (Model HD-A11200P, USA). The equipment has the following specifications; wave length (185 – 900nm), wavelength scan series (300 nm/min), grating (1800 lines/min) with automatic gas control with auto ignition, optimization and change over, heating (transversely heated graphite tube up to 3800 K/s heating rate, sensitivity (2 mg/L Cu: Abs ≥ 0.4, RSD < 0.5 %), standard universal auto-sampler for F/GF and VG random access and 8-lamp 2D motorized array with automatic lamp selection, positioning and alignment.

2.11. Experimental Design and Livestock Management

A total of forty cross bred weaned male pigs (Large white × Landrace) with an initial body weight of 7.33 ± 0.38 weaned at 28 days of age were procured from a reputable farm in Gujarat, India and were individually housed in an open-sided pens with standard dimensions (2.0 × 1.5 × 1.2 m). Animals were transported to the experimental site in the early hours of the morning and given a mixture of water and glucose (10 grams of glucose to 5 liters of water) on arrival to the teaching and research farm. Before the start of the experiment, pigs were placed on two weeks acclimatization period where they were prophylactically treated with antibiotics (Oxytrox L.A) administered subcutaneously on each animal according to the manufacturers recommendation and ivermectin against external and internal parasites (0.1 mL per kilogram of animal). After the adjustment period, the pigs were balanced for their weight to ensure that the initial weight of each group were similar and randomly divided into four treatment group of ten pigs each with one animal per replicate in a completely randomized design. The experiment lasted for 2 months and all necessary management practices were observed according to the ethical guidelines outlined by the ethical guidelines for the use of animal in research.

2.12. Experimental Diet and Design

Basal diet was adequate in all nutrients according to National Research Council (2012) (Table 1). Weaners in treatment one was given basal diet with antibiotics growth promoter (neomycin at 1.5 g/kilogram), and those in treatment two through four were fed basal diet supplemented with 10 grams, 20 grams, and 30 grams of PCCPR respectively.

Table 1. Experimental diets' total composition

Components	Amount (kilogram)
Yellow maize	40.00
Dried cassava peel	4.20
Wheat offal	6.50
Palm kernel cake	21.00
Soya meal	12.00
Groundnut cake	10.00
Fish meal (Imported)	1.00
Bone meal	3.00
Limestone	1.50
Lysine	0.15
Methionine	0.15
*Mineral/Vitamin Premix	0.25
Salt	0.30
Total	100.05
Determined analysis (%)	
Crude protein	19.19
Crude fibre	5.97
Ether extract	3.88
Calcium	1.04
Phosphorus	0.63
Lysine	1.00
Methionine +Cysteine	0.79
Metabolizable energy (kcal/kg)	2865.1

*Mineral/Vitamin premix supplied per kg diet: - vit A, 9,000 I.U; vit E, 8.91 mg; vit D3, 2500I.U, vit K, 3.2mg; vit B2, 5.0mg; Niacin, 40 mg; vit B12, 25 mg; choline chloride, 100 mg; Mn, 5.0 mg; Zn, 35.1mg; Cu, 2.0g; folic acid, 2.5mg; Fe, 5.8g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg (starter's mash)

2.13. Performance Characteristics

The amount of feed ingested was determined by subtracting the feed refused from the feed supplied. The feed conversion ratio, or the quantity of feed necessary to produce one unit of gain, was calculated as the ratio of average feed intake to average body weight growth. The weight gain was calculated using the difference between the initial and final body weights. The average daily body weight was determined by dividing the amount of weight gained for each treatment by the number of trial days.

2.14. Measurement of Blood Sample

Six pigs per treatment were chosen for hemato-biochemical evaluation on the eighth weekday of the study's duration. 4 ml of blood was drawn from the sampled birds using 20 to 100 sterilized metallic needles to take blood from a culinary vein. Two mL were put into vials that had been treated with ethylene diamine tetra acetate for hematological testing, and the other two mL were utilized for serum testing. Using an automated Sysmex analyzer (model XN-3100, China), hamatological variables were measured. For the serum indices, the

remaining 2 mL of blood were drawn and placed in bottles without anticoagulant. Technical details of the apparatus include optical flow (20 L quartz), reaction volume (300–800 L), and photometric range (– 0.10–3.00 abs), and filters (6 interference filters: 380–425–516–508, 600–700 nm).

2.15. Statistical Analysis

Using Statistical Package of Social Sciences (SPSS version 23.0), one way analysis of variance (ANOVA) was used to examine all of the data. Using Duncan's multiple range test of the same package, means were sorted.

3. Results

3.1. Mineral Composition of PCCPR (Novel phytogenic mixture)

Phyto-constituents of PCCPR (novel phytogenic mixture) reveals the major compounds in the sample. Phenols had the highest concentration (2107.18 mg/g) closely followed by flavonoids (1093.86 mg/gram), alkaloids (485.91 mg/gram), tannins (282.10 mg/gram), saponins (112.75 mg/gram), steroids (100.14 mg/gram) and oxalates (12.30 mg/gram) correspondingly (Table 2).^

Table 2. Phyto-constituents of PCCPR (Novel phytogenic mixture)

Constituents	Concentration (milligram /gram)
Flavonoids	1093.86
Alkaloids	485.91
Tannins	282.10
Steroids	100.14
Phenols	2107.18
Saponins	112.75
Oxalates	12.30

Mineral composition of PCCPR (novel phytogenic mixture) (Table 3). Calcium (1712.81 milligram /100 gram), phosphorus (973.68 milligram /100 gram), potassium (877.82 milligram /100 gram), magnesium (509.57 milligram /100 gram), manganese (100.40 milligram /100 gram), zinc (209.38 milligram /100 gram), iron (95.17 mg/100g), sodium (315.60 mg/100g), selenium (80.05 mg/100g), chromium (0.03 milligram /100 gram) and nickel (0.41 milligram /100 gram). Calcium had the highest concentration while chromium had the least value.

3.2. Effects of Supplemented Diet with PCCPR (Novel Phytogenic Mixture) on Growth Performance of Weaned Pigs

Effects of supplemented diet with PCCPR (Novel phytogenic mixture) on growth performance of weaned pigs (Table 4). Initial body weight varies from 7.30 – 7.34 kg, final body weight (19.18 – 25.17 kg), weight gain (11.85 – 17.87 kg), average daily weight gain (0.20 – 0.30 kg), total feed intake (37.14 – 42.05 kg), average daily feed intake (0.60 – 0.75 kg) and feed conversion ratio (2.34 – 3.30). There was effect of treatments on in weight gain, average daily feed intake and feed conversion ratio ($P<0.05$).

Table 3. Mineral composition of PCCPR (Novel phytogenic mixture)

Constituents	Concentrations (milligram /100 gram)
Calcium (Ca)	1712.81
Phosphorus (P)	973.68
Potassium (K)	877.82
Magnesium (Mg)	509.57
Manganese (Mn)	100.40
Zinc (Zn)	209.38
Iron (Fe)	95.17
Sodium (Na)	315.60
Copper (Cu)	87.10
Selenium (Se)	80.05
Chromium (Cr)	0.03
Nickel (Ni)	0.41

Table 4. Effects of supplemented diet with PCCPR (Novel phytogenic mixture) on growth performance of weaned pigs

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Initial body weight (kg /pig)	7.33	7.32	7.34	7.30	0.21
Final body weight (kg /pig)	19.18^{c}	21.40^{b}	25.00^{a}	25.17^{a}	0.89
Weight gain (kg /pig)	11.85^{c}	14.08^{b}	17.66^{a}	17.87^{a}	0.75
Av. daily weight gain (kg/pig)	0.20^{c}	0.25^{b}	0.32^{a}	0.32^{a}	0.02
Total feed intake (kg/pig)	37.14^{b}	40.80^{a}	41.72^{a}	42.05^{a}	1.98
Av. daily feed intake (kg/pig)	0.66^{b}	0.73^{a}	0.74^{a}	0.75^{a}	0.01
Feed conversion ratio	3.30^{a}	2.92^{b}	2.31^{b}	2.34^{b}	0.02
Mortality (%)	-	-	-	-	-

Average in rows having various characters vary markedly ($p< 0.05$); Initial body weight, Final body Weight gain, Average daily weight gain, Average daily feed intake; diet 1: basal diet + 1.5 g/kg Neomycin; PCCPR was added to basal diet in the following: diet 2: 5 g/kg; diet 3: 10 g/kg ; diet 4: 15 g/kg respectively. SEM: standard error of mean.

3.3. Effects of Supplemented Diet with PCCPR (Novel Phytogenic Mixture) on Hematological Indices of Weaned Pigs

Effects of supplemented diet with PCCPR (Novel phytogenic mixture) on hematological indices of weaned pigs (Table 5). Pack cell volume values varies from (29.93 – 34.15 percent), haemoglobin (10.71 to 13.92 gram/dL), red blood cell [4.72 to 8.86 ($x10^6$)], mean corpuscular volume (50.80 to 68.01 fl), mean corpuscular haemoglobin (18.50 to 21.00 pg), mean corpuscular haemoglobin concentration (29.81 to 34.02 g/dL), white blood cell [7.64 to 12.02 ($x10^3$)], monocytes (3.00 to 6.10 percent), lymphocytes (47.80 to 61.27 percent), basophils (1.02 to 1.16 percent) and eosinophil's (0.68 to 0.81 percent). Pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values follow similar pattern as the parameters were highest in diet two through four and lowest in diet one ($P<0.05$). White blood cell, monocytes and lymphocytes values were maximum at diet three and four relative to other treatments ($P<0.05$). Basophils and eosinophils values were not affected by the treatments ($P>0.05$).

Table 5. Effects of supplemented diet with PCCPR (Novel phytogenic mixture) on hematological indices of weaned pigs

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Pack cell volume (%)	29.93^b	33.21^a	34.03^a	34.15^a	0.11
Haemoglobin (g/dL)	10.71^b	12.19^a	13.80^a	13.92^a	0.06
Red blood cell ($x10^6$)	4.72^c	8.10^a	8.51^a	8.86^a	0.02
Mean corpuscular volume (fl)	50.80^b	66.00^a	67.12^a	68.01^a	0.27
Mean corpuscular heamoglobin (pg)	18.50^b	20.80^a	20.40^a	21.00^a	0.09
Mean corpuscular heamoglobin conc. (g/dL)	29.81^b	32.75^a	33.64^a	34.02^a	0.15
White blood cell ($x10^3$)	7.64^c	9.96^b	12.40^a	12.02^a	0.04
Monocytes (%)	3.00^b	3.08^b	5.74^a	6.10^a	0.02
Lymphocytes (%)	47.80^c	50.05^b	60.10^a	61.27^a	0.35
Basophils (%)	1.20	1.02	1.09	1.16	0.03
Eosinophils (%)	0.74	0.68	0.73	0.81	0.02

Values in cells with various characters vary markedly ($P<0.05$); Diet 1: basal diet + 1.5 g/kg Neomycin; PCCPR was added to basal diet in the following: diet 2: 5 g/kg; diet 3: 10 g/kg; diet 4: 15 g/kg respectively. SEM: standard error of mean

3.4. Effects of Supplemented Diet with PCCPR (novel phytogenic mixture) on Serum Biochemical Indices of Weaned Pigs

Table 6 revealed the effects of supplemented diet with PCCPR (novel phytogenic mixture) on serum biochemical indices of weaned pigs. Total protein values varied from (57.18 to 73.25 g/L), globulin (25.10 to 38.01 g/L), albumin (32.08 to 34.24 g/L), urea (3.71 to 5.06 mmol/L), creatinine (68.51 to 73.28 µmol/L), cholesterol (3.06 to 4.02 mmol/L), glucose (2.08 to 3.92 mmol/L), alanine transaminase (30.15 to 45.02 U/L), alanine serum transaminase (42.06 to 61.74 U/L), alanine phosphatase (112.1 to 155.9 U/L), calcium (2.02 to 3.40 mmol/L), phosphorus (1.80 to 2.17 mmol/L), sodium and chloride [98.10 to 120.9 mmol/L; 93.82 to 105.1 mmol/L] respectively. All the values were significantly ($P<0.05$) affected by the treatments.

Table 6. Effects of supplemented diet with PCCPR (Novel phytogenic mixture) on serum biochemical indices of weaned pigs

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Total protein (g/L)	57.18^c	68.04^b	70.16^a	73.25^a	0.56
Globulin (g/L)	25.10^b	36.00^a	37.12^a	38.01^a	0.19
Albumin (g/L)	32.08^b	33.04^b	33.04^b	34.24^a	0.15
Urea (mmol/L)	3.71^c	4.92^b	5.00^a	5.06^a	0.02
Creatinine (µmol/L)	68.51^b	70.83^a	72.19^a	73.28^a	0.55
Cholesterol (mmol/L)	4.02^a	4.00^a	3.00^b	3.06^b	0.01
Glucose (mmol/L)	3.92^a	3.83^a	3.76^a	2.08^b	0.02
Alanine transaminase (U/L)	30.15^b	40.08^a	43.89^a	45.02^a	0.19
Alanine serum transaminase (U/L)	42.06^c	43.72^c	50.70^b	61.74^a	0.42
Alanine phosphatase (U/L)	112.1^c	143.8^b	150.6^a	155.9^a	1.37
Calcium (mmol/L)	2.02^b	2.48^b	3.39^a	3.40^a	0.01
Phosphorus (mmol/L)	1.80^b	1.96^b	2.00^a	2.17^a	0.01
Sodium (mmol/L)	98.10^b	116.8^a	120.2^a	120.9^a	0.38
Chloride (mmol/L)	93.82^b	100.2^a	103.7^a	105.1^a	0.32

Values in cells with various characters vary markedly ($P<0.05$); Diet 1: basal diet + 1.5 g/kg Neomycin; PCCPR was added to basal diet in the following: diet 2: 5 g/kg; diet 3: 10 g/kg; diet 4: 15 g/kg respectively. SEM: standard error of mean.

4. Discussion

The presence of multiple phyto-constituents or phytochemicals in PCCPR gives the sample a higher affinity to function as; antioxidant, anti-fungal, antiviral, anti-helminthic, hepato-protective, immune-modulatory and immune-stimulatory amongst others (Uphadhyay et al., 2010; Igbal et al., 2015). The study's increased flavonoid concentration lends support to PCCPR's anti-inflammatory, immune-stimulating, and antioxidant capabilities. According to Mbaebe et al. (2012), flavonoids exhibits several pharmacological effects and are capable to scavenging free radicals in the body, thus preventing infections. The flavonoid content recorded in this experiment was higher than those recorded for *Hypochaeris radicata* leaves (14.31 mg/g), *Rumex crispus* leaves (130.4 mg/g) and *Mesua ferrea* leaves (140.82 mg/g) by Narender et al. (2012); Jamuna et al. (2012). According to Jamuna et al. (2012), tannins are crucial for both the treatment of inflamed tissues and the prevention of cancer. For example, alkaloids act as agents with analgesics and anti-plasmodic activities whereas saponins operate as antifungal agents (Govindappa et al., 2011). Both alkaloids and saponins are recognized to have ethno-pharmacological benefits (Govindappa et al., 2011).

Minerals are inorganic elements required in animals' feed for adequate development and functioning. Calcium, magnesium and phosphorus values recorded in PCCPR were higher than values reported for *Terminalia sericea* leaf (759.20, 560.70 and 102.1 mg/100g) respectively by Chivandi et al. (2013). Mineral composition of medicinal plants are influenced by age of plant or stage of growth, climatic condition and cultivar within plant species (Oluwafemi et al., 2020). However, PCCPR values recorded were within the NRC (2012) daily requirement for weaned pigs. Potassium are responsible for proper fluid balance, nerve contraction, heart beat regulation and membrane potential (Arinola et al., 2008). Sodium are required for proper fluid and pH balance, nerve transmission and contraction of the muscles (NHWC, 2002). Strong bones, blood pressure regulation and strengthening of the immune system are the role of calcium (Afisu et al., 2016). Copper are needed for the creation of haemoglobin, regulating neuro-transmitters and clean up free radicals (Abu et al., 2016). Zinc is immune boosters, healing of wounds, needed for making protein, blood clotting and genetic materials (NHWC, 2002). Iron is part of haemoglobin which carries oxygen in the body needed for energy metabolism and part of protein (a protein in muscle cells) (Arinola et al., 2008). Selenium and manganese are responsible for the functioning of antioxidant enzymes and metabolism of amino acid, cholesterol and carbohydrates (Alagbe et al., 2023a, Alagbe et al., 2023b). Nickel is responsible for the absorption of iron and other elements in the body (NHWC, 2002).

The results on performance indicated some beneficial effects of the experimental diets on the growth performance of weaned pigs which is reflected in a numerically increased average daily weight gain among animals in diet two (5 g/kg PCCPR), three (10 g/kg PCCPR) and four (15 g/kg PCCPR) and a significant improvement in average daily feed intake and feed conversion ratio among the treatments respectively. The experiment clearly shows that PCCPR has the potential to enhance the growth of animals due its phytogenic constituents. For instance, parsley, peppermint, rosemary, coriander and celery leaves which are the major components of the novel phytogenic material has apiol, menthol, cineole, linalool and borneol as well as phtalides as active compounds with medicinal properties such as; Appetite and digestive stimulant, antiseptic, antioxidant, anti-diarrhoeal, anti-inflammatory amongst others (Luis, 2012). A synergy in these compounds could inhibit the development of pathogenic bacteria, support the proliferation of *lactobacilli spp* as well as improving the secretion of digestive enzymes which in turn positively influence the growth patterns and feed conversion ratio (Singh et al., 2022). Phytogenics have also been reported to support normal liver metabolism and protection of kidney cells against osmotic fluctuations to ensure an outstanding performance in animals (Yang et al., 2015). It can also improve the flavor and palatability of feed as well as its retention time (Singh et al., 2022). The outcome of this research confirms the earlier results by Zhang et al. (2012) when phytogenic consisting of turmeric and clove was fed to weaned pigs at 20 g/kg. Yan et al. (2011) also recorded a positive outcome when *Houttuynia cordata* and *Taraxacum officinale* extract powder was fed to pigs at 15 g/kg.

In situation of nutrient insufficiency and health situation, heamatological data could be used as a point of reference for analysis in animals (Etim et al., 2014). Results of the experiment revealed that the dietary supplementation of PCCPR lead to a numerical elevation in pack cell volume, red blood cell, haemoglobin concentration and a remarkable increase in white blood cell, lymphocytes and monocyte values. Effective distribution of oxygen supply and nutrient round the body will be more prominent among pigs fed diet two through four relative to diet one. Pack cell volume are used for the detection of presence or absence of anemia or polycythemia (Oyawoye and Ogunkunle, 2004). White blood cell and lymphocytes were maximum in diet three and four compared to the other treatments. The outcome helps to strengthen the immune system in these groups. Phytogenic mixture has been reported to exhibit immune-modulatory properties and immune-globulin secretion, thus preventing mortality among animals (Zhou et al., 2013; Olafadehan et al., 2020). Eosinophils and basophils are strong defense mechanisms against parasites and diseases, however, values were not affected by the treatments and are within the normal range specified.

The results on the serum biochemical indices agree that the dietary addition of PCCPR resulted in a significant

increase in total protein (albumin plus globubin). According to Olafadehan et al. (2020), nutrition can greatly influence the total serum protein. Experimental diets were adequate in all nutrients, however, dietary supplementation of PCCPR could stimulate the activities of bile and digestive enzymes (Agubosi et al, 2022; Muritala et al., 2022). This is reflected among pigs fed diet three (10 g/kg PCCPR) and four (15 g/kg PCCPR) compared to the other treatments ($P<0.05$). Urea and creatinine values recorded are within the normal physiological range for pigs. Elevated values are clear sign of renal failure which can lead to death at extreme cases (Alagbe et al., 2022b). Elevated glucose could be triggered during period of stress from starvation or malnutrition, insufficient clean water as well as poor handling during farm operations (Olafadehan et al., 2020). It is important to note that pigs fed on diet three and four had lower values. This could as a result of the active compound in PCCPR especially those with antioxidant properties. Values for cholesterol rose as PCCPR levels improved across the group, this demonstrates unequivocally that PCCPR hypocholesteromic characteristics. As the concentration of PCCPR increased among the group, serum enzyme levels dropped. The existence of harmful substance in the blood causes abnormal readings to be activated (Rafiu et al., 2013). Electrolytes are capable of activating enzymatic activities, helps the cells in the uptake of nutrients and release hormones as well as acting as buffer in the blood (Etim et al., 2013).

5. Conclusion

At the end of the experiment, it was concluded that the dietary inclusion of PCCPR (novel phytogenics) in feed optimizes the performance by competing with harmful gut flora and by stimulating the immune system of the animal and therefore, increasing its resistance to infectious agents. PCCPR may positively impact the secretion of digestive juices and nutrient absorption when included up to 15 g/kg without causing any deleterious effect on the health of the animal.

Author Contributions

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

	A.O.J.
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

Permissions were obtained from the Sumitra Research Institute Ethics Committee (protocol code: 2010/63/EU and date: March 01, 2022).

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Research Article
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EFFECTS OF ORGANIC AND INORGANIC FERTILIZERS ON YIELD AND YIELD COMPONENTS OF BARLEY

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Abstract: This study aimed to assess the impact of various organic and inorganic fertilizers on the yield and yield components of barley varieties. The research was conducted in the greenhouse of the Faculty of Agriculture of Dicle University in 2019-2020. Two barley varieties, Keçiburcu (six rows) and Önder (two rows), and 15 types of organic and inorganic fertilizers were used in this study. All organic fertilizers used in the study were applied at sowing, solid fertilizers were incorporated directly into the soil, and liquid fertilizers were diluted with water and then applied to the soil. Among the fertilizers used in this study, conventional fertilizer (1.48 g/plant) and sheep manure (1.05 g/plant) showed positive effects on grain yield and other traits. As a result, plants benefit from chemical fertilizers in a shorter period because they are absorbed and used more quickly than chemical fertilizers, which are part of traditional agriculture. In addition, organic fertilizers have a positive effect on plant development. For organic barley production, sheep manure is recommended, which yields results similar to those of conventional fertilizers.

Keywords: Barley, Conventional, Fertilizer, Organic, Yield

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1. Introduction

Barley, a member of the Poaceae family, was one of the first crops cultivated. Barley, which is an annual cereal, was initially used more for human consumption, but with the increase in the consumption of wheat and rice, its use as an animal feed and raw material for malt and beer has become more widespread. Barley is the fourth most important cereal in the world after wheat, maize, and rice. While the world barley production is 152 million tons, 5% of barley production is for human consumption, 67% for animal consumption, and 21% for the malting industry (FAO, 2022). Türkiye's barley production is 8.5 million tonnes, of which 86% is for animal feed and 14% for the malting and food industries (TÜİK, 2022).

In Türkiye, barley yield is generally reduced by factors such as decreasing agricultural land, lack of cultural practices, incomplete and incorrect fertilization, and inappropriate variety selection (Şener et al., 2020). Fertilization is an important factor in improving crop quality, reducing crop development time, and improving soil quality (Demirsoy and Aydın, 2020). The use of fertilizers is one of the most important reasons for the 50-75% increase in crop yield. Globally, there is a direct relationship between crop yield and fertilizer use (Polat, 2020). It has been reported in various sources that the unconscious and excessive use of chemical fertilizers in agricultural production from the past to the present has caused the soil organic matter content to decrease over time, which is harmful to soil organisms and human health (Karagöz, 2014; Aydın Can et al., 2019; Bozkurt, 2019). However, the use of organic fertilizers has been reported to increase soil organic carbon and soil fertility, resulting in higher yield trends compared to balanced chemical fertilizers (Zhang et al., 2014; Scaglia et al., 2016).

Organic fertilizers are obtained from different types of animal and plant wastes, such as compost, farm manure, barn manure, and green manure (Demirtaş et al., 2005). According to previous studies, organic fertilizers contain organic components and many beneficial microorganisms, along with plant nutrients (Soba, 2012). Alagöz et al. (2006) investigated the effect of organic matter addition on some chemical and physical properties of soil and reported that leonardite material applied at three different doses had an increasing effect on pH, organic matter, and total N content of soils. The effects of humic acid and different application on plant growth and nutrient uptake have been examined by researchers (Kolsarıcı et al., 2005; Demirkıran and Cengiz, 2010). Yılmaz and Alagöz (2005) investigated the effects of liquid humic acid application on aggregate formation and stability in soils, and reported that the liquid humic acid material used had 0.30% total N, 0.17% organic N, 0.41% CaO, 15% humic and fulvic acid, and 6.5% pH. Arslan (2021) reported that bat manure applied to the soil at different doses showed positive increases in plant height, plant fresh and dry weight, root fresh and dry weight, root length, chlorophyll content, and some microbiological characteristics of the root zone in barley and lentil. Altıntaş et al. (2005) emphasized that

organic and nutrient deficient soils can be made more fertile with the supplementation of bat manure.

Vermicompost, another source of organic fertilizer, facilitates nutrient uptake by plants, has a porous structure, good aeration, high water-holding capacity, and microbial effects (Yılmaz et al., 2017). Chicken manure, such as vermicompost, is an environmentally friendly, economical, and good soil conditioner that makes plants resistant to diseases (Bellitürk, 2016). Another preferred practice to increase the yield and quality of low-fertility soils is the addition of barnyard manure to the soil (Akkaya and Kara, 2018). Barn manure application increases the microbial activity of soil, improves its physical properties, and increases its water-holding capacity (Karayel et al., 2020). In addition, most plant nutrients in barnyard manure are in a water-soluble form that the plant can take up with its roots (Soyergin, 2003). This study aimed to determine the effects of different organic and inorganic fertilizers on the yield and yield components of barley.

2. Materials and Methods

This study was conducted in a greenhouse at the Faculty of Agriculture of Dicle University. The study used 15 organic and inorganic fertilizers and two barley varieties (Keçiburcu-6-row and Önder-2-row). The study was established on 05/12/2019 according to a randomized block design with four replications. The seeds of the varieties were sown in 8-litre pots with four plants in each pot. Table 1 lists the physical and chemical characteristics of the soils used in this study. All fertilizers (solid and liquid) used in this study were applied to pots with sowing. Solid fertilizers were mixed directly into the soil, while liquid fertilizers were diluted with water and applied to the soil. A control group, without fertilizer, was used to compare the fertilizer applied. The organic fertilizers used in this study and their contents are listed in Table 2. The temperature and humidity values under the greenhouse conditions are shown in Figure 1.

Table 1. Some physical and chemical properties of the soil used in the study

Saturation (%)	Salinity (dS/m)	Salt (%, TS 8334)	pH Degree	Lime (%)	Organic Matter (%)	Nitrogen (%)	Phosphorus (ppm)
63.00	0.92	0.04	8.11	11.24	0.71	0.04	4.00
Clay Loam	Without Salt	Without Salt	Light Alkaline	Middle	Low	Low	Low
Potassium (ppm)	Calcium (ppm)	Magnesium (ppm)	Sodium (ppm)	Iron (ppm)	Copper (ppm)	Manganese (ppm)	Zinc (ppm)
314.45	10717.9	471.78	26.65	9.29	1.61	16.5	0.08
High	Very High	Middle	Low	Very High	Middle	Middle	Low

Table 2. Fertilizer sources and contents used in the study

Code	Fertilizer Sources	Advised Dose	Given Fertilizer	Total Nitrogen (%)	Organic Content (%)
FS-1	Conventional Fertilizer (20-20-0)	12 kg/da	3.6 g/pot	20.0	-
FS-2	Commercial Organic Fertilizer-1	100-150 cc/100 lt water	1.5 ml/pot	2.0	30.0
FS-3	Commercial Organic Fertilizer-2	50-60 kg/da	1.2 g/pot	3.0	50.0
FS-4	Commercial Organic Fertilizer-3	50-60 kg/da	1.2 g/pot	7.0	50.0
FS-5	Organic Compost	120-150 kg/da	3 g/pot	2.0	65.0
FS-6	Organic Seed Coating	1000-2000 cc/da	0.004 g/pot	3.0	30.0
FS-7	Raw Leonardite	50-75 kg/da	5 g/pot	1.35	40.0
FS-8	Processed Leonardite	40-60 kg/da	5 g/pot	1.30	40.0
FS-9	Liquid Vermicompost Fertilizer	1000-2000 cc/da	0.004 g/pot	0.80	10.0
FS-10	Liquid Seaweed Fertilizer	2-3 lt/da	0.06 g/pot	0.30	10.0
FS-11	Bat Guano	50-100 kg/da	2 g/pot	5.65	26.4
FS-12	Solid Vermicompost Fertilizer	2000-3000 g/da	2 g/pot	1.50	40.0
FS-13	Farmyard Manure	2 tons	40 g/pot	3.82	61.59
FS-14	Sheep Manure	2 tons	40 g/pot	4.98	68.30
FS-15	Chicken Manure	500 kg/da	10 g/pot	4.09	57.89

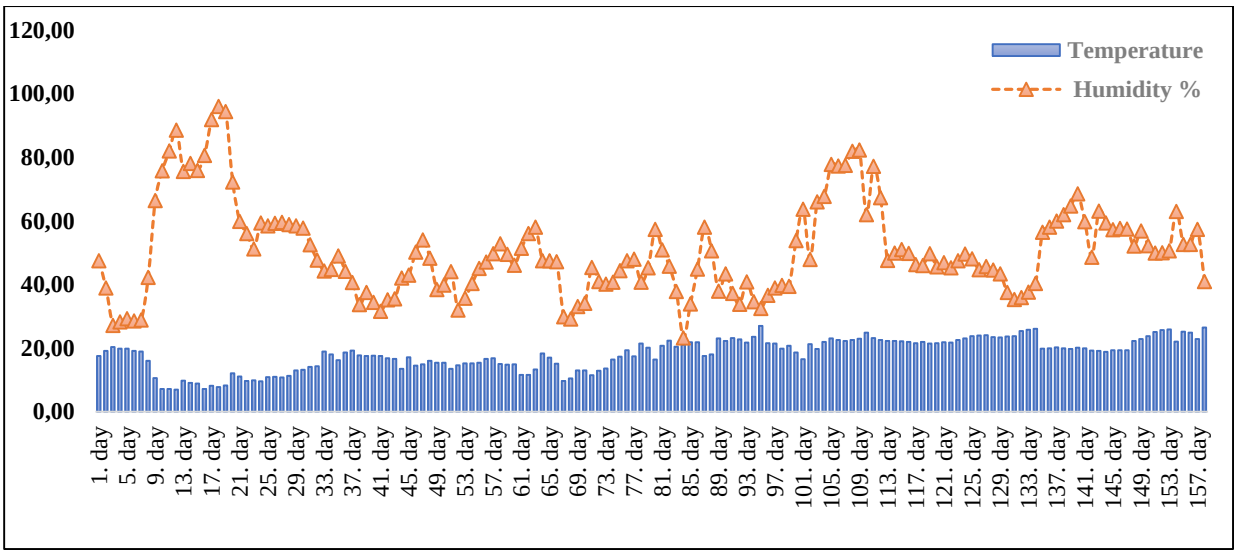

Figure 1. Temperature and humidity values for greenhouse conditions

A drip irrigation system was installed that could be controlled by timer solenoid valves. The traits measured in the study were heading time (days), plant height (cm), maturity time (days), stem diameter (mm), spike length (cm), number of spikelets per spike, number of grains per spike, grain weight per spike, grain yield (g/plant), and biomass (g/plant). The values of the study parameters were analyzed using the JMP Pro 13 statistical package, and statistical differences between the means were determined using the LSD test.

3. Results and Discussion

This study investigated the effects of 15 organic and inorganic fertilizers on the agronomic traits of Keçiburcu and Önder barley varieties grown under greenhouse conditions. The significance status of the traits investigated in the study and the mean values obtained are shown in the tables below.

When the effects of 15 different fertilizers on the heading and maturity times of barley cultivars were studied, no differences were observed between cultivars for maturity and heading time. Sheep manure on the Keçiburcu cultivar resulted in the earliest heading (90.67 days) and maturity (130.33 days) times. Notably, bat manure and processed leonardite delayed the heading and maturity stages of the genotypes (Figure 2-a and 2-b). Previous studies have reported values for days to heading varying from 55.3 to 61.0 days (Bayhan et al., 2022), 111.5 to 112.6 days (Özdemir et al., 2019), and 123.2 to 126.2 days (Akmaz, 2022). Similarly, days to maturity were reported in the range of 90.3 - 93.0 days (Bayhan et al., 2022) and 141.5 - 158.0 days (Akmaz, 2022). The values obtained in this study differ from those of previous studies, probably because of differences in the study conditions and application methods.

There were no significant differences in plant height between the varieties; however, fertilizer application resulted in increased plant height. The highest recorded plant height was obtained with sheep manure (87.00 cm). Among the fertilizer applications, commercial organic fertilizer-3 showed the highest value at 80.33 cm. Notably, processed leonardite, raw leonardite, organic compost, and bat manure did not have a significant effect on plant height (Figure 3-a). Plant height has been found to be affected by various factors such as environmental conditions, soil fertility, sowing density, and cultivar (Akıncı and Yıldırım, 2009; Yaraşır, 2018; Bayhan et al., 2019). Researchers have reported that in cool-climate cereals, increasing fertilizer doses can lead to an increase in plant height (Budaklı et al., 2005; Yang et al., 2008; Kon, 2019), and both organic and inorganic fertilizer applications have been shown to promote greater plant height in barley (Yolcu, 2008; Markoni et al., 2017; Özdemir et al., 2019).

Kiani et al. (2005) found that co-applying organic fertilizer with nitrogen resulted in increased plant height, while farmyard manure resulted in higher plant height values compared to the control group. Çiftçi (2019) found that organic fertilizers increased plant height in barley, with the highest height achieved using vermicompost. Özkan et al. (2021) found that chicken manure significantly increased plant height compared to other organic fertilizers.

The conventional fertilizer (NPK) resulted in a significant increase in stem diameter compared with the other fertilizers. Sheep and chicken manure also affected stem diameter. The mean values ranged from 1.64 to 3.44 mm, with the highest and lowest values observed in the Keçiburcu variety, sheep manure, and raw leonardite (Figure 3-b). Previous studies have reported that the stem diameter values obtained from organic farming are generally lower than those obtained from conventional farming (Bayhan and Yıldırım, 2021; Özkan and Akıncı, 2021). Yolcu (2008) reported stem diameter values ranging from 2.14 to 3.04 mm for barley varieties with farmyard manure application and 2.34 to 2.79 mm for the control group.

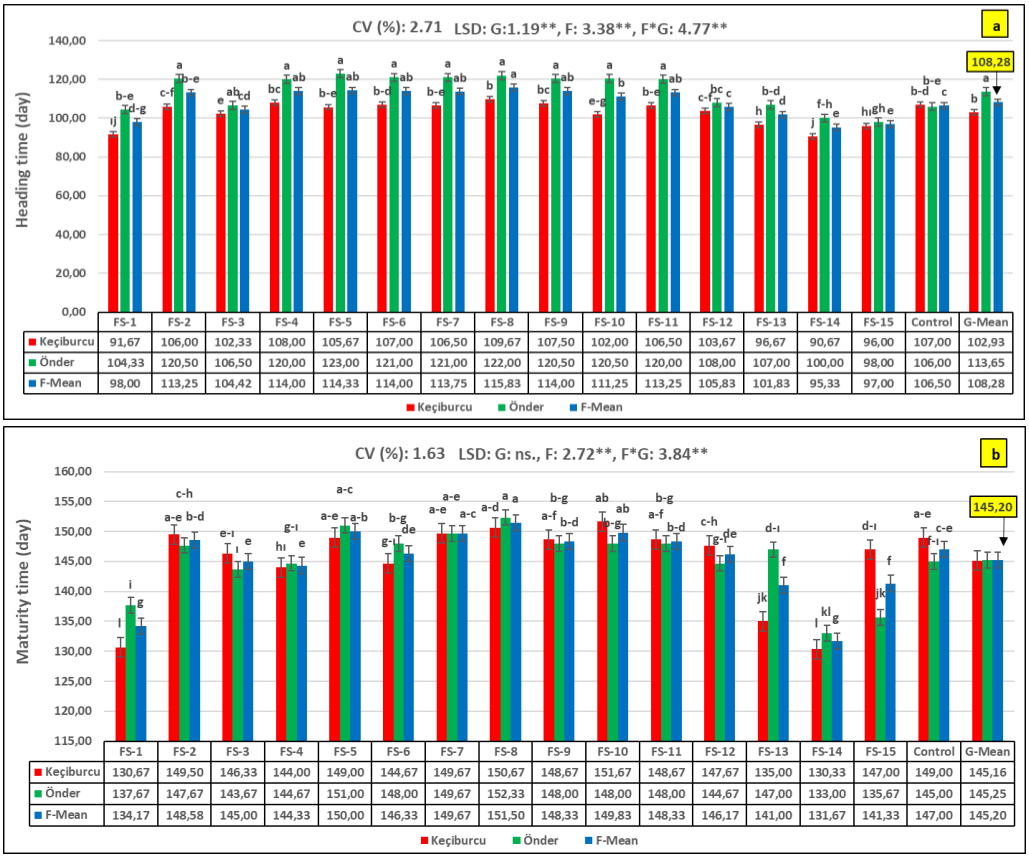

Figure 2. Mean values and groups of heading (a) and maturity (b) times analyzed in the study. ** Significant at P≤0.01, ns= not significant, CV= coefficient of variation, LSD= least significant difference, FS= fertilizer source and G= genotypes, Red= keçiburcu, Green= önder, Blue= fertilizer mean.

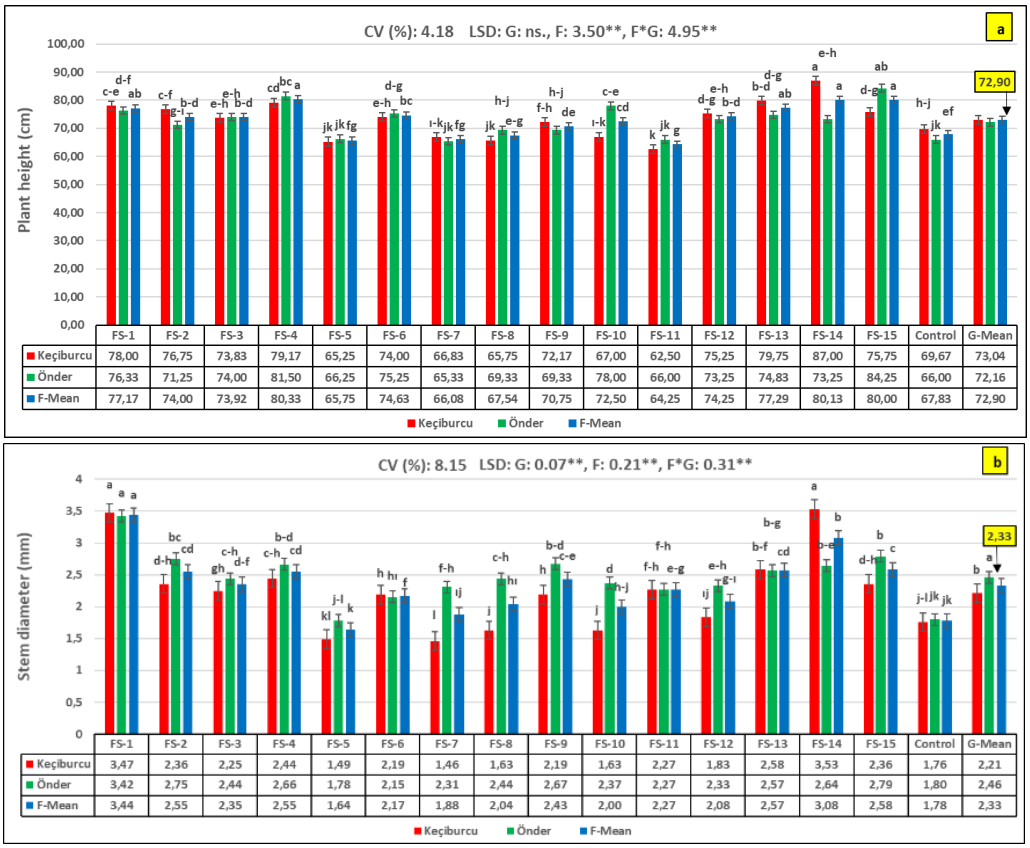

Figure 3. Mean values and groups of plant height (a) and stem diameter (b) analyzed in the study. ** Significant at P≤0.01, ns= not significant, CV= coefficient of variation, LSD= least significant difference, FS= fertilizer source and G= genotypes, Red= keçiburcu, Green= önder, Blue= fertilizer mean.

Significant differences between varieties were observed for all traits except the number of spikelets per spike when assessing the effect of organic and inorganic fertilizer application on spike characteristics in barley plants. In general, the highest values for these traits were obtained from treatments with conventional fertilizer and sheep manure (Figure 4-a, 4-b, 4-c, and 4-d). It is assumed that conventional fertilizer, with its readily available nitrogen and phosphorus in the soil, is more readily taken up by plants, whereas sheep manure is characterized by its high nutrient content. Many studies have reported that the use of organic fertilizers has a positive effect on crop yields and yield components (Ibrahim et al., 2008; Koutrobuas et al., 2016; Markoni et al., 2017). Additionally, vermicompost also increased biomass of plant (Fragaria x ananassa L.) according to the control and chemical fertilizer (Ateş et al., 2019).

İlker (2006) stated that spike length, which has significant direct and indirect effects on the grain yield in barley, can be used as a selection criterion. Gürsoy (2011) stated that the number of grains per spike has a direct effect on grain yield, which varies with spike length and number of spikelets per spike. Mutlu (2018) reported that the use of organic manure and organic manure combined with microbial fertilizer increased spike characteristics in barley, with the highest values obtained from cattle manure with liquid manure and compost with liquid manure. In line with the findings of the present study, other researchers have also reported that the maximum and minimum values of spike traits were attributed to the application of conventional manure and various organic manures (Hammad et al., 2011; Joshi et al., 2013; Kara and Gül, 2013; Aksu, 2017; Mazhar et al., 2018).

Both conventional and sheep manure applications have led to an increase in grain yield and biomass. The maximum and minimum values for both traits were obtained from conventional and sheep manure applications. Keçiburcu had the best yield (1.56 g/plant), while Önder had the highest biomass (3.74 g/plant). In the case of sheep manure, Keçiburcu was superior for both traits. In particular, compared with the control (no fertilizer), raw leonardite fertilizer had no positive effect on grain yield or biomass (Figure 5-a and 5-b). Based on these results, it can be suggested that readily available nitrogen and phosphorus applied to the soil through conventional fertilization are more easily taken up by plants, leading to increased yield. The main disadvantage of organic fertilizers in plant production is the inadequate supply of necessary nitrogen and other essential nutrients for plants. As a result, insufficient nitrogen and nutrient uptake by plants can lead to reduced grain yield. Organic fertilization has been reported to have lower levels of readily available nutrients than conventional fertilization (Hole et al., 2005). Furthermore, owing to the slow release and varying distribution of nutrients in organic fertilizers, yield reductions can occur. The organic nitrogen and phosphorus found in animal manure require mineralization to become readily available to plants (Havlin et al., 2014; Antille et al., 2014). Researchers have reported an increase in yield after 3-5 years of continuous organic fertilizer in the same field, and long-term organic fertilization has a positive impact on yield (Bulluck et al., 2002).

In many studies comparing conventional and organic farming practices have consistently reported lower yields from organic farming (Kaut et al., 2008; Özkan et al., 2021). For instance, Kodaş et al. (2015) found that the highest yield (329 kg/ha) was obtained through conventional farming, whereas the lowest yield (190 kg/ha) was observed in organic farming using farmyard manure. Özdemir et al. (2019) reported the highest grain yield of barley genotypes to be 524.5 kg/da with 160 kg/da vermicompost, while the lowest yield was obtained in the control group. Researchers have emphasized the need to supplement organic fertilizers with mineral fertilizers to enhance both soil fertility and grain yield (Wang et al., 2001; Gopinath et al., 2008). Mutlu (2018) reported a 50-56% increase in barley yield with the use of organic fertilizers in combination with microbial fertilizers. Previous studies have shown positive effects of sheep manure on certain plant nutrients (Hınıslı, 2014), parallel yield increases with increasing fertilizer (Elgin et al., 2006), and yields close to inorganic applications (Beşirli et al., 2004).

Based on the results of correlation analyses, no correlation was observed between ear length and heading time. However, there were significant negative correlations between heading time and the other traits. There were also significant positive correlations between plant height, stem diameter, spike length, number of spikelets per spike, number of grains per spike, grain weight per spike, grain yield, and biomass (Figure 6). These correlation results are consistent with similar results reported in previous barley studies (Akdeniz et al., 2004; Özkan and Akıncı, 2021; Bayhan et al., 2022).

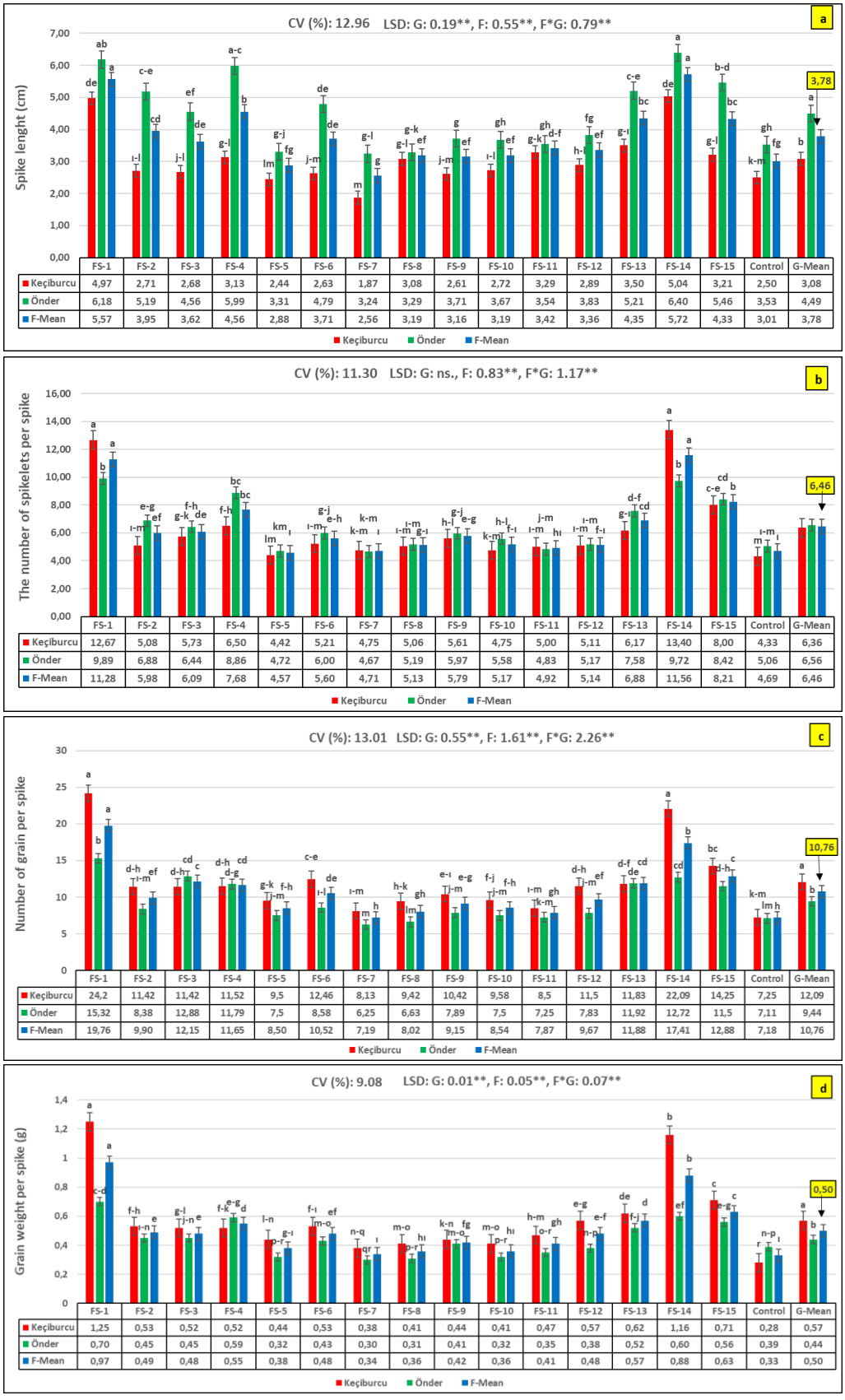

Figure 4. Mean values and groups of spike length (a), number of spikelets per spike (b), number of grains per spike (c), and grain weight per spike (d) analyzed in the study. ** Significant at P≤0.01, ns= not significant, CV= coefficient of variation, LSD= least significant difference, FS= fertilizer source and G= genotypes, Red= keçiburcu, Green= önder, Blue= fertilizer mean.

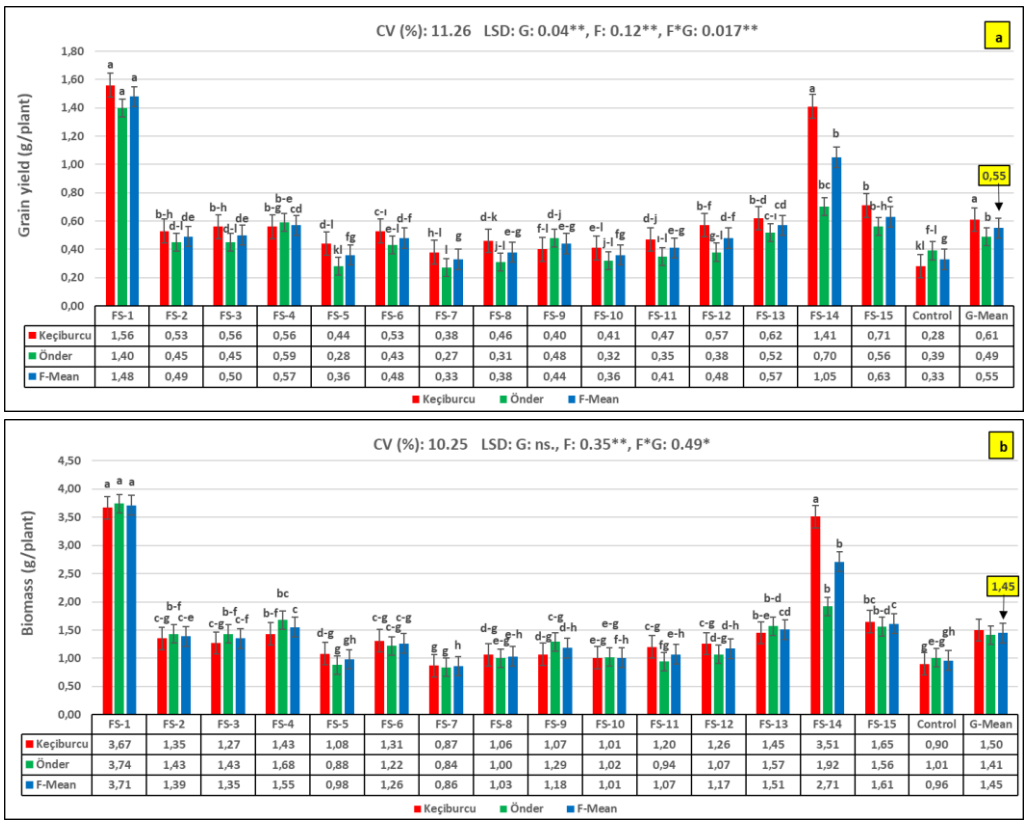

Figure 5. Mean values and groups of grain yield (a) and biomass (b) analyzed in the study. * Significant at P≤0.05, ** Significant at P≤0.01, ns= not significant, CV= coefficient of variation, LSD= least significant difference, FS= fertilizer source and G= genotypes, Red= keçiburcu, Green= önder, Blue= fertilizer mean.

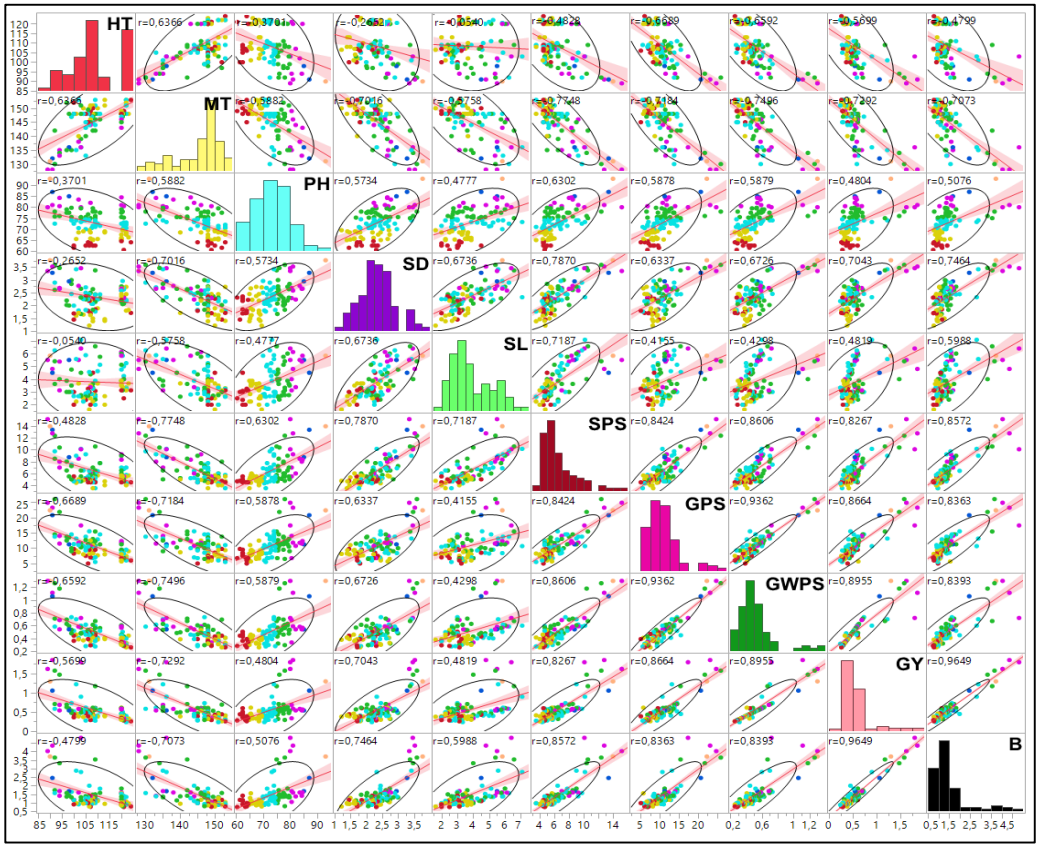

Figure 6. Correlation analysis results of the parameters examined in the study. HT= heading time, MT= maturity time, PH= plant height, SD= stem diameter, SL= spike length, SPS= spkilets per spike, GPS= grain per spike, GWPS= grain weight per spike, GY= grain yield, B= biomass

4. Conclusion

This study determined the effects of different organic fertilizers on the yield and yield components of barley varieties that can be used in organic barley production. In particular, sheep manure competed with conventional fertilizer and led to successful results. In addition, chicken manure also showed a positive increase compared to that of other fertilizers, although not very high. It was concluded that the application of conventional fertilizer, which is part of the conventional production system, is an effective source of fertilizer due to its rapid uptake and use by the crop, and that the application of organic fertilizer has a positive effect on crop development. In addition, it was found that sheep manure, which gives comparable results to conventional fertilizer, can be recommended for organic barley production and that the Keçiburcu variety, which gives promising results, can be used. It was concluded that different doses of sheep manure or the use of sheep manure together with conventional fertilizer should also be tested in future studies on this subject.

Author Contributions

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

	R.Ö.
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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BIBLIOMETRIC ANALYSIS OF SCIENTIFIC STUDIES ON HORSE WELFARE FROM PAST TO PRESENT

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Abstract: Animal welfare studies continue to gain importance over the years. Since horses are bred and cared for many different purposes, welfare studies on horses have a wide scope. Detailed information about the research topic can be obtained by determining many changes such as the fields, researchers and countries in the published studies over the years through bibliometric analysis. For the bibliometric analysis of horse welfare studies, the Web of Science database was scanned and a total of 1983 documents were found between 1983 and 2023. The most studies in this field were found in Animals, Applied Animal Welfare Science and Equine Veterinary Journal. Mc Greevy P.D. was determined as the author with the most articles in this field. The highest number of corresponding authors of articles in horse welfare were from the United Kingdom. By the bibliometric analysis, the change in years of the trend research fields of horse welfare, based on keywords made the changes particularly easy to understand. The results of the present study could easily be used in planning further studies in horse welfare, which could save time and costs.

Keywords: Bibliometrics, Horse, Welfare, Articles, Authors

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1. Introduction

Animal welfare studies could be categorized as a young research field. Animal welfare science is of veterinary origin and has taken a multidisciplinary form covering many fields over time and is now represented in veterinary faculties and animal science departments all over the world (Webster, 2016). The interest in this field has increased with the spread of the emotional approach of the society, political concerns and ethical principles. “Brambell’s Five Freedoms” principles, most known and accepted welfare guidelines have been an advisor for those who were in close contact with animals and even for legal regulations (Lesimple, 2020). However, it was criticized that these principles were actually created by centered on environmental factors and that the welfare of the animal in question was not individually considered (Lesimple, 2020). Furthermore, its lack of integrating the concept of positive welfare and need for more appropriate scientific approaches was emphasized (Webster, 2016). Especially the exposure of horses to very different environments, breeding, work, sports, individual hobby and pet care, and the existence of various factors affecting welfare constitute a serious research area. Since there are many factors affecting well-being, it is difficult to evaluate, so studies in scientific fields such as behavior, immunology, physiology, endocrinology, pathology and neurosciences continue and are used (Marchant-Forde, 2015). It is generally accepted by many authors that welfare refers to the permanent state of the animal concerned over time and depends on the animals’ subjective perception of the environment (Lesimple, 2020). Some of the studies carried out in different fields are accepted as an indicator of welfare, like behavioral indicators, considered to be early indicators of unproper conditions (Keeling and Jensen, 2009). Behavioral indicators include stereotypic behaviors and are reported to be a disease of domestication in horses (Marsden, 2002). Stereotypic behaviors like box walking (Normando et al., 2011; McGreevy, 2012; Devereux, 2019), wood chewing (Albright et al., 2009; Normando et al., 2011), crib biting (Wickens and Heleski, 2010; Nagy et al., 2010), weaving (Cooper et al., 2000; Clegg et al., 2008) and wind sucking (Normando et al. 2011; Escalona et al., 2014). Especially horses have a close relation and a constant exposure to humans in contrast to farm animals. The interaction with humans and their behavior towards humans are reported as welfare indicators (Rivera et al., 2002; Burghardt et al., 2012; Lesimple, 2020). There are many horse behavioral aspects, which were studied in relation to welfare like aggressiveness (Bourjade et al., 2009; Fureix et al., 2010; Pierard et al., 2019), playing habits (Hausberger et al., 2012; Van Dierendock and Spruijt, 2012) and yawning (Górecka-Bruzda et al., 2016; Lesimple, 2020). The search for quantitative measurements such as analysis performed for health screening like hematology, serum biochemistry, endocrinology and different substances indicating the welfare status of a horse are still ongoing

(Massányi et al., 2022; Scholler et al., 2023). However, it has been reported that associating the measurement results made in this way directly with the welfare of the horse may lead to false results and it is necessary to carefully evaluate whether an abnormal result is due to an acute or chronic condition (Lesimple 2020). Otherwise, there are studies that correlate welfare with the evaluation of the environmental conditions of the horses, such as housing and management (Ruet et al., 2019; Rosselot et al., 2019; Kelemen et al., 2021; Mazzola et al., 2021; Mactaggart et al., 2021; Gehlen et al., 2021; de Oliveira and Aurich, 2021; Baumgartner et al., 2023).

Bibliometry also known as bibliometrics is a quantitative method to analyze and measure different features in scientific literature and papers. Bibliometric studies give the chance for researchers how the interested field took shape in a specific time span. Data of which countries, authors and journals were most productive and the most used keywords with time information were shown. All these information could be used for planning a research in the interested field.

Due to the complex fields of study of equine welfare, the aim of this study was to evaluate the development and approaches of this field over time with the use of bibliometric analyzes.

2. Material and Methods

2.1. Data Collection

The present study included 1983 documents, which were obtained from the Web of Science (WoS) database from the years 1985 to May 2023. To ensure publications about horse welfare, keywords and journals were picked up carefully.

2.2. Bibliometric Analysis

This analysis is a sociometric and network analysis method that reveals the social network of scientific studies in a special field with the help of computer technology, thus enabling the determination of the effectiveness of scientific studies (Han et al., 2020; Önder and Tirink, 2022). The analyses provides information in which journals the most articles in the research field were published, which authors studied intensively on this subject, the institutions of the authors and co-authors', also on a country basis. The usage density and distribution over time of the keywords used within the date range included for the bibliometric analaysis are shown. It contributes to determining the impact of specific studies on the field by ranking the most cited articles. Therefore, all these results can directly affect many aspects such as trends, policies, areas of collaboration and financial support for current and future research in a field of scientific research (Ergin et al., 2023).

2.3. Data Analysis

After the data was obtained from the WoS database, it was made ready for data analysis with the help of the "convert2pdf" package of the R software (R Core Team, 2019). Data obtained from horse welfare studies and made ready for establishment were analyzed statistically using the "bibliometrics" package and "bibloshiny" application in R software (Aria and Cuccurullo, 2017; Kaplan and Altay, 2023).

3. Results and Discussion

The results of the main information about collected data had shown that the greater part was compromised of 1593 articles in this field, including 110 proceedings paper, 14 early accesses, and 13 book chapters and followed by 186 reviews, as seen in Table 1. Since 1985 a peak of articles reported in the year 1996, with 70 articles, pointed out, whereas in year 1997 this number dropped to 4 articles (Figure 1). Generally, a trend to increase was seen till 2022, suggesting the interest and importance of welfare in horses. Since 2016, 100 and above articles were observed with the highest number of 200 in 2022, based on the WoS database (Figure 1).

Table 1. Main information of primary data

Document types	Number
Article	1593
Article; Book chapter	13
Article; Early access	14
Article; Proceeding paper	110
Correction	2
Editorial material	33
Letter	6
Note	195
Proceeding paper	24
Review	186
Review; Early access	1

Documents resources were established as 193 and the most relevant source was the journal Animals with 305 articles, followed by the journals Applied Animal Behavior Science, Equine Veterinary Journal, Journal of Veterinary Behavior– Clinical Applications and Research, Pferdeheilkunde and Journal of Veterinary Equine Science with 185, 143, 121, 121 and 112 articles, respectively (Figure 2). A bibliometric study on animal welfare, where documents were scanned in the WoS database between 1968 and 2017, and the most relevant journal was Animal Welfare and Applied Behavior Science (Freire and Nicol, 2019). Besides the semimonthly publications of the journal Animals, special issues about Welfare including horses were available, which could be related to the high number of articles resulted as the most relevant source. Whereas the most local cited journal, which means only in the field horse welfare, was the Journal of Applied Animal Behavior Science (Appl. Anim. Behav. Sci.) with a total of 6584 citations and Equine Veterinary Journal was resulted as the second one with 5291 citations, shown in Figure 3.

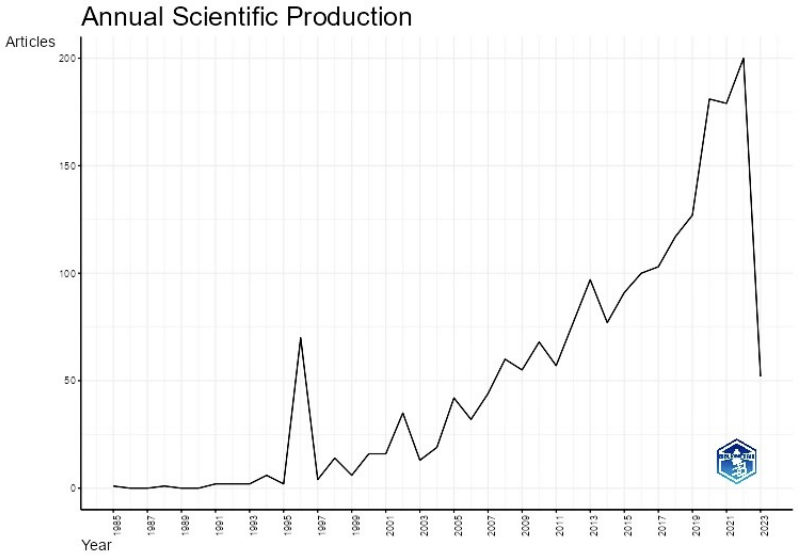

Figure 1. Annual scientific production in horse welfare articles.

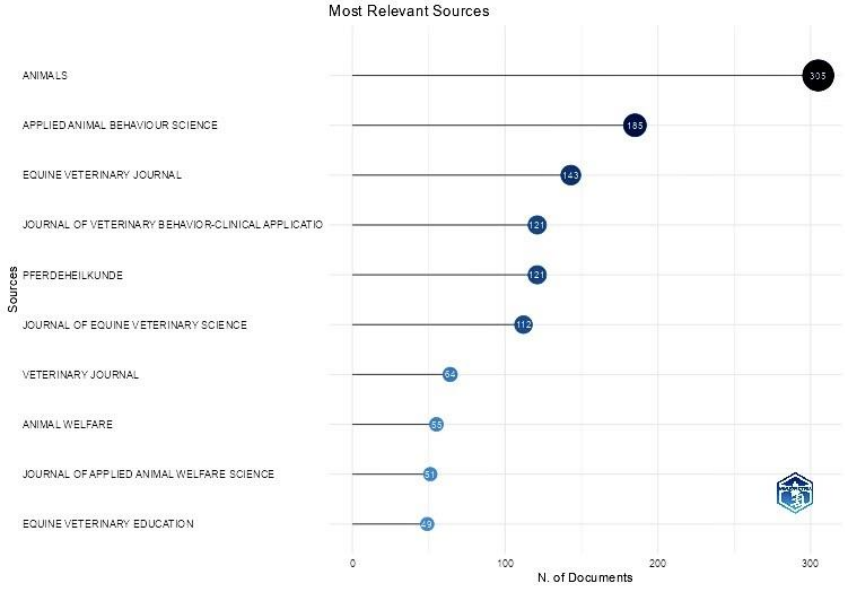

Figure 2. Most relevant sources of articles about horse welfare between the years 1998-2023.

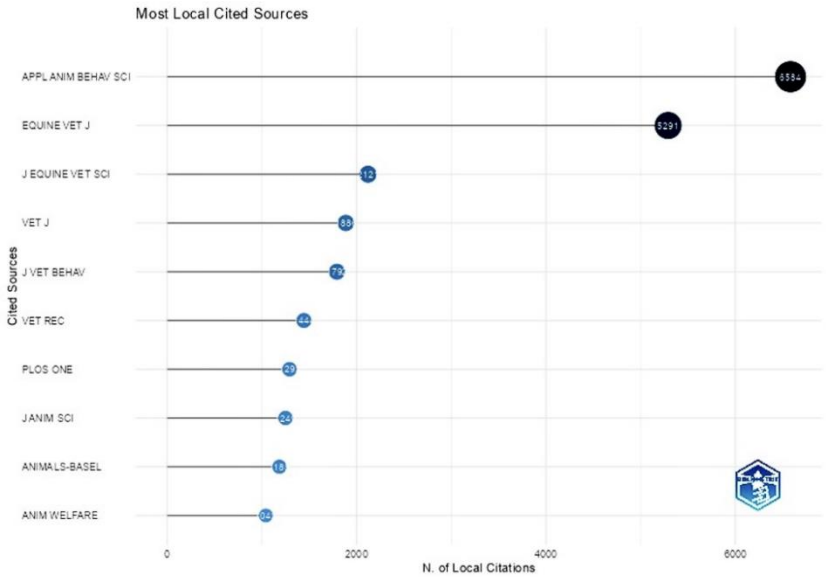

Figure 3. Most cited sources between the years 1998 – 2023.

The most productive authors are rowed in Figure 4. As seen Mc Greevy P.D. and Mc Greevy P. were sorted separately, but as a result of the database review and the examination of the articles, it was revealed that the first two authors were actually the same person. Thus, the author's 73 articles on horse welfare were identified. Padalino B. and Minero M. followed with 34 and 29 articles, respectively, in the field horse welfare.

Documents, which were most cited were represented in Figure 5. The article "Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals -- a review" by Von Borell E., Langbein J., Despres G., Hansen S., Leterrier C., Marchant J., Marchant-Forde R., Minero M., Mohr E., Prunier A., Valance D. and Veissier I., in the journal Physiology and Behavior in 2007, was the most global cited with a total citation of 602, which means the documents cited these review were not only from horse behavior studies. In contrast the most local cited article, only in the topic horse welfare, was "Assessment of the welfare of working horses, mules and donkeys, using health and behavior parameters." by Pritchard J.C, Lindberg A.C., Main D.C.J. and Whay H.R. published in Preventive Veterinary Medicine in 2005, as seen in Figure 6.

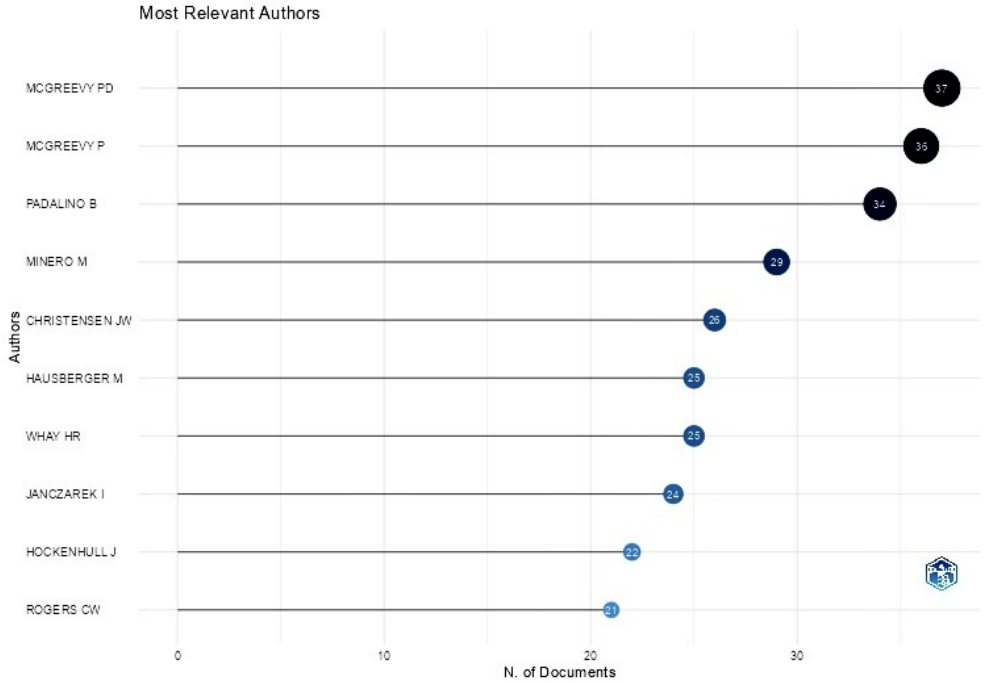

Figure 4. Most relevant authors on horse welfare between the years 1998 – 2023.

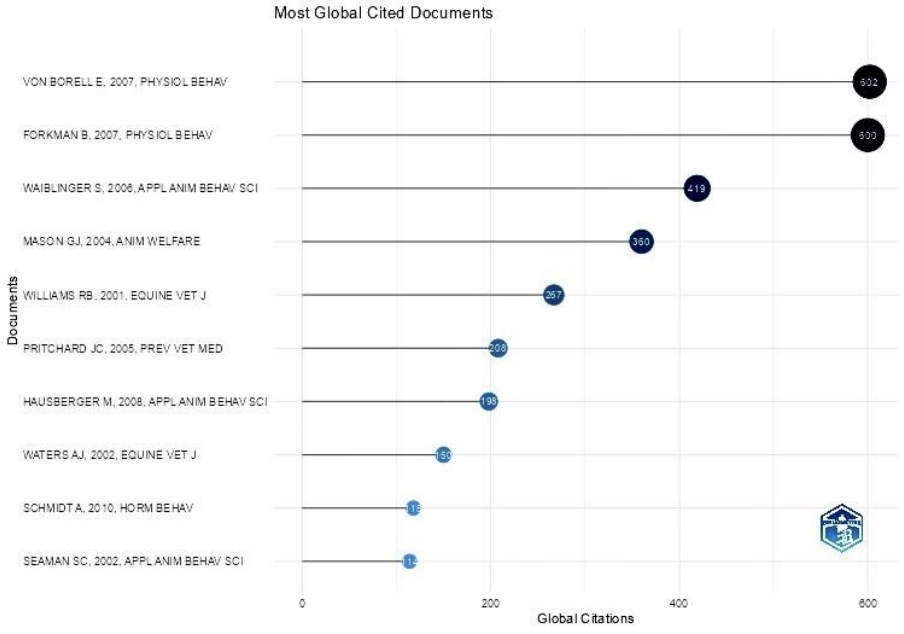

Figure 5. Most global cited documents.

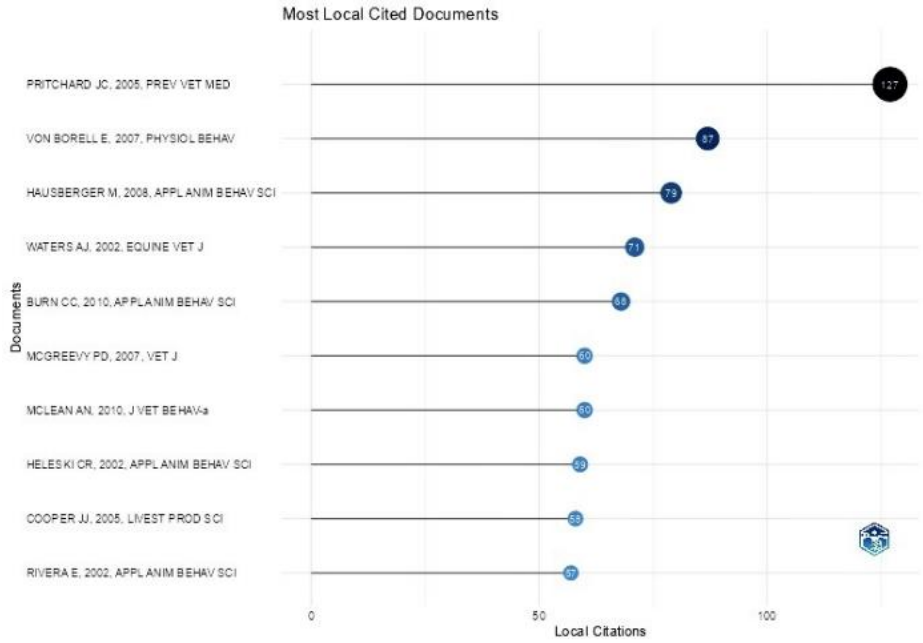

Figure 6. Most local cited documents.

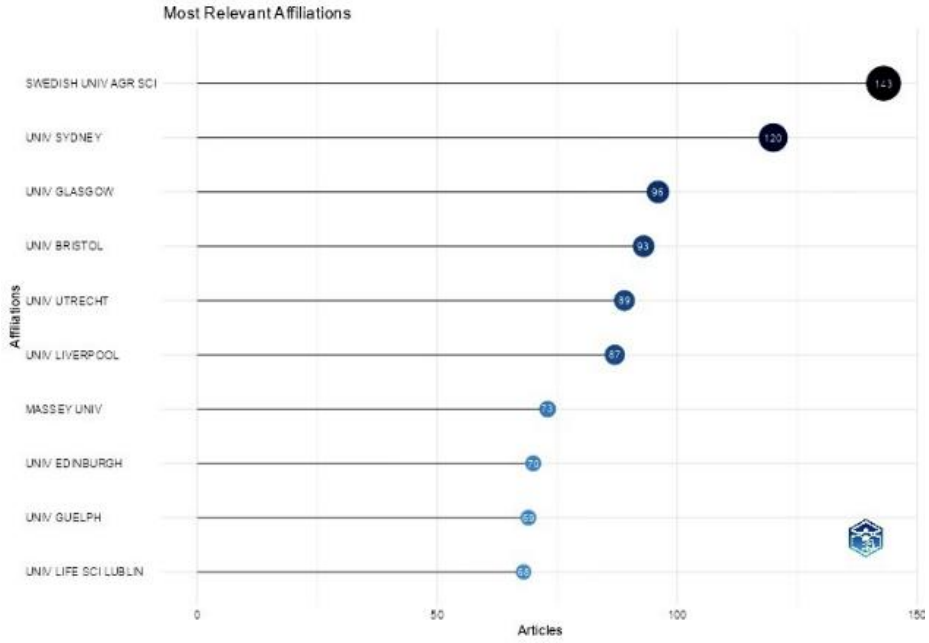

Figure 7. Most relevant affiliations.

The most relevant affiliations on articles themed horse welfare were the Swedish University of Agricultural Science with 143 articles and followed by the University of Sydney with 120 articles, as seen in Figure 7. The 3rd university in the ranking is the University of Glasgow and the number of published articles was determined as 96. Observations on corresponding author's countries had shown 359 single country publications (SCP) and 85 multiple country publications (MCP) from the United Kingdom, with the highest publications. In Table 2 the first ten countries with SCP above 10 articles were demonstrated. It was reported that the highest total paper amount published in Applied Animal Behavior Science were from the USA, England and Australia with 149, 45 and 26 publications, respectively, in a ten year range (2009-2019) (Rousseau and Binfet, 2022). Same countries were the first three ones in the corresponding authors' countries ranking in the field horse welfare in the present study.

Table 2. Corresponding author's countries

Country	Articles	Single Country Publications (SCP)	Multiple Country Publications (MCP)	Frequency	MCP Ratio
United Kingdom	444	359	85	0.22390	0.1914
USA	198	157	41	0.09985	0.2071
Australia	181	119	62	0.09128	0.3425
Italy	133	88	45	0.06707	0.3384
Germany	131	107	24	0.06606	0.1832
Poland	89	73	16	0.04488	0.1798
Sweden	76	44	32	0.03833	0.4211
Netherlands	72	44	28	0.036309	0.3889
Canada	68	44	24	0.03429	0.3529
France	56	41	15	0.02824	0.2679

In the field horse welfare, the ten first most relevant words and their occurrences were as follows; horses 383, behavior 283, welfare 218, stress 212, responses 164, risk-factors 155, prevalence 130, management 120, exercise 95 and heart rate variability 93, which is shown in Figure 8. Beside the mentioned first ten most relevant words, which stand out in the word cloud (Figure 9), the words animal-welfare, physiological responses, donkey, pain and young horses draw attention in the cloud.

The mostly used keywords and their distribution over the years is represented in Figure 10. The keyword horses was mentioned 383 times in articles between 2011 and 2021, whereas in 2017 and the following years the occurrence was higher than before 2017. The word behavior and welfare ranked as second and third most used words were shown between 2013-2020 and were mostly seen in 2017 and the years after. The physiological response term, which stands out in the word cloud but was not in the top ten, appeared 62 times between 2012 and 2019, and was mostly used in 2015 and later. None of the words and terms mentioned in the top ten and stood out in the word cloud were a trend in the last two years. Topics like descriptive epidemiology, riders, communication and therapy, used in different timespans but were mostly seen in 2020 and after were in a trend in the last years. The collaboration world map in Figure 11, had shown that a network between Europe, United Kingdom, USA and Sydney are distinctive. A collaboration frequency of 50 from the United Kingdom to Australia, is the highest one, followed by the collaboration from United Kingdom to USA. Apart from this, working partnerships between the USA and Australia, Sweden and Denmark, and the Netherlands and Belgium are notable.

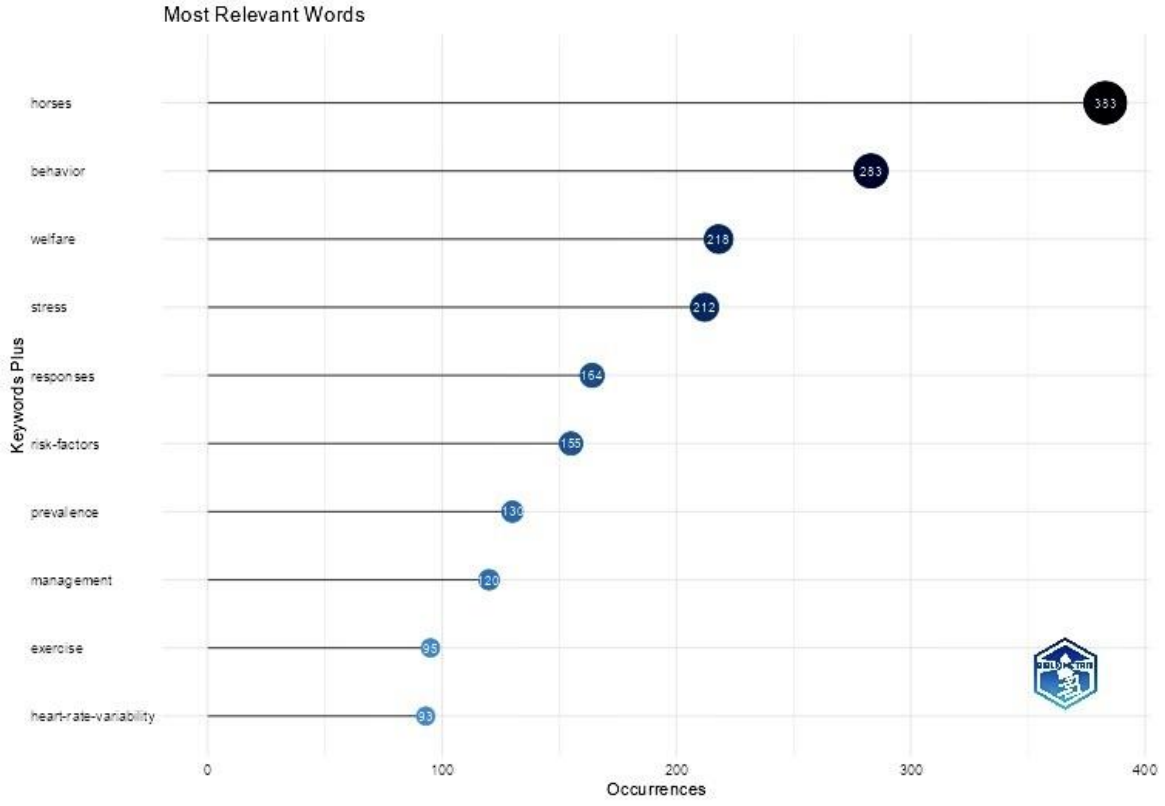

Figure 8. Most relevant words.

Figure 9. Word cloud in the field horse welfare.

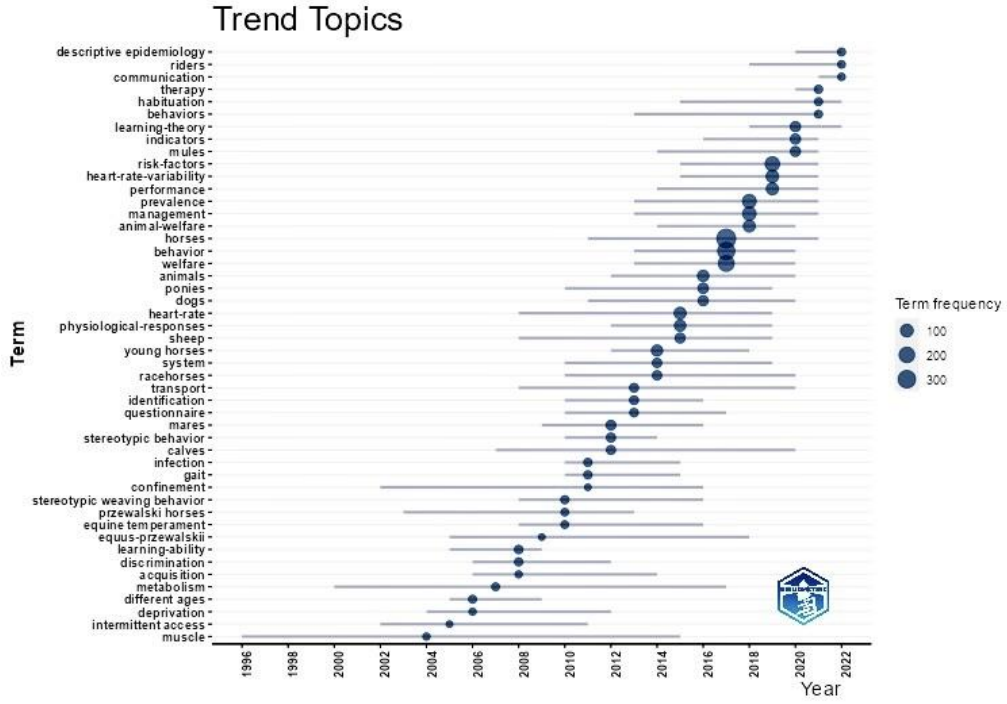

Figure 10. Mostly used keywords and their timespan.

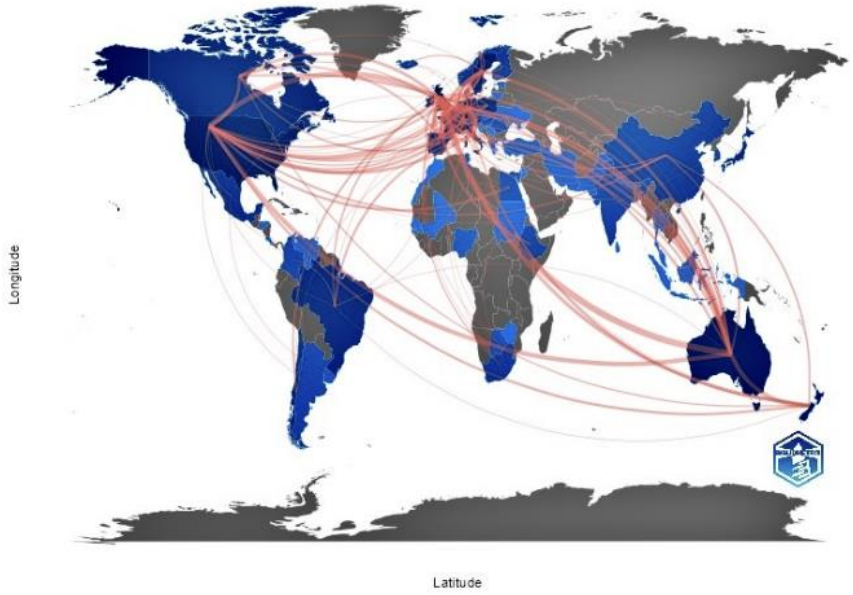

Figure 11. Collaboration world map.

4. Conclusion

Data results had shown an interest in horse welfare including topics like behavior, therapy, communication, riders, habituation and descriptive epidemiology since 2020. Future studies should consider these trends. Thus, scientific studies could have a higher chance to be financed and could lead to new research areas in horse welfare.

Author Contributions

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

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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Research Article
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GENETIC SCREENING OF *FecX*^{G} POLYMORPHISM IN SAANEN GOAT (*Capra Hircus*) BREED IN TÜRKİYE

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Abstract: Determination of fecundity gene mutations and utilizing them in farm animals programs offers the opportunity to improve productivity. The BMP15/FecX gene is one of the candidate genes with significant effects on multiple births in sheep. Studies in small ruminant have shown that BMP15 gene mutations increase the rate of multiple births, although the effect of BMP15 gene mutations varies at the breed level. Although there are many studies on sheep fecundity in Türkiye, there are no studies on goat. Therefore, the objective of the current study was to investigate FecX^{G} mutation in the exon 2 region of BMP15 gene in Saanen goats (*Capra hircus*). A total of 24 samples were used to investigate the FecX^{G} mutation in Saanen goats raised in the Muş Plain of Türkiye. A fragment of 141 bp of BMP15 gene was amplified by PCR and then products subjected to the digestion of restriction enzyme *HinfI*. This preliminary study's findings showed that there is no FecX^{G} mutation in Saanen goats.

Keywords: Goat, Saanen, BMP15, FecX^{G}, PCR-RFLP, *HinfI*

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1. Introduction

The bone morphogenetic proteins (BMPs), which belong to the transforming growth factor β (TGF β) superfamily, have significant influence on cellular processes such as growth, proliferation, and apoptosis within different kinds of cells, with particular relevance to mammalian fertility (Massague, 1998; Galloway et al., 2000). The BMP15 gene was first detected in the Romney sheep and revealed its role in sheep fertility (Davis et al., 1991). The BMP15 gene, located in goat X chromosome (Farhadi et al., 2013), is responsible for regulating the proliferation and differentiation of granulosa cells. It achieves this by stimulating mitosis in granulosa cells, suppressing the expression of FSH receptors, and promoting the expression of ligands. These functions are crucial for maintaining female fertility in mammals (Moore and Shimasaki, 2005).

In Türkiye, as in the world, fecundity gene research has been focused more on native sheep breeds (Karslı et al., 2012; Çelikeloğlu et al., 2022; Gedik 2021; Kırıkçı, 2023a; Kırıkçı, 2023b), while there are significant shortcomings in the research on goat fecundity genes. Recently, there has been an increasing interest in fecundity genes in goats (Maitra et al., 2023; Song et al., 2023; Abuzahra et al., 2023). A study by Maitra et al. (2023) to understand the genetic mechanism of prolificacy in goats found that the genes BMPR1B, BMP15 and GDF9 are highly expressed in goat ovaries. The BMP15 gene and its effects on goat prolificacy were detected in Chinese goat breeds; Funiu white and Taihang black (Wang et al., 2011; Wang et al., 2015).

This study aimed to investigate the Galway (FecX^{G}) mutation on BMP15 gene in Saanen goats, which are well adapted to the ecological conditions of Türkiye (Özkaya et al., 2017).

2. Materials and Methods

A total of 24 blood samples were collected for the study from the enterprise located in the plain of Muş. Genomic DNA was isolated using a kit, IDPURE™ Spin Column (Empire Genomics, Buffalo, NY). Following the manufacturer's instructions, the DNA isolation process was carried out. The PCR reaction for amplifying a 141 bp region of the BMP15 gene was performed in a 25 µl final volume with 1µl of genomic DNA, 12µl of Taq DNA Polymerase 2X Master Mix Red (1.5 mM $MgCl_2$ final concentration, AMPLIQON), 1µl of each primer at a concentration of 10 pmol/µl and water. The PCR analysis was carried out at the following temperatures and times: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of 95 °C for 30 seconds (denaturation), 55 °C for 30 seconds (annealing), and 72 °C for 30 seconds (elongation), with a final extension step at 72 °C for 5 minutes. The goat BMP15 gene exon 2 region was amplified with the primer pairs; 5′ CACTGTCTTCTTGTTACTGTA TTTCAATGAGACG 3′ and 5′ GATGCAATACTGCCTGCTTG 3′ using SimpliAmp thermal cycler (Applied Biosystem). Samples were analyzed for

the presence of the FecX^{G} mutation by using PCR-RFLP technique described by Galloway et al. (2000). Digestion was performed in a final volume of of 30 µL, which included of 1 µL of fast digest enzyme (*HinfI*), 10 µL of PCR product, 2 µL of green buffer, and 17 of µL deionized water. They were incubated at 37 °C for 10 min and inactivated at 65 °C for 20 min. After the PCR-RFLP analysis, all of the samples were run on a 2% high-resolution agarose gel electrophoresis, stained with a green safe dye, and then visualized under a UV transilluminator for evaluation (Kiraz and Ağyar, 2016). A DNA ladder of 100 bp was used as molecular size marker.

3. Results

In the current study, the FecX^{G} mutation was screened in the Saanen goats. A fragment of 141 bp of BMP15 gene was amplified successfully and then all PCR products were subjected to the digestion of *HinfI* enzyme. After digestioning, samples were run on 2% agarose gel electrophoresis (Figure 1).

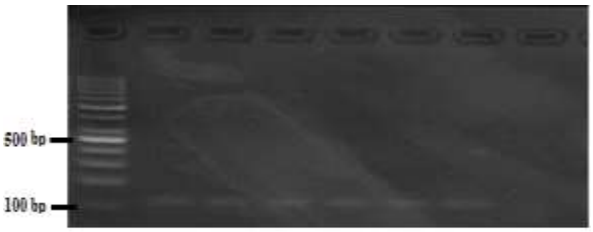

Figure 1. PCR-RFLP result for FecX^{G} locus in Saanen goat.

According to literature, heterozygous mutant individuals were expected to have two fragments of 111 and 54 bp, while individuals that not carrying the mutation were expected to have a single fragment of 141 bp (Basheer et al., 2019) According to the results of the RFLP analysis, not all goats examined carried the FecX^{G} mutation, which displayed a band of 141 bp in length as shown in Figure 1.

4. Discussion

As a candidate gene, BMP15 were well documented in the world for sheep fecundity. In general, the interest in fecundity gene research has been more on sheep in Türkiye, as is the case all over the world (Hanrahan et al., 2004; Wang et al., 2015; Çelikeloğlu et al., 2021; Kırıkçı, 2023a; Kırıkçı, 2023b). The main reason for this may be the consumption of more sheep meat within small ruminants. Recently, the study of the fecundity gene in goats has drawn more interest (Basheer et al., 2019; Dangar et al., 2022; Gujarmale et al., 2023). Nevertheless, more research is need that reveals the genetic structures of goats in terms of fertility genes.

Maskur et al. (2023) reported that the mutations of FecX^{G} (c.718C>T) and FecB (c.746A>G) were in Indonesian Kacang and Boerka goats. Another study examining the S32G mutation in the BMP15 gene in 18 breeds of goats, reported a monomorphic structure in Saanen goats (Feng et al., 2014). This monomorphic result was consistent with the findings of the present study. Similar result was also observed in different goat breeds; Markhoz (Shokrollahi, 2015) and Guizhou (Lin et al., 2007). Hua et al. (2007) reported the same results even when the FecX mutation was examined in a large sample (506) in six goat breeds. Contrary to these findings, Abdel-Rahman et al. (2013) using a total of 25 animals with the highest and lowest litter sizes, reported that goats with the BB genotype for the BMP15 gene produced a larger litter size than goats with other genotype (Abdel-Rahman et al., 2013) According to these findings, prolificacy inheritance differs between sheep and goats, and most likely between breeds (Shokrollahi, 2015).

There are differences between studies in terms of twin birth rates in Saanen goats reared in Türkiye. In a study by Bolacalı and Küçük (2012), the mean number of Saanen goats per birth was given as 1.59 young, while in the studies by Taşkın et al. (2003), Ulutaş et al. (2010) and, Ceyhan and Karadağ (2009) were given as 71.43%, 58.83% and 44.2%, respectively. From the results of these studies it can be concluded that there are large differences in the number of kids per birth. In this study, mean number of kids per birth was 1.64. Apart from its effects on fertility, BMP15 have been reported to be a candidate gene in terms of growth and development in goats (Ahlawat et al., 2016). Therefore, fecundutiy gene mutations should be investigated for economic traits such as growth and fertility in goats. The present study reported that Saanen goats did not carry the mutation of FecX^{G}. In the study, the sample size was small. Therefore, conducting similar studies with more samples and phenotypic data will give more reliable results.

5. Conclusion

The current analysis showed that not all Saanen Goat breed are carriers of the Galway mutation (FecX^{G}). It can be assumed that selected goat individuals have no advantage for the examined mutation. Therefore, this and other fertility genes should be investigated in comprehensive studies.

Author Contributions

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

	O.A.	K.K.
C	60	40
D	100	
S	100	
DCP	50	50
DAI	50	50
L	50	50
W	80	20
CR	80	20
SR	80	20
PM	80	20
FA	80	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

This study was carried out in accordance with the approval (approval date: June 20, 2023, protocol code: 2023/011-E19057416-125.02.02-10311949) of Republic of Türkiye, Ministry of Agriculture and Forestry, Directorate of Muş.

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EFFECT OF THE TIMING OF FUNGICIDE APPLICATION ON YIELD AND QUALITY PARAMETERS OF WHEAT INFECTED WITH *FUSARIUM* CROWN ROT DISEASE

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Abstract: Crown rot caused by *Fusarium culmorum* (FCR) is a common and important pathogen affecting the cereal industry through grain yield and quality losses. In this study, the effects of epoxiconazole plus prochloraz application and several other applications on disease severity and grain quality parameters including thousand-grain weight (TGW), grain yield (GY), protein (GP), Zeleny sedimentation (ZS), wet gluten (WG) and Grain index (GI), were assessed. The efficacy of epoxiconazole plus prochloraz, were determined in the T1 (ZGS25), T2 (ZGS34), and T3 (ZGS45) growth stages of winter wheat with seven alternative spray programs. These programs were based on (i) the application (SF) of seed fungicide to infected seeds (ii) control without fungicide (non-SF) and (iii) three different growth stages of wheat. The interaction between seed fungicide applied and fungicide application time was significant (P≤0.01) for DS, ZS, and WG. The effectiveness regarding the disease severity, TGW, and GY of epoxiconazole plus prochloraz in relation to FCR wheat showed significant (P≤0.01) changes depending on the application time. The disease severity resulted in lower T1-T2 (9.66%) and T1-T2-T3 (9.91%) stages than the other stages. The highest yields were obtained when the fungicide was applied twice at the T1-T2 stages. DS/TGW and DS/GY were negatively correlated and TGW/GY was positively correlated in SF.

Keywords: *Fusarium culmorum*, Fungicides, Grain quality, Wheat

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1. Introduction

Fusarium species such as *Fusarium culmorum* (WG Smith) Sacc. and *F. graminearum* Schwabe the major causal agents of Fusarium crown rot (FCR) causing necrotic lesions in the root, sub-crown, and stem tissues of wheat are also the causal agent of Fusarium head blight (FHB), a globally important wheat disease (Cook, 1992; Burgess et al., 2001; Backhouse et al., 2004; Tunalı et al., 2006). There is resistance to FCR among commercial cultivars, but the disease severity can be high in suitable effects of climatic conditions, even in resistant cultivars (Scherm et al., 2013, Özdemir, 2022). *Fusarium culmorum* has been reported as the most prevalent and virulent pathogen causing crown rot in Türkiye (Demirci and Dane, 2003; Tunalı et al., 2008; Akgül and Erkılıç, 2016; Tok and Arslan, 2016; Gebremariam et al., 2018; Köycü and Özer, 2019). *F. culmorum*, has the lowest requirements for the high relative humidity to infect wheat, (Klix et al., 2008) and shows high disease severity in dry soils and areas with high temperatures (Balmas et al., 2015). Therefore, *F. culmorum* is also the most predominant pathogen and most frequently isolated crown rot disease in the Trakya region (Hekimhan and Boyraz, 2011; Köycü and Özer, 2019).

The grain quality characteristics such as wet gluten (%), particle size index (PSI), Zeleny sedimentation (mL), and gluten index (%) were significantly decreased in infected grains compared to control after Fusarium head blight (Köycü, 2021). Cultural practices such as good tillage for the decomposition of infected plant residues against these seed/soil-borne and residue-borne pathogens, crop rotation, adjusting the planting date, and limiting nitrogen for proper fertilization are recommended for FCR disease control (Montanari et al., 2006; Evans et al., 2010), but these practices are insufficient (Cook, 2010). Seed treatments containing the active ingredients tebuconazole, metalaxyl-M, thiram, difeconazole, fludioxonil, triadimenol, and carboxin alone or in combinations can increase seedling growing and grain yield by decreasing the FHB infections in highly susceptible varieties of wheat (Schaafsma and Tamburic-Ilincic, 2005; Akgül and Erkılıç, 2016; Köycü and Sukut, 2018). However, the application of fungicide to the seed is also inadequate in the prevention of FCR infections in the seedling growth and stem elongation stages (Smiley et al., 2005; Hudec, 2007; Beccari et al., 2011; Akgül and

Erkılıç, 2016). The effectiveness of fungicides may vary depending on whether seedlings are infected with seed/soil-borne disease (Sukut and Köycü, 2019). Few studies have reported the effects of fungicide application against foliar diseases on wheat grain quality (Gooding et al., 2000; Dimmock and Gooding, 2002; Wang et al., 2005; Blandino et al., 2009; Rodrigo et al., 2015). Studies have not been undertaken on changes in the quality parameters of the grain of fungicide application number at different growth stages with *Fusarium* crown rot-infected wheat seeds. Thus, the goals of this study were to determine a) the effectiveness of seed fungicide application/non-fungicide on fungicide applications, b) the effect of fungicide applications and their number on FCR disease, grain yield, and quality parameter characters of grain at the different stage of bread wheat.

2. Materials and Methods

2.1. Fungicides

In the field, we used technical-grade 40 g/L pyraclostrobin plus 80 g/L triticonazole a. i. (Insure® Perform FS BASF, Türkiye; 50 mL 100kg/seed) for the seed coating and 42 g/L epoxiconazole plus 150 g/L prochloraz a.i. (Tocata® TR BASF, Türkiye, 200 g/da), applied with an atomizer, in the control of FCR. The soil was fertilized with 184 kg/ha N and 40 kg/ha P_2O_5. Standard practices for weed control with herbicides were followed.

2.2. Field Experiment

The field experiment was established in the 2017/2018 wheat growing season at the Tekirdağ Namık Kemal University research area (40° 59′ 36.5028" and 27° 34′ 53.0724"). In the experiment, a *Fusarium culmorum* (S-14)-infected Flamura 85 (F-85) cultivar was used. A detailed description of that field experiment using the infected Flamura-85 seed cultivar was provided by Köycü (2018). *F. culmorum*-infected seeds were either coated with seed fungicide (SF) or not (non-SF). Wheat plants were sprayed at the T1 (ZGS25; main shoot and 5 tillers, 14 February), T2 (ZGS34; fourth node detectable, 22 March), and T3 (ZGS 45; boots swollen, 15 April) growth stages (Table1).

Table 1. A winter wheat crown root experiment with three spray timing stages (T1, T2, and T3) at which the plots were untreated or treated with 200 g/da epoxiconazole plus prochloraz with seed fungicide application or non-seed-fungicide application

Treatment	Seed fungicide[a]*	T1 (ZGS 25)	T2 (ZGS 34)	T3 (ZGS 45)
Check	No Fungicide	Untreated	Untreated	Untreated
1	No Fungicide	Fungicide[b]*	Untreated	Untreated
2	No Fungicide	Untreated	Fungicide	Untreated
3	No Fungicide	Untreated	Untreated	Fungicide
4	No Fungicide	Fungicide	Fungicide	Untreated
5	No Fungicide	Untreated	Fungicide	Fungicide
6	No Fungicide	Fungicide	Untreated	Fungicide
7	No Fungicide	Fungicide	Fungicide	Fungicide

a= 40 g/L pyraclorostrobin plus triticonazole 80 g/L; Insure® Perform; b= 42 g/L epoxiconazole plus prochloraz 150 g/L, Tocata® *BASF; commercial fungicide.

Seeds were sown on November 10, 2017, by hand at the rate of 500 seeds per m^2. The experimental design involved a split plot in randomized complete blocks with three replications. The plots were surrounded by 2.5 m of bare-soil borders, which were 5 x 1 m wide. Seed fungicide was applied in the main plot; each other fungicide was applied in the subplot. For disease severity (%), the methodology derived from Townsend and Heuberger (1943) involved the selection of plants with a well-developed sub-crown internode (>2 cm) from each wheat plot during the late milk to early dough stage of development (Zadoks Growth Scale 77–84) (1974). A modified 0-5 scale (0: Healty plant, no color change in the mentioned areas; 1: Necrotic area less than 25%; 2: Necrotic area between 25-50%; 3: Necrotic area between 51-75%; 4: Necrotic area more than 75%; 5: Plant dead) proposed by Wildermuth and McNamara (1994) was utilized for assessment, encompassing a total of 60 plants in each fungicide application program. The winter damage rate was calculated by comparing the count of wheat seedlings before and after winter, utilizing the formula: winter damage rate (%) = (number of seedlings before winter - number of seedlings after winter/number of seedlings before winter) × 100. The grains were

harvested at 13% grain relative humidity (RH) (ZGS 89) using a small-plot harvester (Hege mod. 125B, Maschinenbau, Germany). The thousand-grain weight (TGW) and grain yield (GY) (ton/ha) were determined following the official methods of the Approved Methods of the American Association of Cereal Chemists (AACC 2000).

2.3. Grain Quality Parameters

Grain protein rate (GP) (%) by ICC 1995, Zeleny sedimentation value (mL) (ZS) by ICC method No: 166/1 (ICC 1972)), wet gluten rate (%) (WG) and gluten index (GI) (%) values by ICC method No. 155 (ICC, 1994) were determined in 1 kg seed samples taken from each plot.

2.4. Statistical Analysis

Significant differences between the genotypes were determined based on Fisher's least significant difference (LSD). Data were analyzed with the JMP software version 5.0.1 model.

3. Results and Discussion

3.1. Climatic Condition and Disease Relationship

The climatic conditions (temperature, rainfall, and high RH) were favorable for germination at the end of November and spike emergence at the beginning of May (Figure 1). During this period, the total rainfall was approximately 281 mm, and the average daytime temperature and relative humidity were approximately 14°C, and 79%, respectively. February (T1) was rainy. The natural conditions regarding average rainfall and RH determine the critical period for FCR.

The yield losses can be severe, even for resistant cultivars, when climatic conditions are favorable to disease development, due to the partial resistance in commercial cultivars against FCR (Scherm et al., 2013). In our study, the weather conditions were also suitable for FCR during February and March, which correlated with the T1 and T2 stages. There was abundant rainfall and high RH in February (14th day for ZGS25) and March (20th day for ZGS34) in the Trakya region, and high disease severity at these points. The application of fungicide to the infected wheat seed was also reasonably effective in reducing winter damage in the Trakya region.

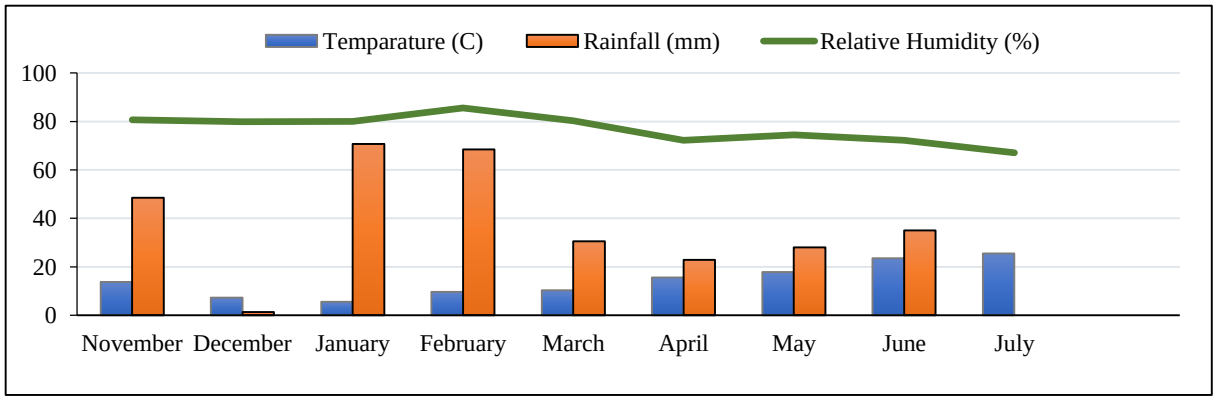

Figure 1. Monthly rainfall, high daytime temperature, and relative humidity at the meteorological station near the experimental site from November 10 to July 15, 2017 (Day of Year 314–365) and 2018 (Day of Year 1–196).

3.2. Field Experiment

This research offers the first comparison of FCR aggressiveness and control, with a focus on the wheat growth stage according to the Zadoks growth scale. The results of our study stand out in several important ways. First, in order to a fungicide application to be successful in reducing the disease severity of root and root collar infections, the fungicide must be applied to the seed. Secondly, depending on the developmental period of the wheat plant, the efficiency of the fungicide applied in the control of FCR may change, affecting yield. Thirdly, applying fungicide to the seed altered the efficiency of the fungicide used to control the FCR, demonstrating that it was effective in terms of FCR disease severity, sedimentation, and gluten. In addition, the application of fungicide to seeds was shown to have a significant effect in terms of reducing the winter damage rate of winter wheat (59.19%) (Figure 2). Brown lesions on the stem of the wheat up to the second node were registered in all plots (Figure 3).

Figure 2. Winter damage (%) of wheat after seed fungicide/non-seed-fungicide application.

Figure 3. Disease severity of FCR in the non-SF plot.

The application of a fungicide against *Fusarium* spp. to seeds, with the active ingredients fludioxonil, fluquinconazole, difenoconazole, maneb, mancozeb, metalaxyl-M, tebuconazole, prothioconazole, triadimenol, triticonazole, and thiram alone or in a mixture, has been reported to increase the seedling emergence, dry weight, and grain yield of *Fusarium*-infected wheat (Dawson and Bateman 2000; Arslan and Baykal 2002; Martin 2005; Schaafsma and Tamburic-Ilincic 2005; Demirci and Maden 2006; May et al., 2010; Köycü, 2018; Correia et al., 2020). Thiabendazole, particularly when applied to cultivars sensitive to crown rot, protects seedlings against pathogen infection by reducing disease development, ultimately leading to increased productivity (Pariyar et al., 2014). It has been shown that the efficacy of seed fungicide application is limited to the early stages of the wheat growth cycle, but it will not maintain its efficiency much beyond the seedling stage (Balmas et al., 2006). Our research, however, shows that the application of fungicide to the seed played a significant role in reducing *F. culmorum* DS, and verifies the effectiveness of fungicide application in terms of FCR management, as greater disease severity developed in non-SF compared to SF plants.

Interactions between the seed fungicide application and fungicide timing for FCR were statistically significant for the DS, ZS, and WG (Table 2). Disease severity was visually scored for crown rot symptoms, and evaluated per subplot. Epoxiconazole plus prochloraz resulted in significant ($P \leq 0.01$) differences in the DS, ZS, and WG values in the different growth stages of SF/non-SF plots. The application of fungicide at the different growth stages of the wheat plants caused no significant effects on protein rate or gluten index. Non-SF plants showed greater disease severity than SF plants, which was significant but still lower. The application of fungicide at different growth stages reduced the infections of FCR compared to the check. A high disease severity (DS) of *Fusarium culmorum* was observed in SF (26.50%) and non-SF (31.33%) plants at the same stage. T1-T2 and T1-T2-T3 showed significantly lower DS values at all stages with epoxiconazole plus prochloraz application in SF compared to non-SF. A significant increase in ZS occurred in the T1-T2 of SF compared to the check. The amount of wet gluten varied between 27 and 31%, and the highest value was found in the T1 stage in SF.

The optimum fungicide application time for FCR management and for ensuring an increased yield at wheat harvest is T1 (ZGS 25), as this prevents high levels of infection in the stem elongation and booting stages. The effect of fungicide application timing on FCR was most clear in the T1-T2 (ZGS25-ZGS34) stage, with the treatment applied twice in wet and humid environments. The critical period for FCR development is between seed germination and spike emergence. The weather conditions during the generative period, in which protein reserves are accumulated, have a significant impact on wheat quality (Finlay et al., 2007). The Zeleny sedimentation value is the most important quality property, as it influences the quality of gluten proteins as affected by the environment (Grausgruber et al., 2000). *F. culmorum* reduces the water-binding capacity of gluten; the quality of proteins and the baking quality of flour during storage can be reduced, causing significant losses in bread quality (Wang et al., 2005; Schmidt et al., 2017). The effects of fungicide application on grain quality parameters may vary depending on the fungicide used. The sedimentation value was significantly lower in Fusarium-infected spikes of wheat, and almost all cultivars contained higher wet gluten levels (Spanic et al., 2021). Epoxiconazole plus fenpropimorph, prothioconazole plus spiroxamine, epoxiconazole plus pyraclostrobin, epoxiconazole plus dimoxystrobine, and cyprodinil plus propiconazole, when applied to septoria leaf blotch, did not significantly affect Zeleny sedimentation when considered across years and locations (El Jarroudi et al., 2015). An increase in Zeleny sedimentation rate was detected in *Fusarium culmorum*-infected spike grains given prothioconazole+trifloxystrobin, compared to a control

(Köycü, 2022). In our study, the Zeleny sedimentation values were affected by the stage of fungicide application and the number of applications in SF and non-SF plants. The Zeleny sedimentation value increased when fungicide was applied to the seed in the T1-T2 period by 52 mL (4%) compared to the check; this was found to be the most effective spray period in terms of reducing FCR disease severity, and >30 mL was considered a high-quality grain. In addition, the gluten contents at different growth stages of wheat grains subjected to fungicide application were determined by the application of SF or non-SF. As a result, monitoring Zeleny sedimentation and wet gluten values after fungicide application under high FCR pressure is important for evaluating wheat flour quality. We determined that epoxiconazole plus prochloraz was more effective when the seed fungicide was applied to seeds infected with *F. culmorum* under the field conditions found in the Trakya region.

Table 2. Means of DS-disease severity ratio, GP-Grain protein content, ZS-Zeleny sedimentation value, WG-wet gluten content, GI-gluten index within seed fungicide/non-seed fungicide experiment conducted to determine the effects of fungicide application timing on *Fusarium culmorum* crown rot

	DS (%)	GP (%)	ZS (ml)	WG (%)	GI (%)
Seed Fungicide					
Check	26.50b*	13.16a	50.33b-e	30.00a-c	94.66a
T1	15.23d-f	13.06a	51.00a-d	31.33a	92.66a
T2	17.83c-e	13.30a	51.67ab	29.33a-d	93.00a
T3	19.17cd	12.70a	49.00c-f	27.67d	94.33a
T1-T2	5.67h	12.76a	52.00a	29.34a-d	93.66a
T2-T3	11.00g	13.16a	49,67b-f	29.67a-d	95.00a
T1-T3	15.33d-f	12.93a	51.00a-d	30.00a-c	94.00a
T1-T2-T3	6.00h	13.00a	51.00a-d	30.33ab	95.00a
Non-Seed Fungicide					
Check	31.33a	12.76a	48.33d-f	28.00cd	94.00a
T1	18.00cd	13.00a	48.00ef	29.00b-d	94.33a
T2	19.67c	13.13a	49.00c-f	28,66b-d	94.66a
T3	27.5ab	13.10a	51.33ab	30.00a-c	94.00a
T1-T2	13.67fg	12.96a	50.33a-d	29.67a-d	94.00a
T2-T3	14.00e-g	12.86a	49.00c-f	28.66b-d	94.00a
T1-T3	15.83c-f	13.23a	50.00a-f	30.67ab	95.00a
T1-T2-T3	13.83fg	12.86a	49.33b-f	29.33a-d	94.66a

*Means followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test at P≤0.01.

The FCR field treatment results summarize the effects of applying epoxiconazole plus prochloraz on GY (ton/ha), and TGW (g) concerning fungicide application time and number in the different growth stages of wheat (Figure 4). The differences between the means of seed fungicide application X timing interaction were not statistically significant for TGW and grain yield. There were significant (P≤0.01) differences in TGW and GY by fungicide application time and number. At all stages, the TGW was higher than in the check (31.18 g). TGW and TGW increase was higher than at the other stages at T2-T3 (39.13-25.53%) and T1-T2-T3 (39.49-26.69%). GY (ton/ha) ranged from 6.4 to 6.78, except for the check (6.47). The highest GY and GY increase was obtained when the fungicide was applied twice at the T1-T2 stage (6.89-6.49%). T3 exhibited lower TGW (36.21) and GY (6.47), according to the other stages. These results suggest that the fungicide application time and a number have differential effects on TGW and GY.

The results of this study suggest that the effective management of FCR depends on seed fungicide application, the timing of said application, and the number of repetitions. All of these factors have been shown to affect FCR disease severity and, together with wheat quality differences, may explain much of the recent fluctuation in the wet gluten and Zeleny sedimentation seen in the growth stages of wheat. Regarding the effects on wheat grain quality, fungicide application can have several positive outcomes. By reducing the severity of *Fusarium* crown rot, fungicides can help maintain the photosynthetic capacity of wheat plants, leading to improved grain filling and increased yield. This, in turn, can positively influence grain quality parameters such as Zeleny sedimentation and wet gluten. However, it's worth noting that the specific effects of fungicide application on grain quality can vary depending on several factors, including the disease pressure, timing and frequency of fungicide application, choice of fungicide, wheat variety, and environmental conditions. Different fungicides may have varying efficacy against *Fusarium culmorum* crown rot in the field, and their application must be carefully timed to achieve optimal results.

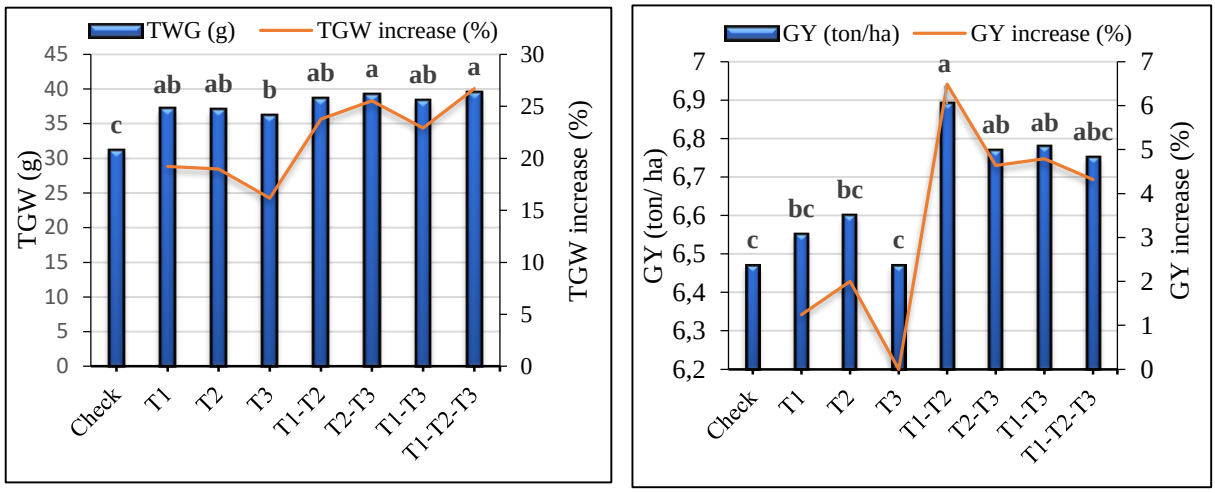

Figure 4. Thousand-grain weight (TGW), and grain yield (GY) of wheat grain after inoculation with *F. culmorum* in the harvest. Different letters above bars indicate significant (P≤0.01) differences according to LSD tests.

3.3. Correlations Between DS, TGW, and GY

Pearson's correlation coefficient between FCR disease severity and other parameters was calculated using the data from SF and Non-SF. In general, a higher correlation was observed in the SF application (Figure 5). FCR disease severity showed a significant (P≤0.01) negative correlation with TGW (r = 0.770) (r = 688) of both SF and non-SF applications respectively. In SF, DS/Yield were negatively correlated (r = 0.629) and GY/TGW were positively correlated (r = 0.406).

Significant positive relationships between TGW and yield (Samar et al., 2019). The results of the current study showed a significant positive relationship between TGW/GY when the fungicide was applicated to the seed. The high correlations were noted in the reduction between disease severity, TKW, and grain yield (Abdallah-Nekache et al., 2019).

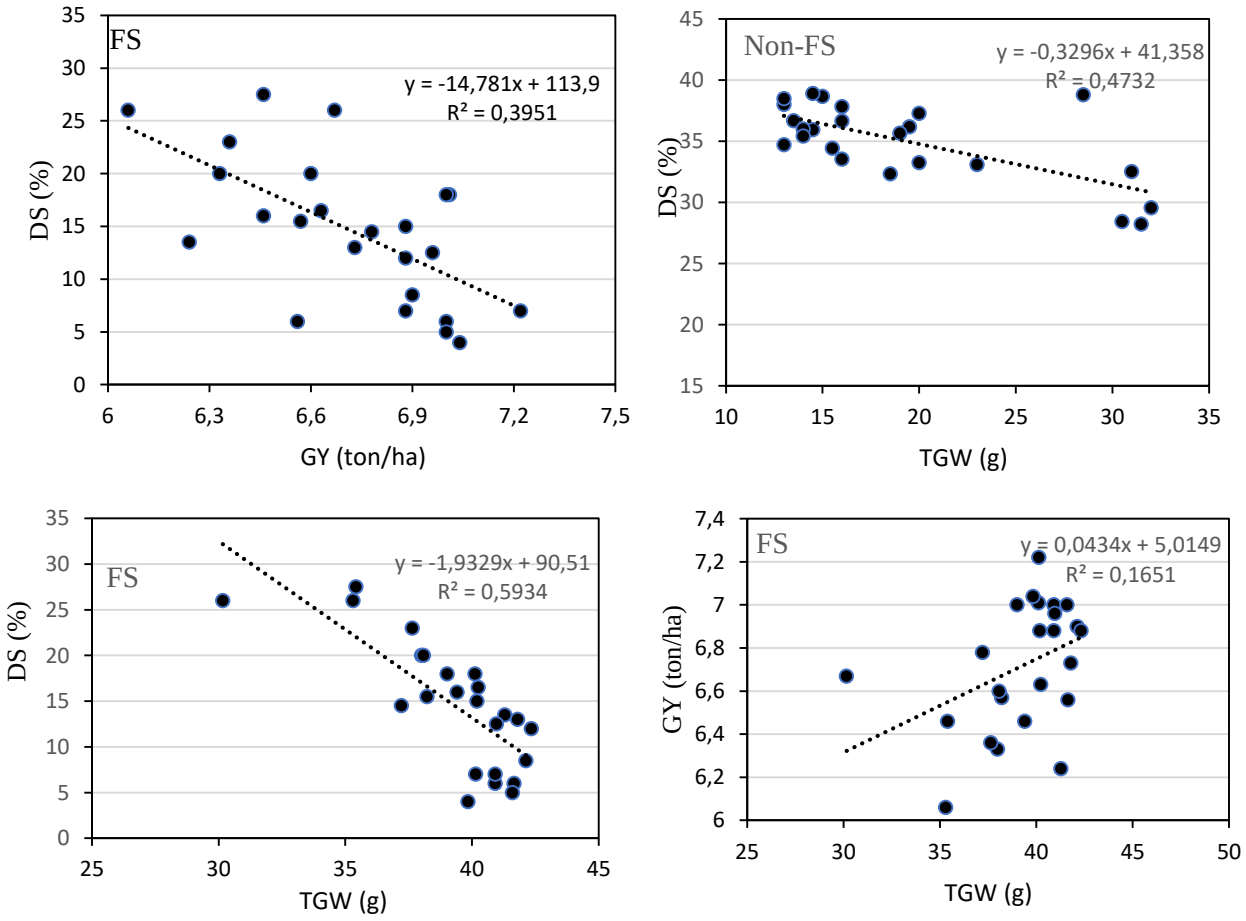

Figure 5. Correlation coefficients for FCR disease severity (DS), thousand-grain weight (TGW), and GY for seed fungicide (SF) and non-seed-fungicide (non-SF) (P≤0.01).

Author Contributions

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

	N.D.K.	F.S.
C	80	20
D	80	20
S	90	10
DCP	40	60
DAI	90	10
L	80	20
W	80	20
CR	80	20
SR	90	10
PM	80	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 there was no study on animals or humans.

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Research Article
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THE EFFECT OF SUPPLEMENTATION OF OAK TANNIN EXTRACT ON DIGESTIBILITY, METABOLISABLE ENERGY, METHANE PRODUCTION AND AMMONIA PRODUCTION IN LAMB DIETS

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Abstract: The aim of current experiment was to determine the effect of supplementation of oak tannin extract on gas production, methane production, digestibility, metabolisable energy and ammonia production of lamb diets using *in vitro* gas production technique. Oak tannin extract was included into total mixed ration at the 0, 2, 4 and 6 % on a dry matter basis. Although supplementation of oak tannin had no significant effect on gas, methane whereas supplementation had a significant effect on ammonia production of lamb diets. Gas and methane production of total mixed rations ranged from 50.25 to 53.25 ml and 7.72 to 8.15 ml respectively. Ammonia concentration of mixed rations ranged from 54.97 to 62.67 mg/100 ml. The decrease in ammonia of lamb diets per g oak supplementation was 0.1263 mg /100 ml. Metabolisable energy and organic matter digestibility of lamb diets ranged from 10.42 to 10.80 MJ kg DM and 70.27 to 73.02 % respectively. This study clearly showed that oak tannin had an anti-proteolytic potential for ruminant animals and supplementation of oak tannin significantly reduced ammonia production without compromising digestibility of diets. Therefore, oak tannin can be used to manipulate the rate and extent of degradation of protein in the rumen. However, before large implication, oak tannin should be further investigated using in vivo experiment to determine the toxic level of oak tannin in ruminant animals.

Keywords: Oak tannin, Methane production, Ammonia production, Total mixed ration

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1. Introduction

Methane and NH_3, which are produced when protein and carbohydrates ferment in the rumen, pose a threat to the environment and deplete dietary energy and nitrogen that could be used to produce milk and meat (FAO, 2006; Eckard et al., 2010; Ozkan, 2016). Methane is created by Archaea from the metabolic H_2 that rumen microbioata produce (Demeyer and Van Nevel, 1975; McAllister and Newbold, 2008). Amino acid deamination and feed protein degradation are caused by hyperammonia-producing bacteria. Nitrous oxide emissions from the rumen's extensive protein and amino acid breakdown produce a significant amount of NH_3 and urea, which may be expelled with urine and contribute to ground water contamination and greenhouse gas emissions (Weimer, 1998).

Significant attention has been paid to tannin, essential oils and saponin, which are derived from plants with antibacterial, antifungal, and antioxidant qualities to manipulate rumen fermentation since the use of antibiotics as growth promoters was outlawed (Cowan, 1999; Waghorn et al., 2002; Kamalak et al., 2005; Kamra et al., 2006; Castillejos et al. 2006; Benchaar et al., 2007; Garcia et al., 2007; Ozkan et al., 2016).

Numerous studies have indicated that dietary tannins may be able to reduce the amount of methane produced in the rumen (Woodward et al., 2001; Waghorn et al., 2002; Kamra et al., 2006; Ozkan, 2016). Tannin blocks the production of methanogens directly or indirectly by inhibiting protozoa. (Animut et al., 2008; Bhatta et al., 2009; Jayanegara et al., 2009). By using tannins at low concentrations, it may be possible to boost the efficiency of microbial protein synthesis and reduce rumen protein breakdown (Makkar, 2000). However, there is limited information about the effect of oak tannin on the fermentation parameters of lamb diets. It was hypothesized that the oak tannin may decrease the methane production and extensive degradation of protein in the rumen.

The aim of current experiment was to determine the effect of supplementation of oak tannin extract on gas production, methane production, digestibility, metabolisable energy, and ammonia production of lamb diets using *in vitro* gas production technique.

2. Materials and Methods

Iso-caloric and iso-nitrogenic lamb diets formulated using the concentrate ingredients namely barley grain, soybean meal and alfalfa hay is given in Table 1. Oak tannin extract was included into lamb diets at the 0, 2, 4 and 6 % on a dry matter (DM) basis.

In vitro gas production experiment was carried out with permission of Animal Ethic committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (Protocol No: 2021/03-2). *In vitro* gas production of lamb diets was determined for 24 h (Menke et al., 1979). Rumen fluid was obtained from three fistula sheep fed with a diet containing alfalfa hay and concentrate before morning feeding. The rumen fluid was transferred into laboratory and combined with buffer solution. The samples were incubated in triplicate in glass syringes with buffered rumen fluid for 24 h in the water bath set at 39 °C.

The ME and OMD of diets were estimated using the equations 1 and 2 as follows (Menke and Steingass, 1988).

$$ME\ (MJ/kg\ DM) = 1.68 + 0.1418*GP + 0.073*CP + 0.217*EE - 0.028*CA \quad (1)$$

$$OMD\ (\%) = 14.88 + 0.889*GP + 0.45*CP + 0.651*CA \quad (2)$$

here, GP = Gas production for 24 h (ml), CP = Crude protein (%), EE: Ether extract (%), CA: Crude ash (%)

Methane content (equation 3) of gas produced was determined using the infrared methane analyzer (Goel et al., 2008).

$$Methane\ production\ (ml) = Total\ gas\ production\ (ml) \times Percentage\ of\ methane\ (\%) \quad (3)$$

After determination of gas production, the content of syringes was transferred into glass bottles for distillation unit to determine ammonia-N content of lamb diets. The Ammonia-N content of diets were calculated as given in equation 4.

$$mg\ N\ (NH3\text{-}N) = 0.1 \times 14 \times (A-B) \quad (4)$$

2.1. Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the effect of oak tannin on *in vitro* gas production, methane production, ammonia production, metabolisable energy and organic matter digestibility of lamb diets. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $P<0.05$.

3. Results and Discussion

The effect of oak tannin on *in vitro* gas production, methane production, ammonia production, metabolisable energy and organic matter digestibility of lamb diets is given in Table 2. Supplementation of oak tannin had no significant effect on gas production, methane production, metabolisable energy and organic matter digestibility of lamb diets. Gas production and methane production ranged from 50.25 to 53.23 ml and 7.447 to 8.15 ml respectively. The percentage of methane gas ranged from 14.90 to 15.32%.

It was suggested that tannin might reduce the methane production through reduction in fibre digestion and inhibition of growth of methanogens (Tavendale et al., 2005). Previous investigation showed that the supplementation of tannin from different sources to diets had a significant effect on gas production and methane production (Jayanegara et al., 2015) whereas supplementation of tannin had no significant reduction in methane production in the current experiment. The differences between these studies are likely to be associated with difference in tannins used (Jayanegara et al., 2015).

Metabolisable energy and organic matter digestibility of lamb diets ranged from 10.42 to 10.80 MJ kg DM and 70.27 to 73.02 % respectively.

Oak tannin supplementation significantly decreased the ammonia production of lamb diets. Ammonia concentration of mixed rations ranged from 54.97 to 62.674 mg/100 ml. This result obtained in the current study is in agreement with findings of Pinski et al. (2015) who found that condensed tannin decreased ammonia production.

Table 1. The chemical composition of diets including oak tannin (%DM)

	Diets			
Ingredients	0%	2%	4%	6%
Barley	600	600	600	600
Oak tannin	0	20	40	60
Oil	1	2	3	4
Soybean meal	109	118	130	142
Alfalfa hay	264	234	201	168
NaCl	10	10	10	10
CaCO3	15	15	15	15
Min-Vit	1	1	1	1
Total	1000	1000	1000	1000
ME (Kcal)	2654	2652	2653	2654
CP	170	170	170	170

CP= crude protein, ME= metabolisable energy.

Table 2. The effect of oak tannin on *in vitro* gas production, methane production, ammonia production, metabolisable energy and organic matter digestibility of lamb diets

	Diets					
Parameters	0	20	40	60	SEM	Sig.
Gas	50.75	51.25	53.25	50.25	1.567	N.S
CH_4(%)	15.22	15.00	15.32	14.90	0.343	N.S
CH_4(ml)	7.72	7.70	8.15	7.47	0.272	N.S
NH_3(mg/100 ml)	62.67[a]	59.00[ab]	56.85[b]	54.97[b]	1.389	***
ME (MJ)	10.42	10.52	10.80	10.40	1.389	N.S
OMD (%)	70.97	71.32	73.02	70.27	1.388	N.S

[ab] same common superscripts letters in the row shows statistical similarity (P>0.05), SEM= standard error mean, Sig= significance level, ***= P<0.001.

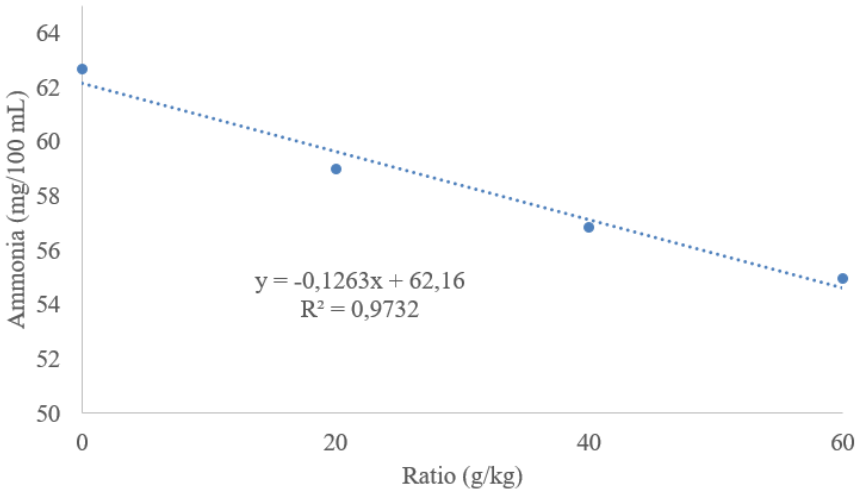

Figure 1. The relationship between oak tannin and ammonia production.

The relationships between oak tannin doses and ammonia production is given in Figure 1. Ammonia production decreased with increasing level of condensed tannin. The decrease in ammonia of lamb diets per g oak supplementation was 0.1263 mg /100 mL.

The clearly showed that supplementation of oak tannin had a significant effect on the degradation of protein in lamb diets without compromising digestibility of diets. Therefore, it can be suggested that oak tannin can be included into lamb diets to prevent the extensive degradation of protein of diets. However, care must be taken into consideration that hydrolysable tannin can be toxic to ruminant animals (Jayanegara et al., 2015). The safe level of oak tannin inclusion should be determined before large implication.

4. Conclusion

This study clearly showed that oak tannin had an anti-proteolytic potential for ruminant animals and supplementation of oak tannin significantly reduced ammonia production without compromising digestibility of diets. Therefore, oak tannin can be used to manipulate the rate and extent of degradation of protein in the rumen. However, before large implication, oak tannin should be further investigated using *in vivo* experiment to determine the toxic level of oak tannin in ruminant animals.

Author Contributions

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

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

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

In vitro gas production experiment was carried out with permission of Animal Ethic committee of Kahramanmaras Sutcu Imam University, Faculty of Agriculture (approval date: March 02, 2021, protocol code: 2021/03-2).

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Research Article
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DETERMINATION OF THE EFFECTS OF DIFFERENT EMS DOSES APPLIED TO SEEDS OF CHICKPEA AND LENTIL VARIETIES ON SOME SEEDLING CHARACTERISTICS

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Abstract: In this study, it was aimed to determine the effects of 11 different doses of Ethyl Methane Sulphonate (EMS) applied to the seed to create variation in the M1 generation of chickpea (Gökçe) and lentil (Şakar) genotypes during germination and seedling development periods and to determine the lethal dose that caused a 50% reduction in plant emergence rate. The research was conducted under the greenhouse and laboratory conditions of the Faculty of Agriculture of Dicle University in 2019/2020. The study was conducted according to a randomized block design with three replicates. For the M1 generation, seeds of each genotype in the elite stage were treated with EMS solution at 0 (control), 10, 20, 30, 40, 40, 50, 60, 60, 70, 70, 80, 90, and 100 mM (1000 seeds for each dose) and then sown in the greenhouse. A total of 132 tubes were sown with 30 seeds for each dose, and the effective EMS dose was determined for each genotype based on the traits examined in the developing seedlings. In the study, it was concluded that 11 different EMS doses applied to the seeds of chickpea and lentil varieties had negative effects on seedling development in the M1 generation, and increasing EMS doses from the control caused a decrease in all traits examined. With increasing EMS doses, plant emergence was observed in both chickpea and lentil up to 60 mM dose, while no germination was observed at 60 mM dose, and the dose rate varied according to species and varieties. The dose that caused a 50% decrease in the plant emergence rate in Gökçe chickpea and Şakar lentil varieties was determined as the LD50 dose. Accordingly, it was determined that the LD50 dose was 30 mM for Gökçe chickpea variety and 60 mM for Şakar lentil variety.

Keywords: Chickpea, EMS, Lentil, LD50, Mutation

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1. Introduction

Legumes, which have been cultivated since ancient times, are of great importance in terms of meeting the protein requirements of plant origin for human nutrition. Grain legumes, which contain high levels of crude protein, are especially rich in essential amino acids, such as Lysine, Leucine, Isoleucine, vitamins A and B, and mineral substances (Şehirali, 1988). Legumes enrich the soil in terms of nitrogen by binding the free nitrogen in the air with Rhizobium bacteria, forming nodosity in their roots (Özdemir, 2002). After the harvest of the plant, the high amount of nitrogenous organic compounds contained in the roots is decomposed by microorganisms in the soil, some of which decompose, and the plants planted later benefit from this nitrogen. In addition, legume roots aerate the soil, prevent soil compaction, improve the physical, chemical, and biological properties of the soil, and contribute to the maintenance of soil fertility (Şehirali, 1988). For these reasons, the cultivation of grain legumes in crop rotation in our country is of particular importance.

As with other crops, it is necessary to develop new varieties of legumes that are resistant to diseases and pests, suitable for machine harvesting, high quality, high yield, and suitable for the demands of domestic consumers and foreign markets. For this purpose, in conditions where genetic problems cannot be solved when developing new varieties with traditional plant breeding methods, one or two characteristics of a productive variety with high adaptability can be increased by mutation breeding. Mutations occur in two ways: spontaneous (natural) and induced (artificial). Artificial mutations are caused by physical mutagenesis (X-rays, gamma rays, ultraviolet radiation, beta radiation, neutrons), chemical mutagenesis (basic analogs and related compounds, antibiotics, alkylated substances, azides) (Spencer-Lopes et al., 2018). Induced mutation or mutagenesis is defined as sudden heritable changes in the genome of an organism that do not result from genetic recombination but are induced by physical, chemical, or biological agents (Roychowdhury and Tah, 2013). Chemical mutagens primarily induce single point mutations and contribute to the development of new varieties with improved traits, such as high yield, short plant height, and disease resistance in breeding programs (Khursheed et al., 2015; Tantray et al., 2017). Among the chemical mutagens, Ethyl Methane Sulphonate (EMS) is the most popular alkylating agent

among plant breeders because it is easily detoxified and used (Pathirana, 2011; Hassan et al., 2021). EMS mutagenesis causes alkylation, so the original base is not physically altered (Kantoğlu and Kunter, 2021).

Mutation breeding studies aim to obtain the highest mutation frequency with the least damage. Mutagen doses and application methods should be selected appropriately for this purpose, and changes in M1 plants and the resulting physiological damage should be determined quantitatively. Generally, doses that kill 50-70% of the seedlings are determined as the appropriate mutagen dose and are called the LD50 dose (Lethal Dose) (Şehirali and Özgen, 1988). According to IAEA (International Atomic Energy Agency) data for 2021, 2652 varieties were registered in physical mutagen applications, 677 varieties in chemical mutagen applications, and 36 varieties in chemical + physical mutagen applications. Worldwide, 1648 mutant varieties have been registered in 15 cereal species and 424 mutant varieties in 18 legume species. In legume species, 27 chickpea (*Cicer arientum* L.) and 18 lentils (*Lens culinaris* L.) mutant varieties have been registered (IAEA, 2021).

In this study, we aimed to determine the effects of 11 different EMS doses applied to the seeds of chickpea and lentil varieties on seedling development in the M1 generation and to determine the appropriate EMS dose.

2. Materials and Methods

The research was carried out under the greenhouse and laboratory conditions of the Faculty of Agriculture of Dicle University in 2019/2020. In this study, eleven different EMS doses, one lentil (Şakar), and one chickpea (Gökçe) variety were used as plant materials. The study was set up in a randomized block design, with three replicates in a greenhouse.

2.1. EMS (Ethyl Methane Sulphonate) Application

EMS application was carried out under the laboratory conditions of the Dicle University Faculty of Agriculture. The EMS amounts calculated for different EMS doses used in this study are listed in Table 1.

EMS was applied to the seeds, according to the method described below. For each dose, 1000 seeds of each variety were first kept in pure water for 6 h, thereby increasing the permeability of the seed coat. The seeds were then thoroughly filtered and kept in 11 different EMS solutions (0 (control), 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mM) for 6 h. The seeds were mixed every hour with a metal spoon for better mutagen penetration. The seeds were rinsed thrice with distilled water to remove EMS mutagens. The washed seeds were dried in the laboratory under air circulation. The dried seeds were planted under greenhouse conditions.

To determine the seedling growth properties of the different plant species, M1 seeds were sown in a controlled greenhouse. Peat (2/3) and perlite (1/3) were used as the soil in the greenhouse, and the seeds were sown on 24.12.2019 according to a randomized plot design with three replications. Thirty seeds were sown per EMS dose. Temperature and humidity values during seedling growth are shown in Figure 1.

The following parameters were examined: plant emergence rate (%), first leaf length (cm), root length (cm), seedling height (cm), fresh seedling weight (g), and dry seedling weight (g), which were determined by measuring the seedlings on the 28th day of M1 plants.

Statistical analyses of the parameter values obtained from the greenhouse experiment were performed using the JMP Pro 13 statistical package program.

Table 1. EMS doses used in the study

EMS doses	Amount of EMS used (ml)	Amount of pure water used (ml)
Control	0.000	500.000
10 mM	0.542	499.458
20 mM	1.084	498.916
30 mM	1.626	498.374
40 mM	2.168	497.432
50 mM	2.710	497.290
60 mM	3.252	496.748
70 mM	3.794	496.206
80 mM	4.336	495.664
90 mM	4.879	495.121
100 mM	5.421	494.579

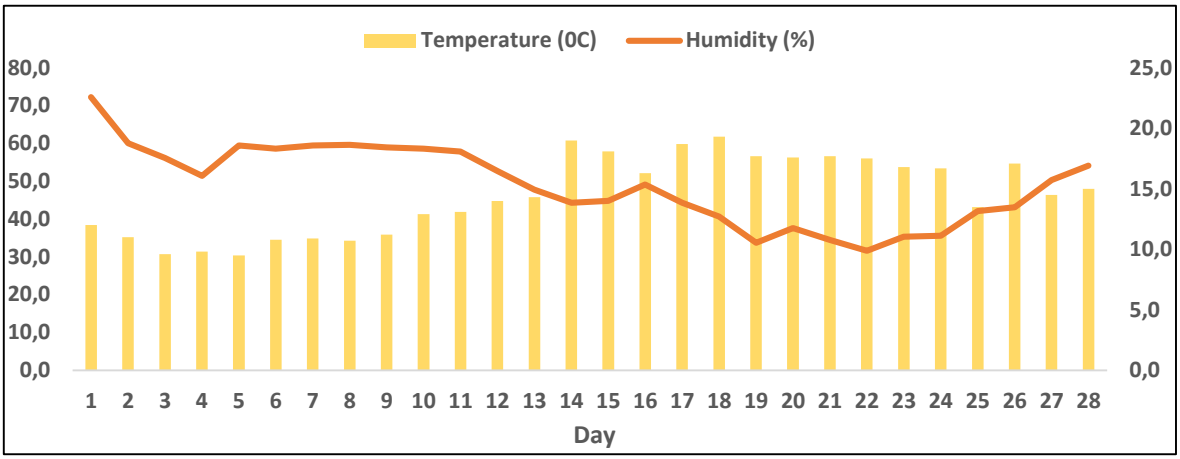

Figure 1. 28-day temperature and humidity data of greenhouse conditions during seedling growth of M1 legume plants.

3. Results and Discussion

The plant emergence rate, root length, seedling height, seedling fresh weight, and seedling dry weight were evaluated in M1 stage legume species generated with eleven different EMS doses under greenhouse conditions. ANOVA revealed that different EMS doses resulted in changes in all properties evaluated across legume species. Regarding the average seedling dry weight, no statistical differences were identified between chickpea cultivars (Figure 2, 3, 4, 5, and 6).

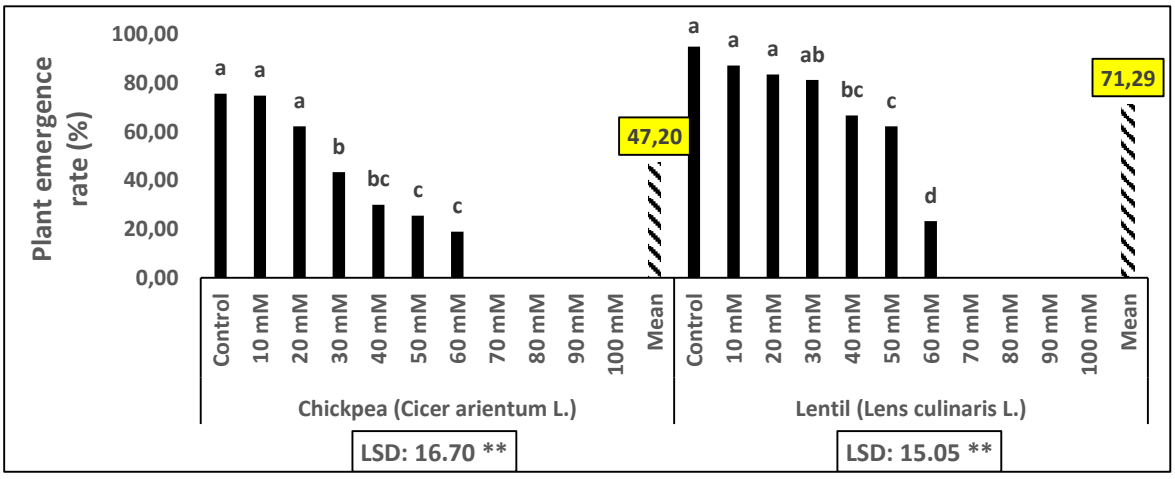

Figure 2. Plant emergence rates (%) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

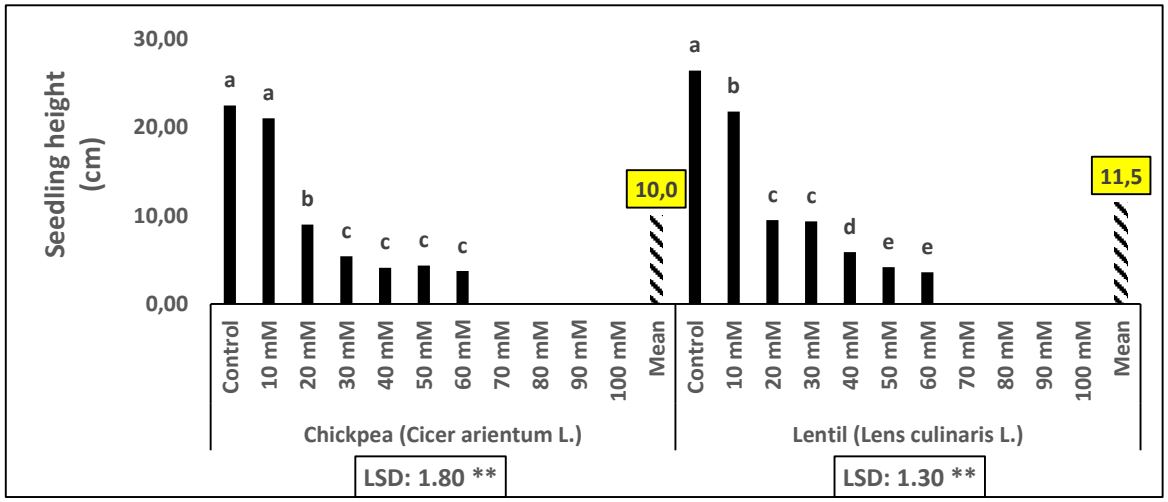

Figure 3. Seedling height (cm) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

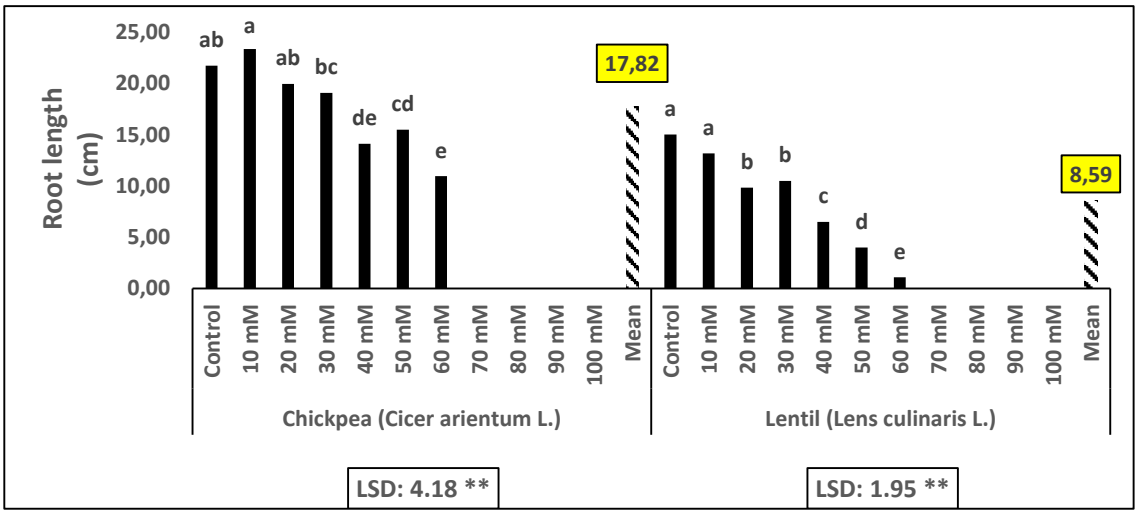

Figure 4. Root lenght (cm) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

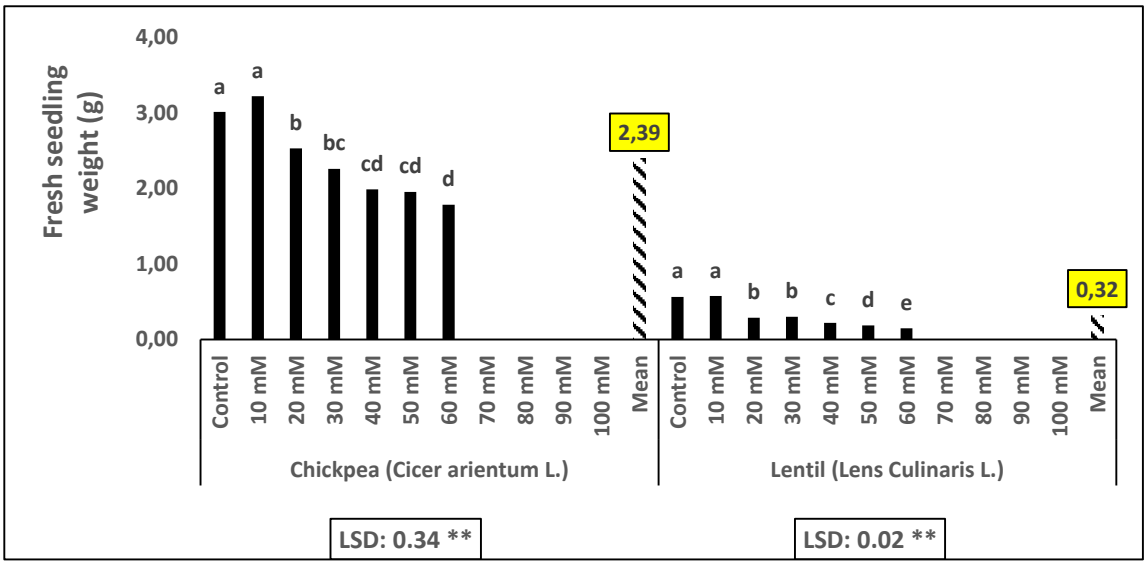

Figure 5. Fresh seedling weight (g) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

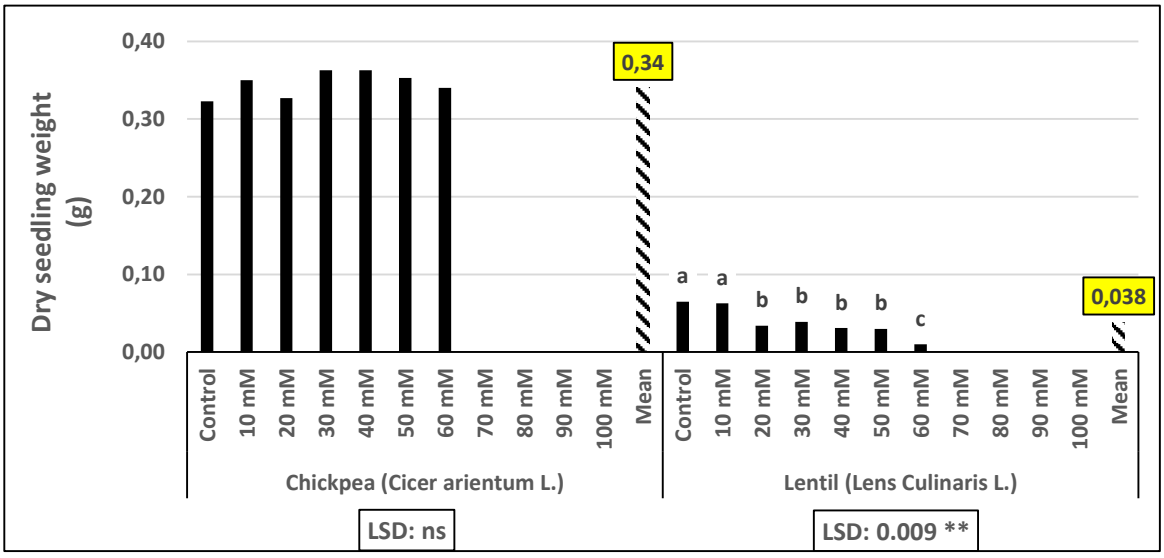

Figure 6. Dry seedling weight (g) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

As shown in Figure 2, while plant emergence was observed up to a 60 mM dose in both chickpea and lentil plant species with increasing EMS doses, no germination was observed at doses of 60 mM. The plant emergence rate decreased with an increase in EMS doses in the Gökçe chickpea and Şakar lentil cultivars. The average emergence rate varied between 18.89% (60 mM EMS dose) and 75.56% (control dose) in the Gökçe chickpea cultivar and between 23.33% (60 mM EMS dose) and 94.97% (control dose) in the Şakar lentil cultivar. A higher plant emergence rate was observed in the lentils than in the chickpeas.

It was concluded that EMS and other mutation sources used as mutagens had different effects on the characteristics examined in M1 plants and that the mutagenic effect increased with an increase in dose (Talebi et al., 2012; Güvercin et al., 2020; Aher and Koche, 2022). Researchers have reported that the success of mutations depends on the efficacy of the mutagen used, duration, frequency, and genotype (Sikora et al., 2011; Anbarasan et al., 2013; Arisha et al., 2015).

Deepika et al. (2016) reported that the reduction in seed germination may be due to the effect of mutagen on the meristematic tissues of the radical/plumula. Kulkarni (2011) reported that one of the physiological effects caused by chemical mutagen application is disorders in the formation of enzymes involved in the germination process. Researchers have found that the highest emergence was observed in the control group in proportion to the increasing EMS doses in the calculation based on days from emergence in M1 plants, and the values obtained decreased significantly compared with the control (Jadhav et al., 2012; Anbarasan et al., 2013; Jagajanantham et al., 2013; Baghery et al., 2016). Similar inhibitory effect of various mutagenic treatments on seed germination has been previously reported in Lentil (Kumar and Sinha, 2003), Cowpea (Gaur et al., 2003), Chickpea (Khan and Wani, 2005; Aher and Koche; 2022), Grass Cowpea (Ramezani and More, 2013) and Cluster bean (Deepika et al., 2016).

Researchers have reported that plant emergence rate in mutagen applications in M1 generation; Akıncı (1999) 16.67 - 98.90% (300 Gy-control), Acar (2010) 38.13 - 76.63% (0.4% EMS-control), Baghery et al. (2016) 55.67 - 97.67% (1,050% EMS-control), Olaolorun et al. (2019), 71.26 - 75.92% (EMS application-control), Güvercin et al. (2020) 47.11 - 89.87% (1% EMS-control), Yorulmaz et al. (2021) 0.00 - 93.33% (30 mM EMS-control) and Aher and Koche (2022) 31.56 - 93.37% (0.3% EMS-control).

The LD50 is used by most researchers to determine the lethal dose of mutagens (Warghat et al., 2011; Talebi et al., 2012; Anbarasan et al., 2013). In every mutation-breeding program, the LD50 was initially determined, which was used as the optimum concentration for induction. If this step is ignored, the mutagen dose may be high or low and mutation frequency may occur. LD50, which is defined as the mutagen dosage resulting in a 50% reduction in seed germination after exposure of seeds to mutagen for a certain period of time and under certain conditions, is often used to compare the effect of mutagens in seeds treated under different conditions (Bharathi et al., 2013; Beyaz et al., 2016). In the study, the dose that caused a 50% decrease in plant emergence rate among 11 different EMS dose applications applied in legume species was determined as LD50 dose. Accordingly, the LD50 dose was determined to be 30 mM for Gökçe chickpea variety and 60 mM for Şakar lentil variety (Figure 2.). Özkan et al. (2021) found that increasing the dose of EMS in chickpea plants decreased the germination rate of seeds, seedling characteristics and the proportion of living healthy plants and that the effective mutation dose on all traits can be obtained from doses between 50-60 mM.

Regarding the seedling characteristics examined in this study, the highest values obtained from control and 10 mM EMS doses for chickpeas, and the control group for lentils. In addition, the highest seedling height in lentil was followed by 10 mM EMS doses. The values for these characteristics decreased proportionally with increasing EMS doses. There were variations in the intermediate doses, despite the fact that the doses at which the highest and lowest readings were attained were the same. At the end of the 28th day, the root length and seedling height of the Gökçe chickpea variety varied between 10.97 - 23.37 cm and 3.73 - 22.43 cm, respectively (Figure 3 and 4). It was determined that seedling fresh weight value varied between 1.78 - 3.22 g and the seedling dry weight value varied between 0.323 - 0.363 g between doses (Figure 5 and 4).

At the end of the 28th day, the root length and seedling height of the Şakar lentil variety varied between 1.10 - 15.03 cm and 3.60 - 26.38 cm respectively (Figure 3 and 4). The seedling fresh weight ranged from 0.147 - 0.573 g, and the seedling dry weight ranged from 0.010 - 0.065 g between the doses (Figure 5 and 4).

Researchers have reported that all traits examined during the seedling stage decrease proportionally with increasing EMS doses, and there are statistically significant differences in the traits examined during the seedling stage (Talebi et al., 2012; Lukanda et al., 2013; Özkan et al., 2021). Seedling height is often used as an index to determine the biological effects of different physical and chemical mutagens on M1 (Bhat et al., 2007). There is a linear dependence between seedling height and dose of physical or chemical mutagens (Talebi et al., 2012). In studies conducted in different plant species, researchers have shown that seedling height decreases owing to the mutagenic effect of EMS (Talebi et al., 2012; Anbarasan et al., 2013; Ambli and Mullainathan, 2014). Researchers reported that the length value measured at seedling stage varied between 8.40 - 33.04 cm (Talebi et al., 2012), 1.27 - 18.04 cm (Atmaca et al., 2012), 25.93 - 33.73 cm (Olaolorun et al., 2019) and 21.87 - 26.83 cm (Yorulmaz et al., 2021) in mutagen treatments. In agreement with these results, our findings indicated that the decrease in seedling height was caused

by an increase in EMS concentration.

The highest root length value of M1 plants was generally obtained from the control group, and the values decreased with increasing doses of EMS (Shah et al., 2012; Ambli and Mullainathan, 2014). Dhakshinamoorthy et al. (2010) reported that 4% EMS treatment caused a 35% reduction in root length compared to 1% EMS treatment, while Anbarasan et al. (2013) reported that 1.8% EMS treatment reduced root length by 46% compared to 0.4% EMS treatment. Talebi et al. (2012) reported that root length decreased with increasing EMS concentration. The values of root length in mutagen treatments were reported to vary between 7.24 - 15.71 cm (Atmaca et al., 2012), 2.23 - 9.66 cm (Talebi et al., 2012) and 5.28 - 7.69 cm (Ambli and Mullainathan, 2014).

In previous studies, it was observed that seedling fresh weight and dry weight values decreased, especially in plants exposed to mutations (Atmaca et al., 2012; Yorulmaz et al., 2021). Saba and Mirza (2002) stated that there was a decrease in fruit weight compared to the control group plants with EMS application at different times and doses. Seedling fresh weight in the M1 generation has been reported to vary between 0.000 - 0.250 g (Başer et al., 2005), 0.010 - 0.630 g (Atmaca et al., 2012) and 0.850 - 0.647 g (Yorulmaz et al., 2021). The values of seedling dry weight in the M1 generation have been reported to vary between 0.025 - 0.037 g (Akıncı, 1999) and 0.071 - 0.051 g (Yorulmaz et al., 2021).

The correlation relationships of the traits measured during the seedling stage in chickpea and lentil varieties treated with 11 different EMS doses are given in Figure 7 and 8. Based on these results, it was determined that there was a significant and positive correlation between all traits examined in both the Gökçe chickpea and Şakar lentil varieties. However, there was a negative and insignificant correlation between seedling dry weight and the other traits examined in the Gökçe chickpea variety. In previous mutation applications, Başer et al. (2005) reported significant and positive correlations between emergence percentage, seedling height, seedling fresh weight, and root length traits measured at the seedling stage. Adebisi et al. (2010) observed a positive relationship between germination rate and other seedling traits measured in mutation treatment. Olaolorun et al. (2019) reported that there was a negative correlation between plant emergence rate, seedling height, and root length because seedlings with early emergence had a better root structure and a higher chance of developing into taller plants.

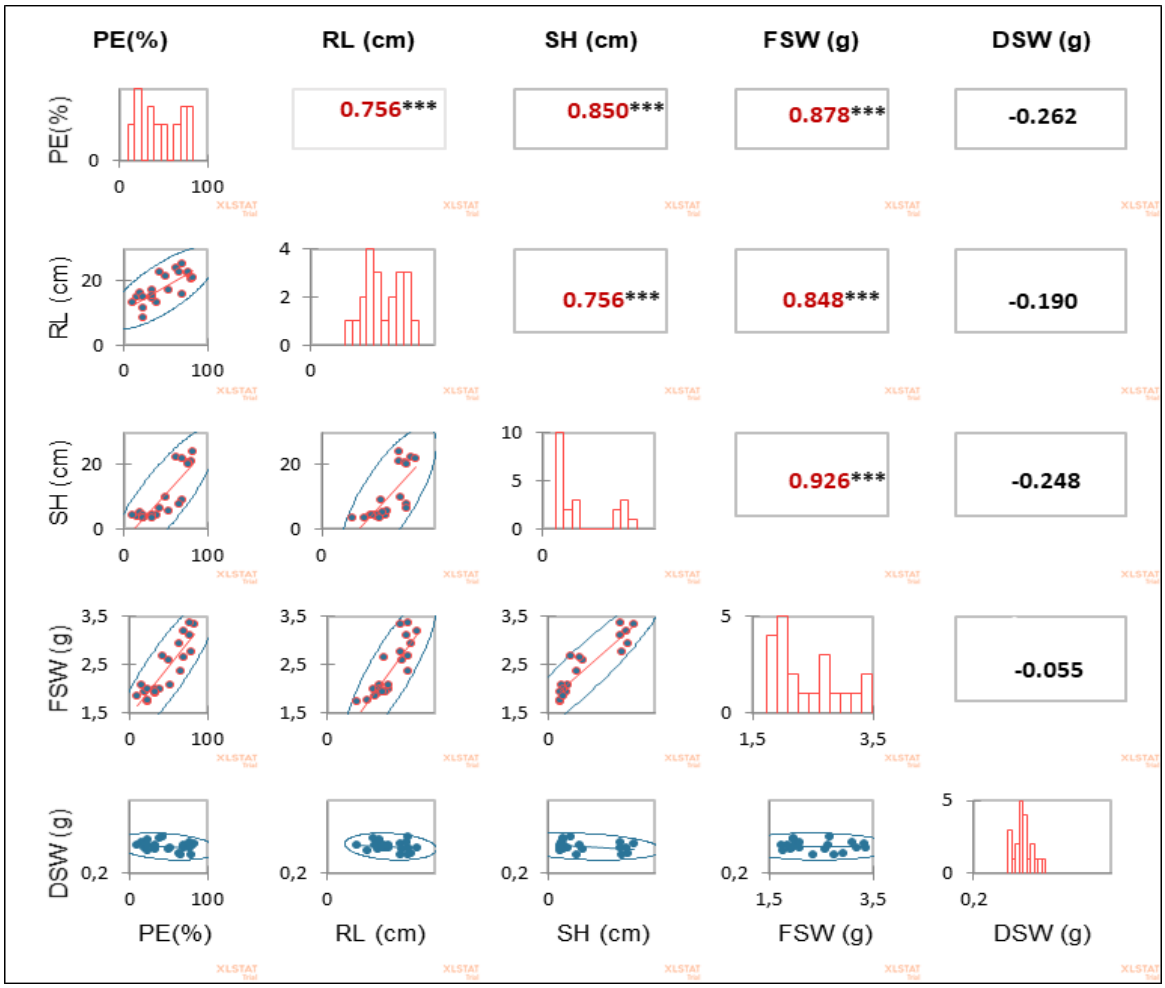

Figure 7. Correlation analysis of the traits analyzed in Gökçe chickpea variety. PE= plant emergence rate, RL= root length, SH= seedling height, FSW= fresh seedling weight, DSW= dry seedling weight.

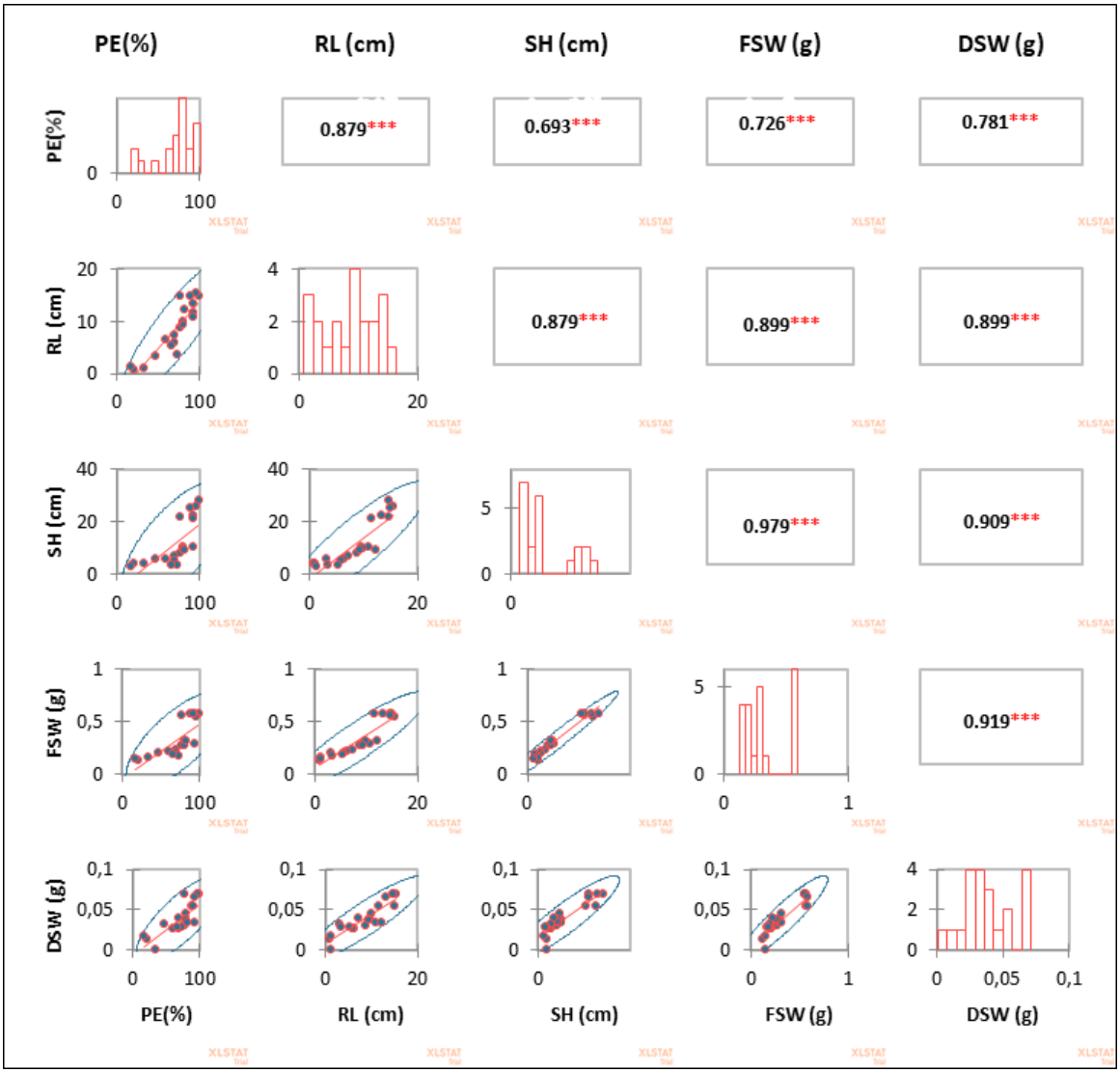

Figure 8. Correlation analysis of the traits analyzed in Şakar lentil variety. PE= plant emergence rate, RL= root length, SH= seedling height, FSW= fresh seedling weight, DSW= dry seedling weight.

5. Conclusion

This Mutation breeding is the easiest and fastest source of variation for plant breeders, and has been widely used worldwide for research purposes. From the past to the present, it has been demonstrated that artificial mutation studies can lead to positive improvements in terms of yield and yield elements, although different plant species may suffer different physiological and chemical damages in different environments and applications. Mutant varieties have been developed and registered in many plants worldwide. In this study, it was concluded that 11 different EMS doses applied to the seeds of some chickpea and lentil varieties had negative effects on seedling development in M1 generation and increasing EMS doses from the control caused a decrease in all traits examined. With increasing doses of EMS, plant emergence was observed in both chickpea and lentil up to 60 mM dose, while no germination was observed at 60 mM dose and the dose ratio varied according to species and varieties. In the Gökçe chickpea and Şakar lentil varieties, the dose that caused a 50% decrease in the plant emergence rate was determined as the LD50 dose. Accordingly, LD50 dose was determined to be 30 mM for Gökçe chickpea variety and 60 mM for Şakar lentil variety.

Author Contributions

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

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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Research Article
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RESPONSE OF WINTER CANOLA VARIETIES TO BORON STRESS DURING GERMINATION AND SEEDLING GROWTH STAGE

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Abstract: The objective of the study was to determine the effects of boron concentrations on germination and seedling growth of winter canola varieties under laboratory conditions. Seeds of four winter canola varieties (KWS Cyrill CL, Miranda, PT264, and NK Caravel) were germinated between papers with different boron levels (0, 20, 40, 60, 80, and 100 mg B L^{-1}) consisting of sodium borate ($Na_2B_8O_{13}.4H_2O$) at 20°C for 7 days. The germination percentage, mean germination time, germination index, seedling growth parameters, and dry matter were measured. The results showed that germination percentage, mean germination time, and germination index were negatively affected by increasing B concentrations. When B levels increased, root and shoot lengths and weights were also inhibited, while the responses of canola varieties differed. B levels had a significant effect on shoot length, which decreased from 5.15 cm to 1.82 cm and root length from 4.99 cm to 2.59 cm. Under boron stress, KWS Cyrill CL germinated higher and developed longer roots and shoots. Differences in both germination and seedling growth among canola cultivars were observed at 80 mg B L^{-1} and higher. It was concluded that there was a genotypic variation among canola varieties concerning boron toxicity and that KWS Cyrill CL was more tolerant to high boron concentrations than the other varieties.

Keywords: *Brassica napus* L., Germination, Seedling growth, Boron, Toxicity

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1. Introduction

Vegetable oils play a crucial role in maintaining a healthy and balanced human diet. The production of edible oils is required to meet the demands of both private households and industries, and at the same time creates promising employment opportunities (Safdar et al., 2023). Canola (*Brassica napus* L.) is a globally important oilseed crop used for human consumption, a source of protein-rich animal feed, and a renewable resource for biodiesel production (Zhao et al., 2020). It contains 40-46% high-quality oil, and its meal has a protein content of 38-40% (Samreen et al., 2022). In 2022, the sowing area of canola in Türkiye was 41.100 ha, and seed production was 150.000 tons (TÜİK, 2023).

Boron (B), an important micronutrient for crop plants, stimulates plant growth and yield. It plays a key role in many physiological processes such as protein formation, cell division, cell wall construction, cell membrane integrity, and root growth (Marschner, 1995; Gupta, 2016). However, high levels of boron (B) inhibit germination and growth of crops, along with other abiotic stresses like extreme temperatures, drought, salinity, flooding, and heavy metal (Mozafar, 1993; Rerkasem et al., 1997; Landi et al., 2019). B requirements vary for different crops, and the optimal concentrations for one species may be toxic to another (Blevins and Lukaszewski, 1998). The concentration of boron in arable soil ranges from 1 to 467 mg kg^{-1}, with a general variation between 0.5 and 5 mg kg^{-1} (Gupta, 2016). In our country, 46.2% of soils suffer from B deficiency, while 19.4% and 3.3% of the total area have excessive and toxic B concentrations (Kıllıoğlu, 2022). However, if a soluble B fertilizer is applied near the seeds, it may cause toxicity in crops, depending on cultivar susceptibility (Metwally et al., 2018). In canola, there are large variations among cultivars in tolerance to B toxicity compared to barley and wheat (Hughes-Games, 1991). Öztürk et al. (2010) found that canola seed yield decreased by 31% at a toxic level of 15 kg B ha^{-1}. In contrast, it supports plant growth and oil content of canola. Ma et al. (2015) found that foliar application of boron increased canola seed yield by 10%, although no improvement was observed with soil B application. Manaf et al. (2019) determined that oil content was increased by application of 2 kg B ha^{-1}, while Eggert and von Wirén (2016) demonstrated that canola seed harvested from B-supplied mother plants produced better seedling growth and development, without improving germination performance. In this study, the response of four winter canola varieties to different concentrations of boron (B) was evaluated during germination and seedling growth stages.

2. Materials and Methods

A laboratory experiment was conducted at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2023. The seeds of winter canola varieties KWS Cyrill CL, Miranda, PT264, and NK Caravel from different seed companies and sodium borate (20.9% $Na_2B_8O_{13}.4H_2O$) were used as materials.

The seeds were germinated at six boron levels of 0, 20, 40, 60, 80, and 100 mg B L^{-1} prepared from Etidot-67 ($Na_2B_8O_{13}.4H_2O$). Distilled water was used as a control. Four replicated fifty seeds (4×50) from each canola variety were spread between three filter paper sheets with a dimension of 20 × 20 cm and each paper was watered with 7 mL for respective boron solutions. They were put into sealed plastic bags after the filter papers were rolled. The packages were incubated at 20°C for 7 days under dark conditions. The appearance of two millimeters of radicle hook was evaluated as the germination criterion. Germination percentage (GP), mean germination time (MGT), and germination index (GI) were also calculated using equations 1 and 2 (ISTA, 2018), and 3 (Salehzade et al, 2009):

$$GP\ (\%)=\frac{Germinated\ seeds\ at\ final\ day}{Total\ seeds}\times 100 \quad (1)$$

$$MGT\ (day)=\frac{\sum Dn}{\sum n} \quad (2)$$

where n represents the seed number germinated on day D, and D refers to the number of days since the initiation of the germination test.

$$GI=\frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ first\ count}+\cdots+\frac{Number\ of\ germinated\ seeds}{Days\ of\ the\ final\ count} \quad (3)$$

On the final day, ten seedlings were randomly selected from each B level to measure the root length, shoot length, the fresh and dry weight of the seedlings. The seedlings were exposed to drying in an air oven at a temperature of 80°C for a period of 24 h.

2.1. Statistical Analysis

The study was designed as a two-factor factorial in a completely randomized design with four replicates. Data were analyzed using the MSTAT-C software, and differences were compared using the Least Significant Differences (LSD) test at a 5% level.

3. Results

There were significant differences among winter canola varieties and boron levels for germination percentage, mean germination time, and germination index (Table 1). KWS Cyrill CL had the highest germination percentage and germination index, while it also had the shortest mean time for germination. Increasing B levels caused a reduction in germination percentage and the lowest germination percentage (88.0%) and index (20.1) were obtained at 100 mg B L^{-1}. Mean germination time was delayed under B stress and reached the maximum at 80 mg B L^{-1}. Increased boron levels significantly inhibited seedling growth parameters of winter canola varieties and the interaction of variety × boron level was significant (Table 1). Seedling growth decreased as boron levels increased: however, an increase was observed in shoot length, and seedling fresh weight at a boron level of 60 mg L^{-1}. No significant effect of boron levels on the seedling dry weight was determined. Among the winter canola varieties, KWS Cyrill CL had a longer shoot and root length and a higher fresh and dry weight of seedlings than the other varieties, while Miranda had a longer root and a higher dry weight. Root growth declined with higher boron levels than 60 mg L^{-1}. Seedling fresh weight also increased up to 60 mg L^{-1} and then decreased.

Table 1. Analysis of variance and main effects of canola variety and boron level on the investigated characteristics.

Factors	GP (%)	MGT (day)	GI	SL (cm)	RL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})	DM (%)
Varieties (A)								
NK Caravel	89.4^{b}	2.59^{a}	19.4^{c}	3.50^{b}	4.03^{b}	451^{b}	36.1^{c}	8.1^{c}†
Miranda	90.1^{b}	2.51^{ab}	22.1^{b}	3.64^{b}	4.89^{a}	388^{c}	37.9^{b}	10.4^{a}
KWS Cyrill CL	95.7^{a}	2.24^{c}	25.2^{a}	4.29^{a}	4.74^{a}	483^{a}	46.0^{a}	9.7^{b}
PT264	95.1^{a}	2.44^{b}	22.3^{b}	3.08^{c}	3.65^{c}	366^{d}	35.7^{c}	9.9^{ab}
Boron doses (B)								
Control	94.6^{a}	2.16^{c}	25.8^{a}	5.15^{a}	4.99^{a}	428^{c}	39.4	9.4^{c}
20 mg B L^{-1}	94.0^{a}	2.49^{b}	23.0^{b}	4.07^{c}	4.79^{a}	459^{b}	38.8	8.7^{d}
40 mg B L^{-1}	94.6^{a}	2.48^{b}	22.4^{b}	3.72^{d}	5.07^{a}	418^{c}	38.3	9.2^{cd}
60 mg B L^{-1}	92.6^{ab}	2.40^{b}	22.1^{b}	4.53^{b}	4.85^{a}	509^{a}	39.3	7.7^{e}
80 mg B L^{-1}	91.6^{b}	2.63^{a}	20.3^{c}	2.47^{e}	3.68^{b}	383^{d}	40.2	10.6^{b}
100 mg B L^{-1}	88.0^{c}	2.51^{ab}	20.1^{c}	1.82^{f}	2.59^{c}	334^{e}	37.5	11.5^{a}
Analysis of variance								
A	**	**	**	**	**	**	**	**
B	**	**	**	**	**	**	*ns*	**
A×B	**	**	**	**	**	**	**	**

**= significant at 1%, *ns*= non-significant, †= Letter(s) connected with the means denote significance levels at P<0.05. (GP: germination percentage, MGT= mean germination time, GI= germination index, SL= shoot length, RL= root length, SFW= seed fresh weight, SDW= seed dry weight, DM= dry matter).

The interaction of variety × boron level on germination and seedling growth parameters is shown in Figure 1. Boron levels significantly affected the germination and seedling growth parameters of winter canola varieties. Germination percentage decreased when B levels increased, but KWS Cyrill CL was the least affected variety (Figure 1A). Surprisingly, Miranda had a germination percentage of 90% in the control, which increased to 96.5% at 40 mg B L^{-1}. Depending on the germination percentage, the germination index was changed. At all B levels, KWS Cyrill CL had the highest germination index, while PT264 had the lowest germination index (Figure 1C). Mean germination time was delayed with increasing B, and the longest mean germination time was observed at 100 mg B L^{-1} (Figure 1B). At a boron level of 60 mg L^{-1}, shoot length was induced, while higher levels inhibited it considerably (Figure 1D). KWS Cyrill CL had the highest shoot length under all boron levels. At the highest boron level, the shoot length was reduced in Miranda by 27%, NK Caravel by 30%, KWS Cyrill CL by 36%, and PT264 by 55%. The root length was depressed by increasing boron levels except for 60 mg L^{-1} at which it was promoted (Figure 1E), except for PT264. Miranda and KWS Cyrill CL had the longest length of root at 60 mg B L^{-1}. The seedling fresh weight fluctuated by changes in shoot and root depletion. Although KWS Cyrill CL had the highest fresh weight at all levels of boron (except for 80 mg L^{-1} boron level), generally seedling fresh weight induced at 60 mg B L^{-1}, but higher levels resulted in a significant decline (Figure 1F). Moreover, a heavier seedling fresh weight was obtained from PT 264 under boron stresses compared to the control. Similarly, KWS Cyrill CL had the highest seedling dry weight at all levels of boron (Figure 1G). All varieties had the lowest dry matter at 60 mg B L^{-1}, while Miranda produced the highest dry matter under all boron levels (Figure 1H).

4. Discussion

This study focused on the toxic effects of boron concentrations on germination and early seedling growth parameters of winter canola varieties. Our results showed that increasing boron levels led to a decrease in germination percentage and germination index. This result confirmed the findings of Turhan and Kuşçu (2021), who determined significant differences among B levels for germination percentage, index, energy, and mean germination time in watermelon, pepper, and eggplant, although they used B doses up to 16 mg L^{-1}. Also, Archana and Pandey (2016) reported that the maximum germination was recorded in a non-boron solution, while a significant reduction was observed especially at 330 mM. Contrarily, Kaya et al. (2023) found no significant reduction in germination percentage of sunflower, soybean, and opium poppy up to 90 mg B L^{-1}. This means that plant species showed sensitivity to B during germination phases and canola was more sensitive than the mentioned species. On the other hand, retarded mean germination time and decreased germination index were recorded under B stresses in this study. Kaya et al. (2023) reported similar results in sunflower and opium poppy.

Seedling growth, including shoot and root length, as well as seedling fresh and dry weight of canola varieties, proved to be more sensitive to boron levels compared to germination because the shoot length of the canola varieties declined significantly. No significant changes were identified in control and at 60 mg B L^{-1}. Our results agree with the findings reported by Metwally et al. (2018), who observed a linear decrease in shoot growth of canola with increasing B levels and found higher levels than 25 mg B kg $soil^{-1}$ to be toxic. In addition, Kaya (2023) reported a promoter effect of 20 mg B L^{-1} on seedling growth of melon cultivars, but higher levels were also found to be toxic. Similarly, Kaya et al. (2023) found a reduction in shoot length of sunflower at 60 and 90 mg B L^{-1}, while a precise B level leading to a decline in root and shoot length of soybean could not be detected, which can be explained by the toxic level of boron depending on the plant species. Our results showed that there were significant differences among canola varieties in terms of B concentrations and KWS Cyrill CL was the least affected variety by high B concentrations. This result confirms the finding of Öztürk et al. (2010) who found that two cultivars were not affected by a toxic B level.

5. Conclusion

The results indicated that boron levels adversely influenced the germination characteristics of winter canola varieties and they declined at 80 mg B L^{-1}. Seedling growth was more sensitive to B stress than the germination traits of canola, and shoot length was more adversely inhibited by boron levels than root length. In addition, there was genotypic variation among canola varieties in terms of tolerance to boron toxicity, with KWS Cyrill CL germinating and growing better than the others. It was concluded that the susceptibility of canola varieties to boron toxicity varies and that tolerant varieties should be preferred on boron-affected soils.

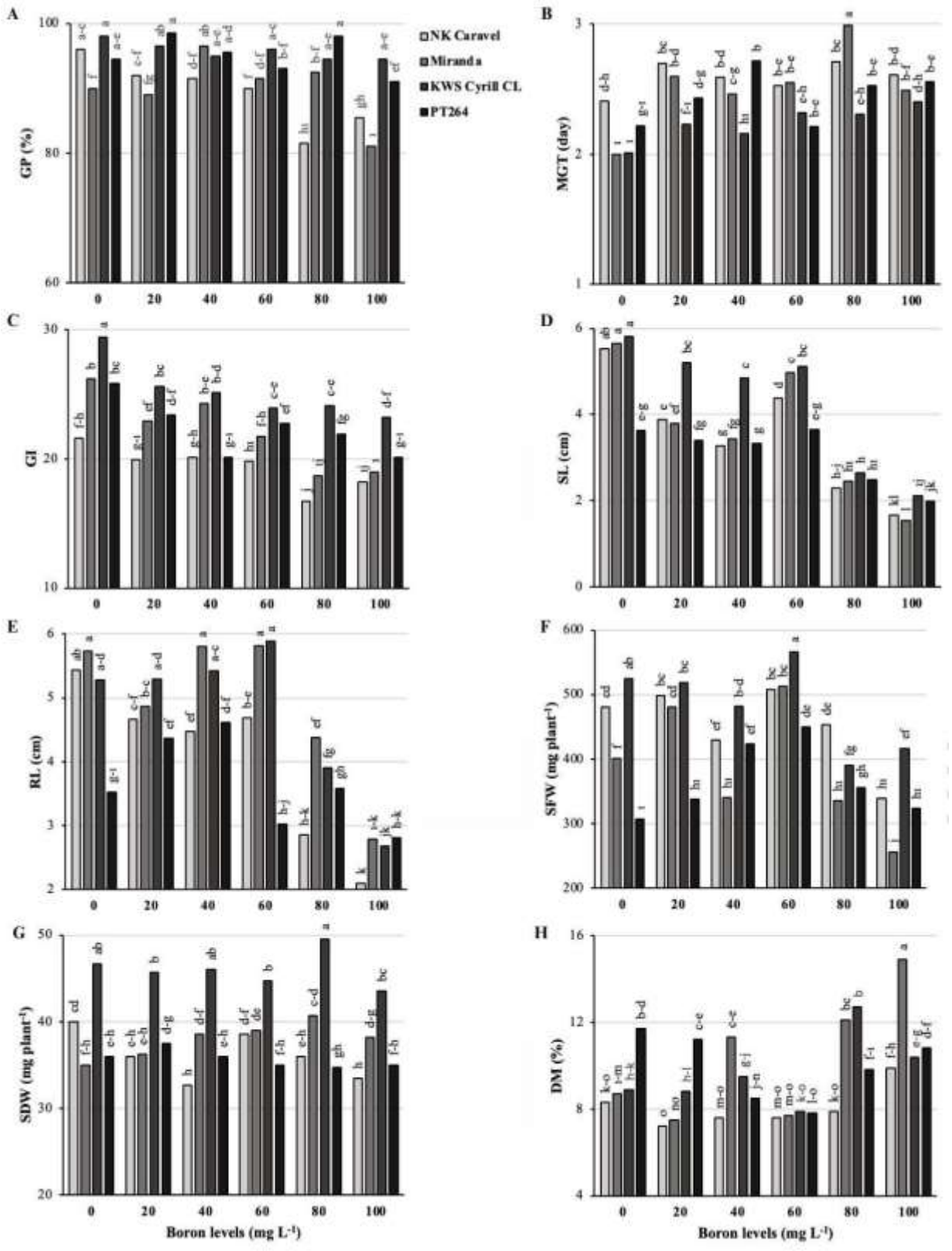

Figure 1. Effects of different boron concentrations on germination percentage (A), mean germination time (B), germination index (C), shoot length (D), root length (E), seedling fresh weight (F), seedling dry weight (G) and dry matter (H) of winter canola varieties. GP= germination percentage, MGT= mean germination time, GI= germination index, SL= shoot length, RL= root length, SFW= seed fresh weight, SDW= seed dry weight, DM= dry matter. Letter(s) on each bar shows significance levels at 5%.

Author Contributions

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

	E.Y.	P.H.	M.D.K.	E.G.K.
C	40	0	60	0
D	40	30	0	30
S	0	0	50	50
DCP	40	40	0	20
DAI	50	0	50	0
L	40	30	20	10
W	50	10	40	0
CR	50	0	50	0
SR	20	20	20	40
PM	20	20	60	0
FA	0	0	80	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 there was no study on animals or humans.

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COMBINED EFFECTS OF DROUGHT AND LOW TEMPERATURE ON GERMINATION AND SEEDLING GROWTH OF MELON CULTIVARS

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Abstract: The study aimed to determine the effects of drought (0.0, -2.0, -4.0, and -6.0 bar PEG 6000) and low temperature (18°C) on the germination and early seedling growth of three melon cultivars (Kırkağaç 589, Hasanbey 1, and Toros Sarıbal). Germination percentage, mean germination time, germination index, root length, shoot length, fresh and dry weight of the seedling, and vigor index of the melon cultivars were investigated. The results showed significant effects of low temperature and drought stress on the germination and seedling growth of melon cultivars. As temperature decreased and drought increased, the germination percentage decreased, and mean germination time was delayed. Drought stress led to a decrease in germination percentage, index, and all investigated seedling growth parameters, while the response of melon cultivars to drought stress varied. Seedling growth was more affected by low temperature than germination. Low temperature decreased germination percentage from 87.2% to 63.7% and seedling fresh weight from 140 mg/plant to 64 mg/plant. Each increase in drought levels resulted in a decrease in germination and seedling growth parameters of melon cultivars. Any seedling growth was not observed in Hasanbey 1 and Toros Sarıbal at -6.0 bar at 18°C. Melon cultivars showed different levels of tolerance to drought stress during germination and seedling growth stages, while they could maintain it up to -4.0 bar. It was concluded that Kırkağaç 589 germinated and grew better under drought stress at both optimum and low temperatures.

Keywords: *Cucumis melo* L., Germination, Genotype, Drought, Cold

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1. Introduction

Melon (*Cucumis melo* L.) is a summer season crop commonly grown for fresh ripe fruit and baby fruit is widely used for pickles in Türkiye (Vural et al., 2000). It is an important alternative crop in arid and semi-arid regions in open-field, where climatic conditions are characterized by drought and low annual rainfall (Aragão et al., 2023). It can be grown under rainfed conditions without irrigation in crop rotation with wheat, barley, and sunflower, while late spring and early autumn frosts limit the vegetation period of melon. For these reasons, it is generally sown in unfavorable seedbed conditions such as suboptimal temperature, salinity, and lack of moisture (Ergin et al., 2021).

Low temperature and drought are the main factors that inhibit moisture uptake by seeds and cause irregular and inadequate seedling emergence, resulting in yield limitations (Copeland and McDonald, 1995). These conditions reduce and delay germination and emergence, and inhibit seedling growth (Finch-Savage, 2010). In melon, Pinheiro et al. (2017) found that increasing drought stress led to a reduction in germination and seedling growth in four seed lots. Also, Edelstein and Kigel (1990) found that water uptake by melon seeds was inhibited by low temperature, and no germination was observed at 14°C in Noy Yizre'el accession, suggesting genotypic variation among melon genotypes in response to low temperature (Hutton and Loy, 1991). Similar observations were identified in pea (Okçu et al., 2005), sunflower (Ergin and Kaya, 2020), muskmelon (Xu et al., 2017), and maize (Khaeim et al., 2022) under drought stress. However, it remains unclear which stresses have a greater impact on melon germination. This study aimed to determine the combined effects of drought and suboptimal temperature on the germination and growth of melon seedlings and to determine which stress is more harmful than the other.

2. Materials and Methods

An experimental study under laboratory conditions was planned to determine the response of melon (*Cucumis melo* L.) cultivars to different drought levels under optimum and low-temperature conditions during germination and early seedling growth. The experiment was carried out in 2023 at the Seed Science and Technology Lab., Department of Field Crops, Eskişehir Osmangazi University, Türkiye. The seeds of three melon cultivars (Kırkağaç 589, Toros Sarıbal, and Hasanbey 1)

were germinated under different levels of drought stress, using various concentrations of polyethylene glycol 6000 (PEG) with 0.0 (distilled water), -2.0, -4.0, and -6.0 bar under optimum (25°C) and low (18°C) temperatures according to Michel and Kaufmann (1973).

2.1. Germination Conditions

Germination procedures were performed according to the ISTA (2003) rules. A total of two hundred (4×50) seeds of each melon cultivar were used for each treatment, and 50 seeds were placed on two layers of filter paper and then covered with a sheet. They were moistened with 21 mL of the respective solutions. After rolling, the samples were placed in sealed plastic bags to prevent water evaporation. The papers were replaced with new ones every 2 days during incubation to prevent PEG accumulation. The packages were transferred to incubators set at optimum 25°C and low 18°C temperatures in the dark. Germination was determined by a 2 mm radicle elongation and recorded every 24 hours for 8 days. The mean germination time (MGT), calculated following the ISTA (2003) guidelines, was used to assess the germination speed. MGT= Σ(Dn)/Σn, where n denotes the number of seeds that germinated on day D, and D indicates the number of days from the beginning of the germination test. The germination index (GI) was determined by implementing the formula, GI= number of germinated seeds/days of first counting +. . .+ number of germinated seeds/days of final counting (Ahmad et al., 2009). On the eighth day, ten seedlings were randomly selected from each treatment to measure seedling growth characteristics including root length (RL), shoot length (SL), seedling fresh weight (SFW), and seedling dry weight (SDW). To calculate the vigor index (VI), the germination percentage (%) was multiplied by the seedling length (cm).

2.2. Statistical Analysis

The data analysis involved a three-factor factorial (drought × temperature × cultivar) using a completely randomized design (CRD) with 4 replicates. Analysis of variance and mean comparison was performed using Duncan's multiple range test ($P<0.05$) with the Mstat-C v. 2.10 software program.

3. Results and Discussion

The main effects of cultivar, temperature, and drought stress on germination and seedling growth characteristics of melon are shown in Table 1. It is not interesting that low temperature resulted in reduced germination and seedling growth of melon. However, melon cultivars responded differently to drought and low-temperature stress. In general, Kırkağaç 589 generally performed better results under these stress conditions. Similar findings were reported by Pinheiro et al. (2017), who found that drought stress reduced germination and seedling growth in four seed lots of melon.

Table 1. Main effects of cultivar, temperature, and drought stress on germination and seedling growth of melon.

Factor	GP %	MGT day	GI	RL cm	SL cm	SFW mg/plant	SDW mg/plant	VI
Temperature								
18°C	63.7^{b}	3.49^{a}	7.8^{b}	3.31^{b}	0.83^{b}	64^{b}	20.8^{b}	335^{b}*
25°C	87.2^{a}	2.57^{b}	18.5^{a}	9.32^{a}	3.54^{a}	140^{a}	24.6^{a}	1150^{a}
Cultivar								
Kırkağaç 589	91.2^{a}	3.06	16.8^{a}	7.15^{a}	2.88^{a}	126^{a}	26.6^{a}	944^{a}
Toros Sarıbal	68.9^{b}	3.03	11.5^{b}	6.28^{b}	1.95^{b}	92^{b}	22.2^{b}	621^{b}
Hasanbey 1	66.2^{b}	3.00	11.2^{b}	5.52^{c}	1.73^{c}	88^{b}	19.4c	661^{b}
Drought (bar)								
Control	89.5^{a}	2.74^{c}	17.5^{a}	7.19^{b}	5.39^{a}	203^{a}	24.0^{b}	1137^{a}
-2.0	88.4^{a}	3.09^{b}	16.2^{b}	8.59^{a}	1.92^{b}	109^{b}	24.3^{b}	943^{b}
-4.0	74.1^{b}	3.71^{a}	12.4^{c}	6.71^{b}	0.78^{c}	64^{c}	25.4^{a}	626^{c}
-6.0	49.8^{c}	2.58^{d}	6.6^{d}	2.77^{c}	0.65^{c}	31^{d}	17.4^{c}	262^{d}

*Means connected with the same letter(s) in each column are not significant at $P<0.05$. GP= Germination percentage, MGT= mean germination time, GI= germination index, RL= root length, SL= shoot length, SFW= seedling fresh weight, SDW= seedling dry weight, VI= vigor index.

The effects of different drought levels on germination characteristics and root length of melon cultivars under optimum (25°C) and low (18°C) temperatures are shown in Table 2. A three-way interaction of temperature × drought × cultivar was significant ($P<0.05$) for germination percentage, mean germination time, germination index, and root length. At optimum temperature (25°C), germination percentage of melon cultivars was different, and Hasanbey 1 had the lowest germination percentage in control. As drought stress increased, the difference between melon cultivars increased. No significant reduction in germination percentage of Kırkağaç 589 was observed up to -6.0 bar. Also, MGT was delayed with decreasing water potential of the solution, and the fastest germination was obtained from Kırkağaç 589 at all drought levels. Our results were confirmed by the findings of Kaya et al. (2006) and Toscano et al. (2017) in sunflower, Magar et al. (2019) in corn, Muscolo et al. (2014) in lentil, and Sadeghian and Yavari (2004) in sugar beet, who determined that drought, caused a reduction in germination and retardation in mean germination time. Germination index was decreased depending on drought stress and melon cultivars and Kırkağaç 589 had the highest germination

index. Root length was negatively affected by drought stress, while the root length of Hasanbey 1 enhanced up to -4.0 bar. Under low temperature (18°C) stress, drought stress influenced germination and root length more severely than optimum temperature. This finding aligns with the results observed in zucchini (*Cucurbita pepo* L.) by Gülşen et al. (2022), revealing notable differences in germination traits among 15 breeding lines under low temperatures of 12°C and 15°C. However, Kırkağaç 589 germinated better than the other cultivars at all drought levels. Germination percentage of Toros Sarıbal and Hasanbey 1 at -4.0 bar dropped below 50%. Also, their MGT could not be calculated due to insufficient germination percentage at -6.0 bar, Kırkağaç 589 had the lowest MGT at all drought levels. The germination index was higher under optimum temperature conditions compared to lower temperature. Among the melon cultivars, Kırkağaç 589 had the highest germination index. Drought affected severely the root growth of melon cultivars under low temperature, and the longest root was also measured in Kırkağaç 589. Muscolo et al. (2014) reported that root growth of lentil decreased when drought stress increased.

Table 2. Effects of different drought stresses on germination characteristics and root length of melon cultivars under optimum (25°C) and low (18°C) temperatures

Drought (bar)	Cultivar	GP (%)		MGT (day)		GI		RL (cm)	
		25°C	18°C	25°C	18°C	25°C	18°C	25°C	18°C
Control	Kırkağaç 589	94.5^{abc}	98.5^{a}	1.97^{i}	2.93^{f}	24.3^{a}	16.3^{d}	13.21^{a}	4.22^{fg}*
	Toros Sarıbal	91.0^{a-e}	89.0^{c-f}	2.10^{hi}	3.54^{de}	21.7^{b}	12.4^{f}	9.11^{cd}	4.72^{efg}
	Hasanbey 1	83.5^{e-h}	80.5^{gh}	2.21^{gh}	3.70^{cd}	19.6^{c}	11.1^{fg}	8.33^{d}	3.56^{gh}
-2.0	Kırkağaç 589	97.0^{ab}	95.5^{abc}	2.01^{hi}	3.45^{e}	24.2^{a}	14.1^{e}	13.28^{a}	5.31^{ef}
	Toros Sarıbal	90.5^{b-e}	81.5^{fgh}	2.23^{gh}	4.27^{b}	21.2^{b}	9.0^{hi}	9.70^{c}	4.31^{efg}
	Hasanbey 1	85.0^{d-g}	81.0^{gh}	2.36^{g}	4.23^{b}	19.3^{c}	9.3^{hi}	13.67^{a}	5.28^{ef}
-4.0	Kırkağaç 589	92.5^{a-d}	95.5^{abc}	2.15^{ghi}	3.89^{c}	22.2^{b}	12.3^{f}	9.22^{cd}	4.47^{efg}
	Toros Sarıbal	90.5^{b-e}	30.5^{k}	2.88^{f}	5.33^{a}	16.3^{d}	1.6^{k}	8.80^{cd}	2.53^{hi}
	Hasanbey 1	89.0^{c-f}	46.5^{j}	2.74^{f}	5.30^{a}	17.5^{d}	4.6^{j}	11.79^{b}	3.46^{gh}
-6.0	Kırkağaç 589	96.5^{abc}	59.5^{i}	2.88^{f}	5.25^{a}	16.7^{d}	4.1^{j}	5.65^{e}	1.83^{i}
	Toros Sarıbal	77.0^{h}	1.5^{l}	3.88^{c}	- ^{j}	10.2^{gh}	- ^{l}	4.99^{ef}	- ^{j}
	Hasanbey 1	59.5^{i}	4.5^{l}	3.52^{de}	- ^{j}	8.7^{i}	- ^{l}	4.15^{fg}	- ^{j}

*Means connected with the same letter(s) in each character are not significant at P<0.05. GP= Germination percentage, MGT= mean germination time, GI= germination index, RL= root length.

The effects of different drought levels on seedling growth characteristics of melon cultivars at optimum (25°C) and low (18°C) temperatures are shown in Table 3. The seedling growth of the melon cultivars was significantly different, and Kırkağaç 589 produced the longest shoot, the greatest fresh and dry seedling weight, and the highest vigor index. At 25°C, the shoot length of melon cultivars was significantly depressed at -2.0 bar and reached the minimum values at -4.0 bar. Depending on the reduction in shoot and root length, seedling fresh weight decreased with increasing drought stress. It was reduced by 52% in Kırkağaç 589, by 55% in Toros Sarıbal, and by 61% in Hasanbey 1. However, changes in seedling dry weight were not related to fresh weight. The highest dry weight of seedling (29.6 mg/plant) was recorded in Kırkağaç 589 under drought stress of -6.0 bar. Pinheiro et al. (2017) reported that seedling growth of melon was inhibited by increasing drought stress. These results are in line with findings in maize (Liu et al., 2017), in lentil (Muscolo et al., 2014) and in carrot, eggplant, and watermelon (Steiner and Zuffo, 2019). Vigor index, a valuable screening criterion for genotypes against stress, combines germination performance and seedling growth. In this study, melon cultivars in the control treatment showed that there was a clear difference among melon cultivars; furthermore, each increase in drought stress caused a reduction in the vigor index of melon cultivars. The highest vigor index was calculated in Kırkağaç 589 at all drought stress levels. Under low-temperature stress, a remarkable drop in seedling growth was observed. Shoot length of Kırkağaç 589, Toros Sarıbal, and Hasanbey 1 declined from 12.31 cm to 1.84 cm, from 7.56 cm to 1.72 cm, and from 7.87 cm to 1.82 cm, respectively. No shoot length was measured in Toros Sarıbal at -4.0 bar and -6.0 bar at 18°C, indicating its sensitivity to low temperature, because this cultivar had shoot growth at the same drought levels under 25°C. However, there were no significant differences in seedling fresh weight among the melon cultivars in each drought, and a similar response was observed. Seedling dry weight was not altered by increasing drought stress, while Hasanbey 1 had the lowest dry weight under all drought stresses. Low temperature caused a reduction in the vigor index of melon cultivars. Among the melon cultivars, Kırkağaç 589 had the highest index value at all drought levels and was the least affected cultivar by low temperature and drought.

Table 3. Effects of different drought stresses on seedling growth characteristics of melon cultivars at optimum (25°C) and low (18°C) temperatures

Drought (bar)	Cultivar	SL (cm)		SFW (mg/plant)		SDW (mg/plant)		VI	
		25°C	18°C	25°C	18°C	25°C	18°C	25°C	18°C
Control	Kırkağaç 589	12.31^{a}	1.84^{e}	365^{a}	127de	24.7^{b-e}	27.5^{abc}	2412^{a}	598^{hı}*
	Toros Sarıbal	7.56^{b}	1.72^{e}	265^{b}	126^{def}	23.0^{cde}	26.2^{a-d}	1517^{c}	574^{hı}
	Hasanbey 1	7.87^{b}	1.02^{f}	234^{c}	100^{efg}	20.5^{e}	22.0^{de}	1356^{d}	370^{jk}
-2.0	Kırkağaç 589	4.06^{c}	1.00^{f}	177^{c}	81^{ghı}	25.5^{a-d}	26.7^{a-d}	1685^{b}	603^{hı}
	Toros Sarıbal	1.95^{e}	0.58^{f}	120^{def}	69^{g-k}	25.0^{a-e}	24.4^{b-e}	1055^{ef}	399^{jk}
	Hasanbey 1	2.98^{d}	0.96^{f}	138^{d}	67^{h-k}	22.1^{de}	22.4^{de}	1416^{cd}	505^{hıj}
-4.0	Kırkağaç 589	0.88^{f}	0.94^{f}	96^{fgh}	57^{ıjk}	28.6^{ab}	25.4^{a-d}	934^{fg}	517^{hıj}
	Toros Sarıbal	0.98^{f}	- ^{g}	62^{ıjk}	46^{k}	25.2^{a-e}	27.5^{abc}	884^{g}	80^{mn}
	Hasanbey 1	0.95^{f}	0.94^{f}	77^{g-j}	48^{jk}	22.8^{cde}	23.1^{cde}	1132^{e}	211^{lm}
-6.0	Kırkağaç 589	0.99^{f}	0.99^{f}	57^{ıjk}	44^{k}	29.6^{a}	25.6^{a-d}	642^{h}	165^{lm}
	Toros Sarıbal	1.04^{f}	- ^{g}	45^{k}	- ^{l}	26.5^{a-d}	- ^{f}	467^{ıj}	-n
	Hasanbey 1	0.90^{f}	- ^{g}	40^{k}	- ^{l}	22.4^{de}	- ^{f}	301^{kl}	-n

*Means connected with the same letter(s) in each character are not significant at P<0.05. SL= shoot length, SFW= seedling fresh weight, SDW= seedling dry weight, VI= vigor index.

4. Conclusion

Drought and low-temperature stresses are common in seedbed conditions after sowing. Thus, the germination and early seedling growth performance of melon cultivars were negatively limited by them. In this experiment, significant differences were found among melon cultivars in terms of tolerance to drought and low temperature; however, melon was more sensitive to low temperature than to drought during germination and subsequent growth stage of the seedlings. Among the melon cultivars, Kırkağaç 589 appeared to be more tolerant to drought stress under optimum and low temperature because it germinated and grew better than the others. It was concluded that low temperature was more detrimental to melon cultivars than drought, and the selection of a suitable melon genotype was a critical factor in achieving higher and faster germination in drought-stricken fields.

Author Contributions

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

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

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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GRAPE BERRY MORPHOLOGY IN SEMI-ARID CLIMATE OF TEKIRDAĞ: EVALUATING THE EFFECTS OF ENVIRONMENTAL FACTORS AND STRESS APPLICATIONS

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Abstract: The growth and development of grapes are influenced by various biotic and abiotic stresses. The presence of *Vitis vinifera* L. on Earth is threatened by the increase in abiotic stresses and biotic stresses due to global warming. On the other hand, grape quality and, consequently, berry characteristics can also be negatively affected by these stress factors. The hypothesis of this experiment is to determine the effects of biotic and abiotic stresses applied five days before harvest on the berries of live grapevines under field conditions. For this purpose, for two years (2016 and 2017), Cabernet-Sauvignon and Merlot grape varieties grafted onto the SO4 rootstock at Te-Ha Corp. vineyard were used. In the late pre-harvest period (five days before harvest), seven stress applications, including control, were implemented. The stress application methods included control, shock action (1 minute with a plastic hammer at 08:00 and 19:00), leaf removal (removing all leaves), leaf injury (injuring all leaves by hitting with a stick), UV-C (1 minute at 08:00 and 19:00), vibration (1 minute of vibration at 08:00 and 19:00), and *Botrytis cinerea* Pers ex. Fr (once). The measurements of the features performed are as follows, in order: berry width-length (mm), bery volume (cm^3), berry skin area (cm^2/grain), berry skin area/berry flesh volume ratio (cm^2/cm^3), berry fresh-dry weight (g), 100 berry fresh weight (g), berry density (g/cm^3), and % dry weight. As a result, it was observed that the applied abiotic and biotic stress treatments did not negatively affect berry characteristics in two years, especially in the second year. Therefore, the application of Shock action, UV-C, Vibration, Leaf injury, Leaf removal, and *Botrytis cinerea* for improving grape quality was found not to be objectionable.

Keywords: Abiotic stress, Biotic stress, UV-C, Cabernet-Sauvignon cv., Merlot. cv.

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1. Introduction

Due to the increase in greenhouse gases in the atmosphere, changes are occurring at the biogeochemical levels, leading to an increase in temperature, drought, and an associated rise in CO_2 concentration. In short, the viability of *Vitis vinifera* L. on Earth will be jeopardized in the future due to the increase in abiotic stresses and biotic stresses associated with climate change (Aguilera et al., 2022).

Grapes are subject to various biotic and abiotic stresses that affect their growth and development. Among abiotic stresses, drought (Bianchi et al., 2023; Hewitt et al., 2023), salinity (Aazami et al., 2023), extreme temperatures (Biniari et al., 2023; Hewitt et al., 2023), toxic chemicals, heavy metals, high UV radiation, atmospheric CO_2, and oxidative stresses can be listed (Ma et al., 2012; Lopez-Fernandez et al., 2016). As is known, living organisms such as pathogens, insects, viruses, viroids, bacteria (Ma et al., 2011), and fungi (Cosseboom and Hu, 2022) constitute biotic factors in the ecosystem (Candar, 2023; Darriaut et al., 2023). To practice resilient viticulture against biotic and abiotic stress factors, healthy soil (Biasi et al., 2023), and appropriate clone-rootstock selection to reduce environmental stress effects are important (Ferrandino et al., 2023). The use of mycorrhiza as a biostimulant is effective in reducing abiotic stresses caused by climate change since it enhances the uptake of water and plant nutrients from the soil (Kara and Erdogan, 2010; Aguilera et al., 2022).

Debastiani et al. (2023) found that the use of the S26 isolate obtained from *Bacillus sp*., as a bioinoculant in Cu-contaminated soils, led to an increase in plant growth to reduce biotic and abiotic stresses in grapevines. Additionally, it was determined that controlled application of UV-C radiation improved grape quality. However, the impact of UV radiation on berry size is not known (Del-Castillo-Alonso et al., 2021). In winemaking, the application of thermovinification + UV-C has been found to enhance the composition of wine compared to traditional winemaking (Tahmaz and Soylemezoglu, 2017). Klarner et al. (2015), using UV-C radiation to eliminate *Bortytis cinerea* in vineyards, reported an 82% reduction in fungicide usage. Lorenzini et al. (2010) expressed that microorganism formation in grape juice

and wine can be sterilized with UV-C. Jung et al. (2018) indicated that exposure to mechanical vibration (vibration) stress through simulation could result in weight loss, rapid ripening, and spoilage in grapes. On the other hand, the timing and intensity of leaf removal should be adjusted considering the local climate (Mucalo et al., 2021).

Sabır et al. (2015) observed that in the Italia grape variety, berry weights were higher under full irrigation. On the other hand, Tardaguila et al. (2010) determined that late-season leaf removal did not alter the number of berries in the cluster. However, Palliotti et al. (2011) stated that early-season leaf removal increased the ratio of pulp to skin in the berries. Additionally, Poni et al. (2009) found that leaf removal before flowering and Korkutal et al. (2017) leaving all basal leaves increased the berry skin area. Romero et al. (2022) expressed that water stress leads to smaller berry size and increased berry skin ratio. Similarly, it was noted that berries from vines subjected to water stress and leaf removal were lighter and had a higher skin ratio compared to the control (Alatzas et al., 2023). These practices are considered by researchers to enhance grape quality (Roby and Matthews, 2004). It should be noted that excessive leaf removal can negatively affect berry color formation (Price et al., 1995).

Dai et al. (1995) reported that berry weight is influenced by environmental and viticultural practices, while Barbagallo et al. (2011) mentioned that quality decreases when berries are large. This is because in red wine grape varieties, a small berry size is desired, and it has been emphasized by Matthews and Anderson (1988) that wines from small berries are of higher quality. In contrast, Kasimatis et al. (1985) and Johnstone et al. (1995) reported that there is no such relationship between berry size and wine quality.

Berry size is emphasized to be influenced by the position of the berry in the cluster, the number of berries in the cluster, and the balance between the production center and the consumption center of the vine (Ollat et al., 2002; Dai et al., 2009). In addition, the effect of temperature on berry shape has been known for many years, with lower temperatures leading to longer berries (Harris et al., 1968). Furthermore, it has been noted that berry size can also vary with different pruning practices (mechanical, 2-buds, and 4-8 buds+2 buds) (Holt et al., 2008). As is known, there is a close relationship between quality and the composition of grape berries in grapes and wine. Therefore, quality is primarily dependent on the distribution of substances in its composition, such as grape variety, total soluble solids, organic acids, pH, and phenolic compounds. Quality is also influenced by factors such as berry skin thickness, berry skin area, the ratio of skin area to berry volume (Roby and Matthews, 2004), ecological conditions, maturity time, the impact of diseases, and practices such as rootstock and canopy management (Ribéreau-Gayon et al., 2000; Blouin and Guimberteau, 2000; Karanis and Çelik, 2002; Keller, 2010).

In the scope of this study, two types of stress applications, abiotic and biotic, were applied to live grapevines. Shock action, UV-C, vibration, leaf injury, leaf removal, and *Botrytis cinerea* stress applications were performed in the field conditions five days before harvest. The study aimed to determine whether these late-season stress applications had a negative impact on berry characteristics. Because the abiotic stress factors tested in this research are actually factors that could endanger the vitality of grapevines. Among these factors, especially, the application of UV-C can result in the loss of plant life. Or the winter buds for the following year may not emerge due to UV-C radiation. Practices such as impact, vibration, removal of all leaves, and leaf injury, while not as impactful as UV-C radiation, can still have negative effects on the next year's bud yield. As a result of this study, it will be revealed whether the applications performed five days before harvest affect berry characteristics (quality) in the year of stress occurrence and the subsequent year. Similarly, it will be observed whether the biotic stress of *Botrytis cinerea*, conducted in a similar manner, contributes to this effect.

2. Materials and Methods

2.1. Trial Site

The Merlot (VIVC number 7657) and Cabernet-Sauvignon (VIVC number 1929) grape varieties of *Vitis vinifera* L. were used as plant material. These varieties are grafted onto SO4 rootstock in a vineyard established in 2007. The vineyard is located in Tekirdag province at an altitude of 235 m above sea level. Rows are planted in a north-south direction with a spacing of 2,6 m between rows and 0,9 m between vines on the row. The vineyard is trained in a double cordon Royat system. In a Randomized Complete Block Design, the experiment consists of 2 grape varieties, 7 treatments [Control, *Botrytis cinerea* (biotic), UV-C, shock action, leaf injury, leaf removal, and vibration (abiotic)], 3 replicates, and a total of 126 vines with 3 vines in each replicate.

When the soil characteristics of the trial area (0-30 cm) were examined, it was determined that the soil structure of cv. Cabernet-Sauvignon and cv. Merlot vineyards is clay-loamy. In the cv. Cabernet-Sauvignon vineyard, it was found that potassium, calcium, iron, copper, and manganese elements are sufficient; organic matter, total nitrogen, and zinc are low. In the cv. Merlot vineyard, it was observed that phosphorus, potassium, calcium, iron, copper, and manganese elements are sufficient; organic matter, total nitrogen, and zinc are also low. The soil analysis results revealed that magnesium is very high in both vineyards.

2.2. Stress Applications

The grape harvest was conducted on Sept 18, 2016, and Sept 27, 2017. Trial clusters were selected as homogeneously as possible. Biotic and abiotic (such as UV-C) stresses that negatively affect and can even be lethal to the plant were planned, and these applications

were carried out five days before harvest. Special attention was given to the short time remaining until harvest when determining the durations of these stress applications. The aim of these applications conducted five days before harvest was to preserve the vitality of the plant in the trial year and the following year, and to enhance the grape quality in the trial year. The stress applications conducted are outlined below:

- Control: No stress was applied.
- Shock action: The trunk and arms of the vines were subjected to shock action twice a day (08:00 and 19:00) for 1 minute each, for 5 days, using a plastic hammer.
- UV-C irradiation: A cabin covering an entire vine was created, and UV-C radiation (Langcake and Pryce, 1977) was applied twice a day (08:00-19:00) for 1 minute each, over 5 days.
- Vibration: Mechanical vibration was applied to trunk, arm junction, and arm of the vine using a drill with an isolated tip.
-Leaf injury: Leaves were wounded by striking the vines in two directions with a flexible rod. This application was performed for 5 days at 1-minute intervals (08:00 and 19:00).
- Leaf removal: All leaves on a cluster were manually defoliated.
-*Botrytis cinerea* Pers ex. Fr. inoculation: The *Botrytis cinerea* isolate's 14-day conidia were placed in sterile distilled water. A sterile $2.5x10^5$ conidia/ml spore suspension was sprayed onto the clusters using a hand sprayer, and the clusters were covered with a PE bag.

2.3. Berry Measurements

Berry characteristics are listed as qualitative (color, shape, etc.) and quantitative (size, weight, etc.) determinants in the OIV descriptor (Bodor-Pesti et al., 2022 and 2023; Szűgyi-Reiczigel et al., 2022).

For all berry measurements, sampling for each repetition in each application combination was conducted with two clusters from each vine, and 12 berries were selected from each cluster. According to OIV (2009), berry width (mm) and length (mm) were measured using a digital caliper (Leo brand, 150 mm, Zhejiang Leo Co. Ltd., China). The sampled berries were used to record the volume of water displaced by the berries in a ranked cylinder using the water displacement method (cm^3). To calculate berry skin area (cm^2/berry), the radius was found from the berry volume formula (equation 1), and the calculation was performed as follows (Barbagallo et al., 2011).

$$Berry\ surface\ area\ (\frac{cm2}{berry}) = 4\pi r2 \quad (1)$$

The berry skin area was then ratioed to the berry volume (BSA/BV) (cm^2/cm^3). In this way, BSA/BV was determined (Palma et al., 2007). Berry fresh weight (g) was measured on a precision scale sensitive to 0.01 g (Knmaster, MT 200 model, Karun Teknoloji, Türkiye). The fresh weight of 100 berries (g) was also measured in the same manner. The berries used to determine berry dry weight (g) were dried in an oven (Nüve, EN300 model, Nüve Sanayi Malz. İmalat ve Tic. A.Ş., Türkiye) at 65-70°C for 72 hours and weighed on the same scale. Berry density (g/cm^3) was calculated by dividing berry fresh weight (g) by berry volume (cm^3). % Dry weight (Bahar et al., 2011) was calculated using the following formula (equation 2).

$$\%\ \text{Dry weight} = \text{Berry dry weight (g)x100/ Berry fresh weight (g)} \quad (2)$$

2.4. Statistical Analysis

The data obtained from the experiment were evaluated using the MSTAT-C program, and the LSD test was employed to reveal the differences that emerged. The two-year impact of these stress applications has been assessed.

3. Results and Discussion

3.1. Climate data forTekirdağ

Tekirdağ is located in a semi-arid climate zone. According to long-term climate data (1939-2017), the average temperature during the vegetation period is 18.99°C. The hottest months are July and August (Table 1). The total precipitation during the vegetation period has been recorded as 247.60 mm over the years.

Table 1. Long term and 2016 and 2017 vegetation period weather conditions in Tekirdag

Month/Year	Mean daily temperature, °C			Rainfall, mm		
	2016	2017	1937-2017	2016	2017	1937-2017
April	15.61	11.10	11.80	25.50	51.80	40.90
May	17.94	16.80	16.80	28.10	16.70	36.70
June	23.57	21.90	21.30	35.70	36.80	37.90
July	25.58	24.10	23.80	0.10	52.20	22.80
August	24.65	25.10	23.80	0.10	14.60	13.30
September	21.64	21.60	20.00	3.90	11.20	33.60
October	15.95	15.00	15.40	35.40	111.20	62.40
Mean temperature, °C	20.71	19.37	18.99			
Cumulative rainfall, mm				128.80	294.50	247.60

In the long-term average, rainfall in the months of April, May, June, September, and October has been determined to be above 30 mm. In the first year of the experiment, 2016, the average temperature was 1.72°C higher than the long-term average. Additionally, the total precipitation was 118.8 mm below the long-term

average. In 2016, with these values, the year was hotter and drier than the long-term averages. The temperature in 2017 was 0.38°C higher than the long-term average, with the total precipitation being 46.9 mm above the long-term average. When comparing the averages of the two experimental years, it is observed that 2016 was 1.34°C warmer and had 165.7 mm less precipitation than 2017 (MGM, 2016; MGM, 2017a; MGM, 2017b).

3.2. Berry Width

The berry width values for the years 2016 and 2017 are presented in Table 2. All main effects and interactions on berry width values were found to be statistically insignificant. When the interaction between berry width values and berry length was examined, it was determined that in both 2016 and 2017, berry width and berry length increased together, indicating a linear interaction between the two values (Figure 1).

Melo et al. (2015) stated that with an increase in berry size, berry weight, volume, and berry surface area also increased. Bahar and Öner (2016) reported berry width values for the Cabernet-Sauvignon variety ranging from 11.06 mm to 12.07 mm, while Candar (2023) indicated that these values varied between 11.20 mm and 14.7 mm for the Merlot grape variety. The measured berry width values in the study fall within a similar range. Thus, it can be observed that berry width was not affected by the late-season stress factors.

Table 2. Change in berry width for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	11.10	11.07	11.09		Control				
	Sa	11.26	11.22	11.24	10.96	10.96	10.96			
	UV-C	11.40	11.43	11.42		Sa			Ca	
Ca	Vib	11.11	11.06	11.08	11.24	11.24	11.24	11.20	11.18	11.19
	Li	11.18	11.14	11.16		UV-C				
	Lr	11.25	11.17	11.21	11.46	11.46	11.46			
	Bc	11.12	11.16	11.14		Vib				
	Control	10.80	10.88	10.84	11.15	11.15	11.15			
	Sa	11.32	11.14	11.23		Li				
	UV-C	11.48	11.51	11.50	11.21	11.21	11.21		Me	
Me	Vib	11.18	11.26	11.22		Lr		11.19	11.18	11.18
	Li	11.34	11.17	11.26	11.33	11.33	11.33			
	Lr	11.46	11.45	11.46		Bc				
	Bc	10.71	10.83	10.77	10.95	10.95	10.95			
YE		11.19	11.18							

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

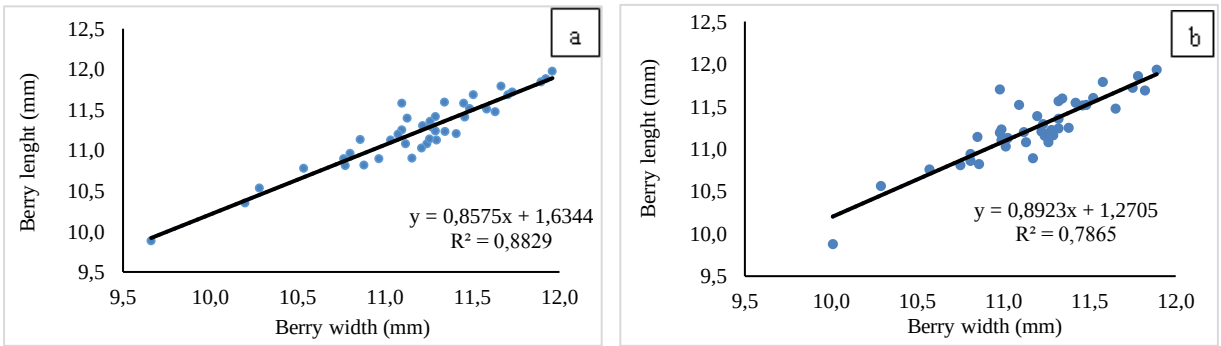

Figure 1. Interaction of berry width and berry length values in 2016 (a) and 2017 (b).

3.3. Berry Length

According to the statistical analysis, no significant difference was observed between the berry length values for the years 2016 and 2017. The conducted stress applications did not alter the berry length values for both grape varieties. When examining the data for the year 2017, it is particularly noteworthy that practices with the potential to cause damage to the vine, such as UV-C, Vib, Sa, Li, Lr, and Bc, did not affect the berry length. This finding is considered important in demonstrating that potentially harmful practices did not impact berry length (Table 3). The main effects of Cultivar Main Effect (CE) and Year Main Effect (YE) are not statistically significant.

According to OIV (2009), the berry size of the Cabernet-Sauvignon cv. is approximately in the "short" category, with a size around 13 mm. However, the obtained results

indicate values smaller than this reference. The findings of the research align with the results of previous studies, where Bahar and Öner (2016) reported Cabernet-Sauvignon berry length ranging from 11.60 mm to 12.28 mm, and Candar (2023) found that the berry length of the Merlot cv. varied between 11.90 mm and 14.60 mm, demonstrating a similar range to the research values. Similar to the findings stated by Melo et al. (2015), an increase in berry size is associated with an increase in berry volume. In line with this, a linear interaction between berry length and berry volume was observed in 2016 and 2017 (Figure 2).

Table 3. Change in berry length for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	11.07	11.14	11.11		Control				
	Sa	11.40	11.41	11.41	11.06	11.09	11.08			
	UV-C	11.40	11.44	11.42		Sa			Ca	
Ca	Vib	11.12	11.11	11.12	11.34	11.35	11.34	11.24	11.24	11.24
	Li	11.30	11.24	11.27		UV-C				
	Lr	11.24	11.16	11.20	11.44	11.46	11.45			
	Bc	11.16	11.16	11.16		Vib				
	Control	11.05	11.04	11.05	11.19	11.18	11.18			
	Sa	11.28	11.30	11.28		Li				
	UV-C	11.47	11.47	11.47	11.33	11.40	11.37		Me	
Me	Vib	11.26	11.23	11.24		Lr		11.22	11.26	11.24
	Li	11.36	11.56	11.46	11.27	11.24	11.25			
	Lr	11.30	11.32	11.31		Bc				
	Bc	10.85	10.90	10.85	11.01	11.03	11.02			
YE		11.23	11.25							

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

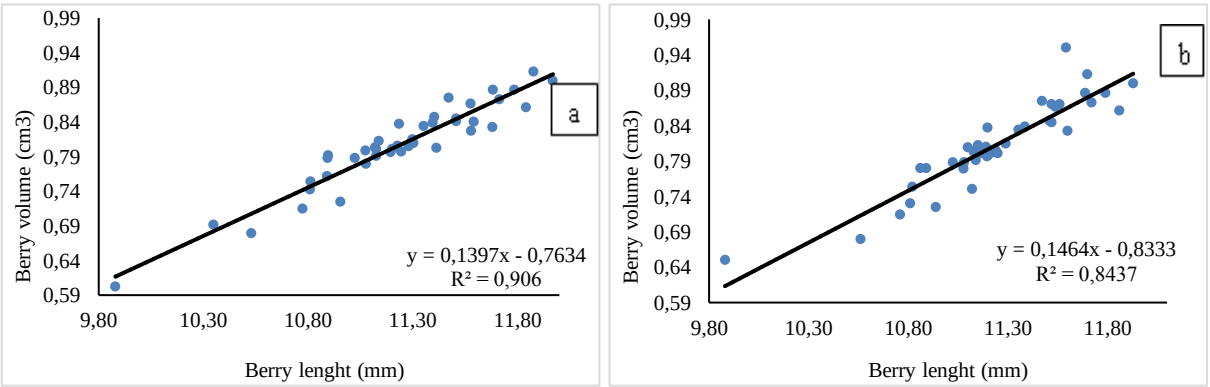

Figure 2. Interaction of berry length and berry volume values in 2016 (a) and 2017 (b).

3.4. Berry Skin Area

The effects of applications and interactions of these applications on berry skin area values in Cabernet-Sauvignon and Merlot grape varieties in 2016 and 2017 were examined. Only the main effect of the YE was found to be statistically significant at the P<0.01 level (Table 4). Melo et al. (2015) stated that with the increase in berry size, the berry skin area also increases. In their research, Bahar and Öner (2016) determined that the berry skin area of the Cabernet-Sauvignon variety ranged from 3.86 cm²/berry to 4.17 cm²/berry. The research findings are consistent with the researcher's results. Candar (2023) reported a berry skin area value ranging from 4.83 cm²/berry to 6.61 cm²/berry in the Merlot grape variety. The research findings are lower than the researcher's findings, and this is thought to be due to differences in the year and soil structure of the vineyard.

Table 4. Change in berry skin area for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	4.15	3.87	4.01		Control				
	Sa	4.27	4.02	4.15	4.09	3.82	3.95			
	UV-C	4.32	4.11	4.22		Sa			Ca	
Ca	Vib	4.16	3.89	4.03	4.24	3.99	4.11	4.20	3.95	4.08
	Li	4.14	3.91	4.03		UV-C				
	Lr	4.20	3.91	4.05	4.30	4.13	4.00			
	Bc	4.20	3.91	4.05		Vib				
	Control	4.03	3.78	3.90	4.18	3.92	4.22			
	Sa	4.21	3.95	4.08		Li				
	UV-C	4.27	4.15	4.21	4.18	4.00	4.05			
Me	Vib	4.20	3.94	4.07		Lr			Me	
	Li	4.24	4.09	4.16	4.23	3.99	4.10	4.16	3.95	4.06
	Lr	4.26	4.07	4.16		Bc				
	Bc	3.95	3.70	3.83	4.07	3.81	3.94			
YE		4.18A	3.95B							
P< 0.01		0.148								
YE P< 0.01= 0.148										

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

3.5. Berry Volume

It has been observed that the stress applications on berry volume values for the years 2016 and 2017 did not have statistically significant effects in terms of main effects and interactions of years and varieties (Table 5).

Melo et al. (2015) stated that there is a proportional relationship between berry size increase and berry volume. It should be noted that the increase in accumulated sugar and water content also plays a role in the increase in berry volume (Ağaoğlu, 2002). The berry volume for Cabernet-Sauvignon is reported to range from 0.88cm^3 to 1.37cm^3 by Bahar and Öner (2016). Ünlüsoy (2019) also mentioned that this value varies between 1.08cm^3 and 1.25cm^3 for the Merlot grape variety. In this study, it is observed that these values are in a similar range.

In the years 2016 and 2017, as the berry volume increased, the berry weight also increased, and a linear interaction between these two values was observed (Figure 3). It is a scientific fact that these two characteristics are highly correlated with each other (Roby and Matthews, 2004; Houel et al., 2013).

Table 5. Changes in berry volume for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	0.79	0.80	0.80		Control				
	Sa	0.82	0.83	0.83	0.77	0.80	0.78			
	UV-C	0.86	0.85	0.86		Sa			Ca	
Ca	Vib	0.79	0.80	0.80	0.81	0.83	0.82	0.81	0.81	0.81
	Li	0.80	0.79	0.80		UV-C				
	Lr	0.80	0.81	0.81	0.84	0.84	0.84			
	Bc	0.81	0.80	0.81		Vib				
	Control	0.76	0.77	0.77	0.80	0.82	0.81			
	Sa	0.81	0.82	0.82		Li				
	UV-C	0.83	0.83	0.83	0.81	0.81	0.81			
Me	Vib	0.81	0.83	0.82		Lr			Me	
	Li	0.82	0.83	0.83	0.82	0.82	0.82	0.80	0.83	0.82
	Lr	0.83	0.83	0.83		Bc				
	Bc	0.74	0.83	0.79	0.77	0.82	0.80			
YE		0.81	0.82							

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

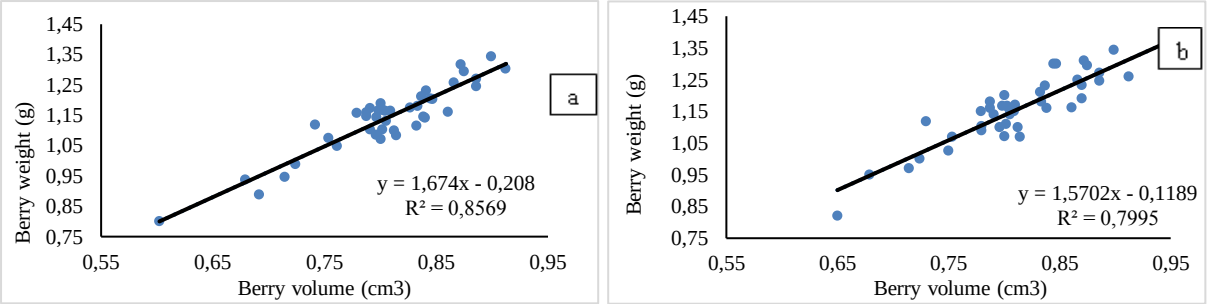

Figure 3. Interaction of berry volume and berry weight values in 2016 (a) and 2017 (b).

3.6. Berry Skin Area/Berry Volume

The difference between the two years in terms of the ratio of berry skin area to berry volume (cm^2/cm^3) was found to be statistically significant at P<0.01 level (Table 6). The size of grape berries is known to be an important quality characteristic in wine grape varieties due to its association with the ratio of berry skin area to berry pulp volume (Roby et al., 2004; Barbagallo et al., 2011). On the other hand, as the berry size increases, the ratio of berry skin area to berry volume decreases (Melo et al., 2015), and therefore, it is known that smaller berries have a higher ratio of berry skin area to berry volume (Roby and Matthews, 2004). The research results revealed that both varieties had a similar ratio of berry skin area to berry pulp volume. The values for the ratio of berry skin area to berry volume for the Cabernet-Sauvignon grape variety ranged from 3.03cm^2/cm^3 to 3.09cm^2/cm^3, as reported by Bahar and Öner (2016). For the Merlot grape variety, Ünlüsoy (2019) reported values ranging from 3.91cm^2/cm^3 to 6.97cm^2/cm^3. The measured values in this study fell within a similar range as the findings of the researchers.

Table 6. Changes in the ratio of berry skin area to berry volume in 2016 and 2017.

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	5.22	4.85	5.04		Control				
	Sa	5.15	4.84	4.99	5.26	4.88	5.07			
	UV-C	5.12	4.86	4.99		Sa			Ca	
Ca	Vib	5.21	4.84	5.02	5.16	4.82	4.99	5.19	4.86	5.02
	Li	5.24	4.94	5.09		UV-C				
	Lr	5.19	4.84	5.01	5.13	4.93	5.03			
	Bc	5.19	4.86	5.03		Vib				
	Control	5.30	4.91	5.10	5.20	4.86	5.03			
	Sa	5.18	4.81	5.00		Li				
	UV-C	5.15	4.99	5.07	5.21	4.91	5.06		Me	
Me	Vib	5.20	4.88	5.04		Lr		5.22	4.87	5.04
	Li	5.18	4.88	5.03	5.17	4.88	5.03			
	Lr	5.15	4.93	5.04		Bc				
	Bc	5.37	4.70	5.03	5.28	4.78	5.03			
YE		5.20A	4.87B							
P<0.01		0.094								
Yıl X Çeşit X Uygulama P<0.01= 0.094										

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

3.7. Berry Fresh Weight

The applications on berry weight of the varieties, year, and their interactions did not have a significant effect (Table 7). However, it should be noted that berry weight is a variety-specific genetic trait (Houel et al., 2013). The values for berry weight in the Cabernet-Sauvignon variety, ranging from 0.94g to 1.30g (Bahar and Öner, 2016) and 0.95g to 1.08g (Roby and Matthews, 2004), are in line with the trial findings. Similarly, in the Merlot grape variety, the values for berry weight ranged from 1.12g to 1.35g (Ünlüsoy, 2019), which aligns with the research. In addition, berry weight was observed to have a positive relationship with berry volume (Gray and Coombe, 2009; Houel et al., 2013), berry skin area (Melo et al., 2015), and berry size (Chen et al., 2018), similar to the findings of other researchers.

In the year 2016, as the berry fresh weight increased, the berry dry weight also increased, and in the year 2017, an increase in berry weight was associated with an increase in berry density (Figure 4). There is a linear relationship

between these values.

Table 7. Berry weight changes in 2016 and 2017.

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Ca	Control	1.15	1.17	1.16	Control			1.15	1.15	1.15
	Sa	1.17	1.15	1.16	1.12	1.13	1.12			
	UV-C	1.21	1.25	1.23	Sa					
	Vib	1.10	1.11	1.11	1.17	1.18	1.17			
	Li	1.12	1.13	1.13	UV-C					
	Lr	1.14	1.14	1.14	1.21	1.24	1.22			
	Bc	1.11	1.12	1.12	Vib					
Me	Control	1.08	1.09	1.09	1.13	1.14	1.13	1.14	1.16	1.15
	Sa	1.17	1.20	1.19	Li					
	UV-C	1.20	1.23	1.22	1.12	1.14	1.13			
	Vib	1.15	1.16	1.16	Lr					
	Li	1.12	1.15	1.13	1.17	1.17	1.17			
	Lr	1.20	1.21	1.21	Bc					
	Bc	1.03	1.09	1.06	1.07	1.11	1.09			
YE		1.14	1.16							

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

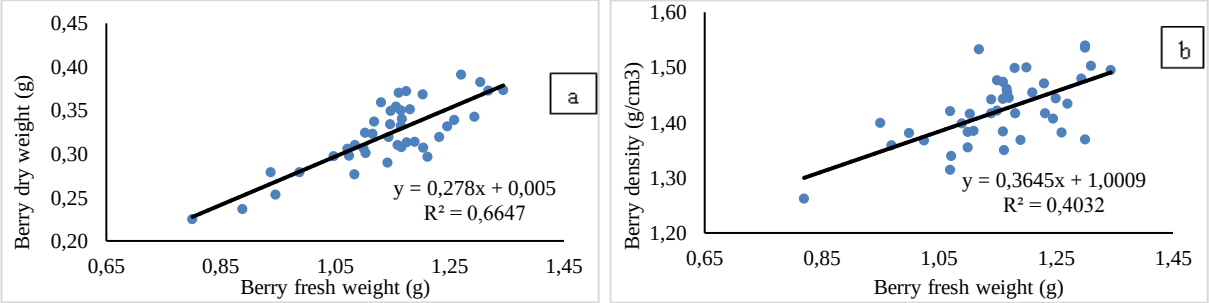

Figure 4. Interaction between berry weight and berry dry weight in 2016 (a) and berry weight and berry density in 2017 (b).

3.8. 100 Berry Weight

Similarly, there was no statistical difference observed among the values of 100-berry weight, which varied in the same way as berry weight. In the study of Kotseridis et al. (2012) on Merlot grape variety, where they collected all leaves in the cluster region and obtained an average of 100g, 107g by removing the basal leaves, and a control value of 109g, the findings were similar to the values obtained for the same variety in this research (114.96g). Likewise, a similarity was observed with Candar (2023), who reported this value between 145.17-158.47g. For Cabernet-Sauvignon variety, the 100-berry weight was reported as 89g by collecting all leaves in the cluster region, 98g by removing basal leaves, and 105g for control (Kotseridis et al., 2012), which parallel the research findings (114.91g). The study findings align with Drenjančević et al. (2023), who reported that the 100-berry weight slightly decreased compared to the control during flowering and berry drop, but this difference was not statistically significant.

3.9. Berry Dry Weight

In 2016, as the berry dry weight increased, the percentage of dry weight also increased (Figure 5). The same positive relationship is present with berry fresh weight (Melo et al., 2015). In 2016 and 2017, AE (Application Main Effect) was found to have a significant effect on berry dry weight ($P<0.01$). It was observed that Ultraviolet-C application had the highest effect on berry dry weight (0.34g), while Bc application (0.30g) had the least impact (Table 8). Other applications took values between these two applications. The measured berry dry weight value for Cabernet-Sauvignon variety (ranging from 0.10g to 0.37g) was found to be similar to the findings of Bahar and Öner (2016) and an average of 0.23g reported by Cooley et al. (2017).

3.10. Dry Weight Percentage

According to the combined year data for the percentage of dry weight in 2016 and 2017, the year 2016 was observed to have the highest value with an average of 28.24%. Statistical differences were detected among varieties at a 5% LSD level. The percentage of dry weight was highest in Cabernet-Sauvignon, reaching 28.61% (Table 9).

In a study involving different leaf removal and soil cultivation practices, the percentage of dry weight values for Cabernet-Sauvignon ranged from 27.35% to 28.14%

(Bahar and Öner, 2016). In another study involving different shoot treatments in Merlot grape variety, the percentage of dry weight values ranged from 23.57% to 24.42% (Candar, 2023). These values from previous studies are within a similar range as the measured percentage of dry weight values in this research.

3.11. Berry Density

In the years 2016 and 2017, as the berry weight increased, the berry density also increased (Figure 6).

2016 and 2017 average berry density data indicate that 2017 had the highest average berry density with 1.42 cm^3 (Table 10). LSD at a 1% level of significance was observed among applications in the combined years (Table 10).

In a study where different shoot applications were applied to Merlot grape variety, the berry density values ranged between 0.84 g/cm^3 and 1.44 g/cm^3 (Candar, 2023). The values obtained in this study fall within a similar range as the measured berry density values in this research.

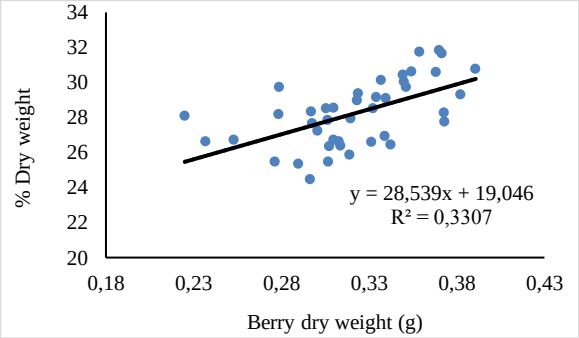

Figure 5. Interaction between berry dry weight and dry weight % values in 2016.

Table 8. Berry dry weight values for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	0.33	0.32	0.32		Control				
	Sa	0.33	0.33	0.33	0.31	0.31	0.31 AB			
	UV-C	0.37	0.34	0.35		Sa			Ca	
Ca	Vib	0.32	0.32	0.32	0.32	0.32	0.32 AB	0.33	0.33	0.33
	Li	0.33	0.32	0.32		UV-C				
	Lr	0.33	0.34	0.34	0.35	0.34	0.34 A			
	Bc	0.31	0.31	0.31		Vib				
	Control	0.30	0.30	0.30	0.31	0.31	0.31 AB			
	Sa	0.31	0.31	0.31		Li				
	UV-C	0.34	0.33	0.33	0.33	0.33	0.33 AB		Me	
Me	Vib	0.30	0.31	0.31		Lr		0.32.	0.32	0.32
	Li	0.33	0.33	0.33	0.33	0.34	0.34 AB			
	Lr	0.34	0.34	0.34		Bc				
	Bc	0.28	0.30	0.29	0.30	0.30	0.30 B			
YE		0.31	0.32							
P < 0.01							0.040			
AE P < 0.01=0.040										

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

Table 9. Changes in percentage of dry weight values for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	28.24	27.75	28.00		Control				
	Sa	27.91	28.77	28.34	28.03	27.74	27.89			
	UV-C	30.15	27.33	28.74		Sa			Ca	
Ca	Vib	28.86	28.50	28.68	27.13	27.26	27.19	28.75	28.46	28.61a
	Li	28.96	28.83	28.89		UV-C				
	Lr	28.80	29.99	29.40	29.07	27.07	28.07			
	Bc	28.31	28.07	28.19		Vib				
	Control	27.82	27.74	27.78	27.69	27.62	27.65			
	Sa	26.34	25.76	26.05		Li				
	UV-C	27.99	26.81	27.40	29.11	28.89	29.00		Me	
Me	Vib	26.52	26.74	26.63		Lr		27.74	27.21	27.47b
	Li	29.27	28.94	29.11	28.50	29.01	28.75			
	Lr	28.19	28.03	28.11		Bc				
	Bc	28.02	26.44	27.23	28.17	27.25	27.71			
YE		28.24	27.83							
P < 0.01										0.930
CE P < 0,01=0.930										

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

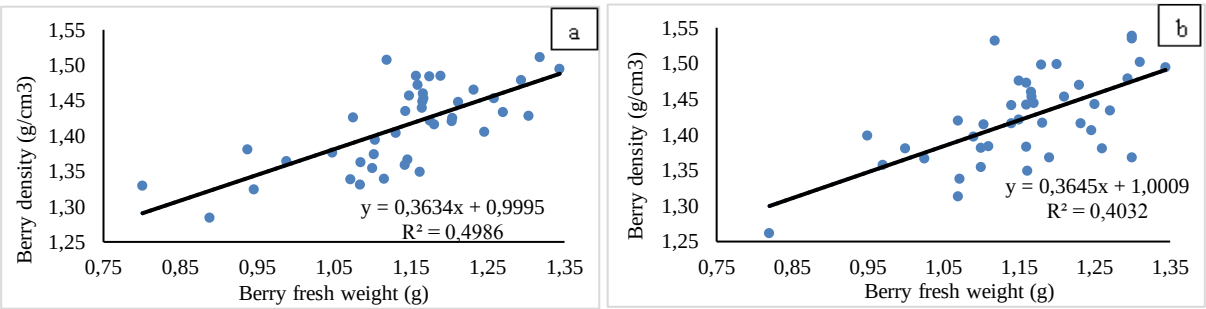

Figure 6. Interaction of berry density and berry weight values in 2016 (a) and 2017 (b).

Table 10. Berry density values for the years 2016 and 2017

C	A	C x A int.			AE			CE		
		2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
	Control	1.45	1.46	1.46		Control				
	Sa	1.41	1.39	1.40	1.43	1.44	1.43 AB			
	UV-C	1.44	1.47	1.46		Sa			Ca	
Ca	Vib	1.41	1.38	1.40	1.42	1.42	1.42 AB	28.75	28.46	28.61a
	Li	1.42	1.43	1.42		UV-C				
	Lr	1.38	1.41	1.40	1.44	1.48	1.46 A			
	Bc	1.37	1.39	1.38		Vib				
	Control	1.42	1.42	1.42	1.43	1.41	1.42 AB			
	Sa	1.44	1.46	1.45		Li				
	UV-C	1.44	1.48	1.46	1.39	1.40	1.39 B		Me	
Me	Vib	1.45	1.43	1.44		Lr		27.74	27.21	27.47b
	Li	1.37	1.40	1.36	1.41	1.44	1.42 AB			
	Lr	1.42	1.45	1.45		Bc				
	Bc	1.39	1.35	1.38	1.38	1.38	1.38 B			
YE		1.41	1.42							
P < 0.01							0.063			
AE P < 0.01=0.063										

C= Cultivar, Ca= Cabernet-Sauvignon, Me= Merlot, CE= Cultivar main effect, AE= Application main effect, YE= Year main effect, A= Applications, Sa= Shock action, Vib= Vibration, Li= Leaf injury, Lr= Leaf removal, Bc= *Botrytis cinerea*, C x A int.= Cultivar x Application interaction.

4. Conclusion

In the years 2016 and 2017, the effects of some biotic and abiotic stresses applied were examined on berry characteristics in this study. Considering that Cabernet-Sauvignon and Merlot varieties, which were the subject of the experiment, have different genetic structures, differences between the two varieties are expected. Therefore, the data of the two varieties have not been compared with each other. However, as is known, the berries of the Merlot grape variety are larger than the berries of Cabernet Sauvignon.

When examined in terms of the year effect, it is observed that the year 2016 was hotter and drier compared to the year 2017. However, in the year 2017, the criteria of berry skin area and berry skin area/berry volume slightly decreased compared to the year 2016. It is believed that this might be attributed to the abundant rainfall experienced in the year 2017. Nevertheless, this decrease is noteworthy as it indicates that the applied stress factors did not have a very negative impact.

In terms of the application effect, the UV-C application, expected to be destructive in terms of berry weight and berry density values, was slightly elevating compared to other stress applications, and the Bc application was slightly lowering these two values. However, neither of the stresses has caused a significant negative impact on the plant and the berries.

It was observed that abiotic and biotic stress applications applied to clusters in the late period (5 days before harvest in the vineyard) did not have a negative effect on berry characteristics in both the application year and the following year. Therefore, it was not considered problematic to perform Shock action, UV-C, Vibration, Leaf injury, Leaf removal, and *Botrytis cinerea* applications to improve grape quality. When the principles of the stress applications tested in the study were adhered to, it was revealed that the berry characteristics of the next year did not change.

Author Contributions

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

	E.B.	İ.K.	C.T.A.
C	40	30	30
D	100		
S	100		
DCP	20	5	75
DAI	50		50
L	10	50	40
W	10	80	10
CR	40	30	30
SR		100	
PM	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.

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 there was no study on animals or humans.

Acknowledgments

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THE EFFECTS OF PRE-HARVEST MELATONIN APPLICATIONS ON PHYTOCHEMICAL PROPERTIES OF CRIMSON SEEDLESS GRAPE VARIETY (*V. vinifera* L.)

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Abstract: Foliar melatonin applications are crucial for grape quality as they can enhance skin color development, increase antioxidant capacity and nutritional value of grapes. The effectiveness of preharvest melatonin applications may change depending on fruit species, variety, application time, and dose. In the current study, it was utilized from various doses of melatonin application, including 0, 0.25, 0.50, and 1 mmol l^{-1} for improving the phytochemical attributes of Crimson Seedless table grape variety. The results of the principal component analysis showed that different doses of foliar melatonin application had different effects on the yield and biochemical attributes of grape variety. But particularly, 1 mmol l^{-1} and 0.50 mmol l^{-1} of melatonin doses had significant effects on total phenolic compounds content and antioxidant capacity from the phytochemical properties of Crimson Seedless table grape variety.

Keywords: Table grape, N-acetyl-5-methoxytryptamine, Phenolic compounds, Antioxidant capacity, CIELAB color system

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1. Introduction

Crimson Seedless is a widely cultivated late-ripening grape type with firm, crisp berries and exceptional flavor (Martinez-Gil et al., 2013; Amaro et al., 2020). A wide range of phytochemicals and natural metabolites found in grapes such as phenolic acids, ascorbic acids, carotenoids, and flavonoids, significantly contribute to their quality, appearance, and flavor as well as a host of other biological functions and health benefits (Waterhouse, 2002; Cosme et al., 2018; Averilla et al., 2019; Albuquerque et al., 2021; Yakhchi et al., 2023). As a result, one of the main areas of focus in grape production has been on enhancing the phytochemical properties of grapes. Numerous variables, including ecological circumstances, cultivar, rootstock, and various viticultural techniques used in vineyards, have an impact on the phytochemical concentrations and compositions in grapes (Obreque-Slier et al., 2010; Kok, 2022 a, b).

All living things as well as certain plant species like *Rosaceae, Poaceae, Apiaceae, Brassicaceae* and *Vitaceae,* contain the chemical melatonin, which has a low molecular weight and an indole-based structure (Dubbels et al., 1995; Nawaz et al., 2016). The physiological processes of ripening, aging, and defense in fruits and vegetables are all regulated by the endogenous bioactive chemical melatonin (Li et al., 2017). However, the effects of preharvest melatonin applications on fruit ripening on the plant and quality at harvest have been evaluated in very few studies, and different effects have been reported depending on the fruit types or application doses and times.

Shang et al. (2021) state that melatonin induction triggers the accumulation of total phenolic compounds, flavonoids, and anthocyanins in blueberry fruits and increases the antioxidant capacity of the fruits during the physiological processes of the fruits.

A study conducted by Xia et al. (2021) disclosed that melatonin applications increased soluble solids content in grapes by enhancing sucrose phosphate synthase activity, which is important for the synthesis of anthocyanin.

Vitallini et al. (2011) stated that, as a result of their study, there was a synergistic effect between melatonin and grape polyphenols, resulting in high antiradical activity.

This study primarily aimed to determine the impacts of various doses of pre-harvest melatonin application on the yield and biochemical properties of Crimson Seedless table grape variety.

2. Materials and Methods

The study was performed in an experimental vineyard located in Tekirdağ Viticulture Reseach Institute, Türkiye (40°58'18.56″ N; 27°28″ E) in the 2019 vegetation period. In the current research, 5-year-old Crimson Seedless grapevines grafted onto Kober 5 BB (*V. berlandieri* x *V. ruspetris*) rootstock were employed as plant materials. Grapevines were trained to shoot vertically on a trellis (VSP). During the conduct of research, different cultural practices such as fertilization, winter pruning, and green pruning practices were carried out in the vineyard. A standard control program was also used against diseases and pests during the growing period. The climate feature of the research region is a Mediterranean-like warm-temperate climate, and the annual average temperature, daily sunshine duration, relative humidity and total precipitation were recorded as 15.60°C, 4.34 hours, 70.49%, and 334.6 mm, respectively for the 2019 year.

With the aim of improving the phytochemical characteristics of Crimson Seedless table grape variety, powder melatonin (N-acetyl-5-methoxytryptamine) (Sigma-Aldrich, >98%) was used in this research. For this purpose, aqueous solutions of melatonin were prepared in 4 different doses, including 0, 0.25, 0.50, and 1.00 mmol l^{-1}.

In the vineyards, uniform grapevines were identified before the research started. In the study, foliar melatonin applications were implemented twice at 10-day intervals, including 10 days before the vérasion period (first time) and at the vérasion period (second time) by means of a back pump. As the surfactant, tween 20 (0.1 ml l^{-1}) was added to all spray solutions of melatonin to increase its effectiveness. Later, various doses of foliar melatonin were sprayed on all grapevines until run-off in the early morning period between 6:00 AM and 9:30 AM, when the temperature was not yet effective.

Berry weight (g), berry length (mm), berry width (mm), bunch weight (g), bunch length (cm), bunch width (cm), and berry firmness (N) were measured as yield variables in the research. In addition, total soluble solids (TSS) (%), titratable acidity (TA) g l^{-1}, maturity index, must pH, chromatic parameters like a*, b*, c*, L*, hue angle (h°), total phenolic compounds (mg GAE 100 g^{-1} fw) and antioxidant capacity (µmol TE 100 g^{-1} fw) were also found as biochemical variables.

The grapes on control grapevines of the Crimson Seedless type were harvested in 2019 during the vegetative period when the berries on the control grapevines reached about 16% TSS. After harvest, measurements and analyses for yield and biochemical properties were hastily conducted. Initially, 250 berries were used to determine TSS, TA, and pH. For chromatic measurements and analysis of total phenolic compounds and total antioxidant capacity, samples consisting of 350 berries were separated and stored at -25°C until analysis. The maturity index was calculated as the ratio of TSS (%) to TA content (%) (Perralta-Ruiz et al., 2020).

Grape skin colors were measured as CIE L*, a*, b* color system by using a Hunter-Lab Colorimeter (Hunter Lab DP-9000 Color, USA) (McGuire, 1992).

The total phenolic content of berries was determined using a calorimetric assay and Folin-Ciocalteu reagent (Sigma) through the reagent method (Slinkard and Singleton 1977). Total phenolic compound content was calculated using the spectrophotometer at 765 nm and the results were explained in mg of gallic acid equivalent (mg GAE 100 g^{-1} fw).

Antioxidant capacity was assessed via the 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method as described by Brand-Williams et al. (1995) with some modifications. A dose-response curve was generated using Trolox as a standard and the antioxidant capacity was expressed as Trolox equivalent (µmol TE 100 g^{-1} fw).

2.1. Statistical Analysis

The research was carried out in a completely randomized block design with four replicates. For all data, SPSS statistical software (version 20.0) was used and significant differences were assessed using one-way analysis of variance (ANOVA). Statistical differences for average comparisons were evaluated using the LSD multiple range test at the 5% level.

3. Results and Discussion

3.1. Yield Properties

The physical properties of fruit species are one of the most important factors that affect fruit quality (Minas et al., 2018). Overall, doses of melatonin application exhibited the same pattern of increase in berry characteristics in the current study, (Table 1). Diverse doses of foliar melatonin applications have no statistical effects on berry length ($P<0.05$) and the means of berry length varied from 2.12 mm (0.25 mmol l^{-1}) to 2.21 mm (0 mmol l^{-1}).

Table 1. Influences of diverse doses of foliar melatonin applications on yield parameters of Crimson Seedless grape variety

Applications (mmol l^{-1})	Berry Length (mm)	Berry Width (mm)	Berry Weight (g)	Berry firmness (N)	Bunch Length (cm)	Bunch width (cm)	Bunch weight (g)
M 0.00	2.21	1.69	4.18	5.91^{b}	18.60	14.53	521.33
M 0.25	2.12	1.61	3.69	5.69^{b}	18.20	15.93	603.13
M 0.50	2.15	1.64	3.89	6.12^{ab}	18.53	15.26	586.40
M 1.00	2.19	1.65	3.84	6.89^{a}	18.80	14.13	490.66
$LSD_{5\%}$	N.S.	N.S.	N.S.	1.33	N.S.	N.S.	N.S.

Different letters stand for significant differences in each column at 5% level in accordance with the LSD multiple range test. M 0.00: 0 mmol l^{-1}, M 0.25: 0.25 mmol l^{-1}, M 0.50: 0.50 mmol l^{-1}, M 1.00: 1.00 mmol l^{-1} N.S.: Non-significant

Concerning berry width (Table 1), there are no significant differences among the diverse doses of melatonin application (P<0.05). In the study, melatonin-applied grapevines had lower berry width means, including 1.61 mm (0.25 mmol l^{-1}), 1.64 mm (0.50 mmol l^{-1}), and 1.65 mm (1.00 mmol l^{-1}) compared to 0 mmol l^{-1} (1.69 mm).

As far as berry weight is concerned shown in Table 1, berry weight is not significantly influenced by diverse doses of foliar melatonin application (P<0.05). In the research, the highest berry weight mean was recorded for 0 mmol l^{-1} (4.18 g), followed by 0.50 mmol l^{-1} (3.89 g), 1.00 mmol l^{-1} (3.84 g) and 0.25 mmol l^{-1} (3.69 g).

As shown in Table 1, berry firmness is significantly influenced by the diverse doses of foliar melatonin application (P<0.05). In the study, the lowest berry firmness means were respectively 5.69 and 5.91 N for 0.25 mmol l^{-1} and 0 mmol l^{-1}, whereas the highest berry firmness mean was 6.89 N for 1.00 mmol l^{-1}.

With regard to bunch length (Table 1), diverse doses of foliar melatonin application have no significant effects on bunch length (P<0.05) and the lowest bunch length mean was 18.20 cm for 0.25 mmol l^{-1}, whereas the highest bunch length mean was 18.80 cm for 1.00 mmol l^{-1}.

As regards bunch width, it is seen in Table 1 that diverse doses of foliar melatonin application do not significantly affect bunch width (P<0.05). While the highest bunch width mean was 15.93 cm for 0.25 mmol l^{-1} in the study, the lowest bunch width mean was 14.13 cm for 1.00 mmol l^{-1}.

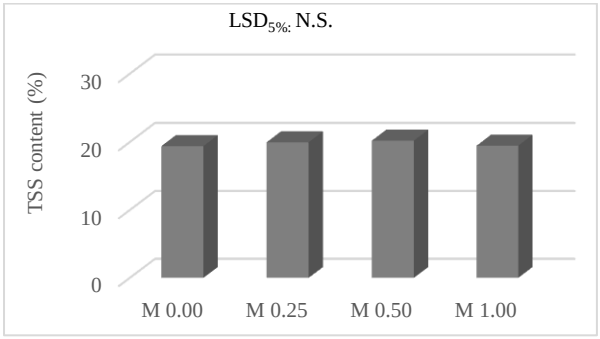
In view of the bunch weight, it is apparent in Table 1 that diverse doses of foliar melatonin applications have no statistical effects on bunch weight (P<0.05). In the present study, bunch weight means altered from 490.66 g (1.00 mmol l^{-1}) to 603.13 g (0.25 mmol l^{-1}).

3.2. Biochemical Properties

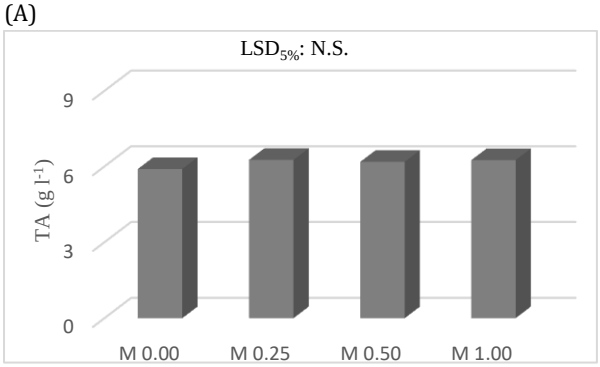
Sugar is the main soluble solid in grape musts and sweet wines and the quantity of soluble solids is a leading indicator of grape maturity (Jackisch, 1985**)**. With regard to TSS content demonstrated in Figure 1A, there are no differences among the diverse doses of foliar melatonin application (P<0.05). In the current study, the lowest TSS content mean was 19.40% for 0 mmol l^{-1}, whereas the highest TSS content mean was 20.20% for 0.50 mmol l^{-1}.

There are numerous organic acids such as malic acid, tartaric acid, and citric acid in grapes and these organic acids greatly contribute to the organoleptic quality of grapes and wines (Silva et al., 2015). In connection with TA pointed out in Figure 1B, TA is insignificantly affected by diverse doses of foliar melatonin application (p< 0.05) and TA means changed from 5.90 g l^{-1} (0 mmol l^{-1}) to 6.26 g l^{-1} (0.25 mmol l^{-1}). While melatonin treatments were not clearly altering titratable, tartaric, and malic acids in a study led by Xu et al. (2017), the contents of titratable acids in another study led by Meng et al. (2019) were increased by melatonin treatments. But melatonin treatments had no significant effects on TSS content.

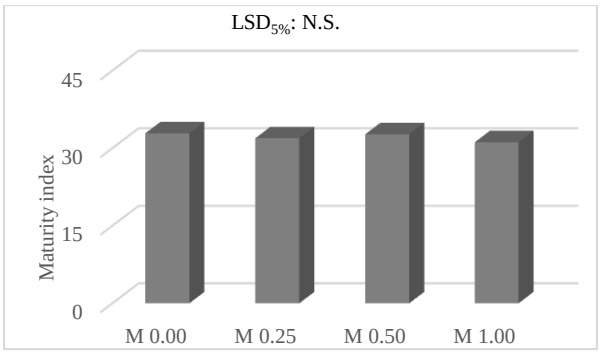
The TSS/TA ratio is considered the maturity index employed to establish the ideal harvest time for grapes (Liang et al., 2005). On the subject of the maturity index represented in Figure 1C, it is noteworthy that diverse doses of foliar melatonin application have no effects on the maturity index (p <0.05). While the highest maturity index mean was 32.88 for 0 mmol l^{-1}, the lowest maturity index mean was 31.16 for 1.00 mmol l^{-1} in the study.



(A)



(B)



(C)

Figure 1. Influences of diverse doses of foliar melatonin applications on TSS (A), TA (B) content and maturity index (C).

The pH of grape juice must normally vary between 3.0 and 4.0 depending on metabolic activities (Bisson et al., 2007). In relation to must pH exhibited in Figure 2, there are no significant differences among the diverse doses of foliar melatonin application (p<0.05) and pH means ranged from 3.39 (0.25 mmol l^{-1}) to 3.44 (0 mmol l^{-1}).

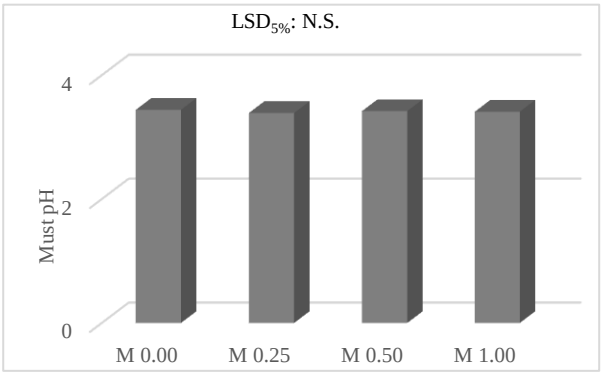


Figure 2. Influences of diverse doses of foliar melatonin applications on must pH.

Skin color is remarkable for table grape quality and consumer acceptance and the CIELAB color system is used for color description in various fruit species (Sahin and Sumnu, 2006). In the CIELAB color system, a* value represents redness (+a) and greenness (-a). When it comes to a* values of grape skin denoted in Table 2, it is viewed that a* values are significantly influenced by diverse doses of foliar melatonin application ($p< 0.05$). In the present study, the lowest a* value mean was recorded for 0.25 mmol l^{-1} (4.96), whereas the highest a* value means were respectively recorded for 1.00 mmol l^{-1} (6.25), 0 mmol l^{-1} (6.39) and 0.50 mmol l^{-1} (6.76).

In the CIELAB color system, the b* value indicates the yellowness (+b) and blueness (-b) (Sahin and Sumnu, 2006). Regarding b* values of grape skin manifested in Table 2, diverse doses of foliar melatonin applications have no statistical effects on b* values ($p <0.05$) and b* value means in the study varied from 2.27 (0.25 mmol l^{-1}) to 2.77 (0.50 mmol l^{-1}).

Chroma is considered the quantitative property of color and is used to determine the degree of difference between a color tone and a gray color with the same lightness (Pathare et al., 2013). Regarding the c* values of grape skin illustrated in Table 2; it is notable that diverse doses of foliar melatonin application have significant effects on c* values ($P<0.05$). In the study, the highest c* value means were successively obtained from 0.50 mmol l^{-1} (7.44), 0 mmol l^{-1} (6.96), and 1.00 mmol l^{-1} (6.75) when compared with 0.25 mmol l^{-1} (5.67).

In the CIELAB color system, the L value shows lightness from black (0) to white (100) (Sahin and Sumnu, 2006). Concerning L* values of grape skin demonstrated in Table 2, there are no differences among the diverse doses of foliar melatonin application ($P<0.05$), and L* values in the current study differed from 36.19 (1.00 mmol l^{-1}) to 37.13 (0.50 mmol l^{-1}).

Hue angle is considered the qualitative property of color and is employed to define the difference between a certain color and a gray color with the same lightness (Pathare et al., 2013). In reference to hue angle (h°) attributes of grape skin demonstrated in Table 2, diverse doses of foliar melatonin application have no significant effects on hue angle ($P<0.05$). The lowest hue angle mean was 21.14 (0.50 mmol l^{-1}) in this study, whereas the highest mean was 36.31 (0.25 mmol l^{-1}).

Table 2. Influences of diverse doses of foliar melatonin applications on chromatic attributes of gape skin

Applications (mmol l^{-1})	a*	b*	c*	L*	h°
M 0.00	6.39[a]	2.58	6.96[a]	36.37	24.68
M 0.25	4.96[b]	2.27	5.67[b]	36.41	36.31
M 0.50	6.76[a]	2.77	7.44[a]	37.13	21.14
M 1.00	6.25[a]	2.41	6.75[a]	36.19	24.21
$LSD_{5\%}$	1.23	N.S.	1.14	N.S.	N.S.

Different letters stand for significant differences in each column at 5% level in accordance with LSD multiple range test. M 0.00= 0 mmol l^{-1}, M 0.25= 0.25 mmol l^{-1}, M 0.50: 0.50 mmol l^{-1}, M 1.00= 1.00 mmol l^{-1}. N.S. = non-significant

Phenolic composition in grapes is broadly influenced by different factors, including viticulture practices, ecological conditions, and grape variety (Downey et al., 2006). As for the total phenolic compound contents displayed in Figure 3, it is discerned that diverse doses of foliar melatonin applications significantly influence the total phenolic compound contents ($P<0.05$). In the existing study, the highest total phenolic compound means were recorded for 1.00 mmol l^{-1} (283.96 mg GAE 100 g^{-1} fw) and 0.50 mmol l^{-1} (259.10 mg GAE 100 g^{-1} fw) compared to 0 mmol l^{-1} (195.96 mg GAE 100 g^{-1} fw). According to results of a study conducted by Meng et al. (2015), it has been concluded that melatonin treatments of pre-veraison grape berries alter wine aroma components of Merlot grape variety.

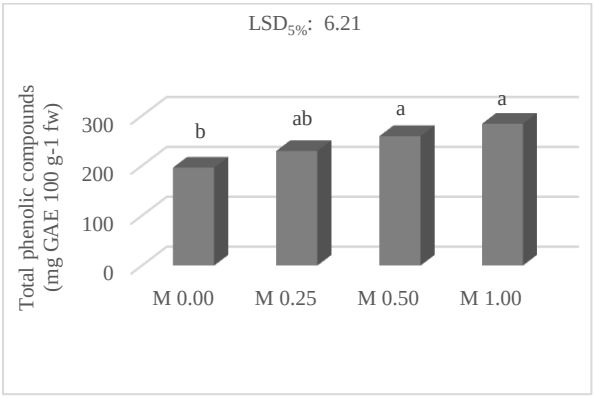


Figure 3. Influences of diverse doses of foliar melatonin applications on total phenolic compounds.

The grape has high levels of antioxidant capacity and is an important fruit species that contributes the most to antioxidant capacity (Gross, 2016). With respect to the antioxidant capacity indicated in Figure 4, it is clear that antioxidant capacity is significantly affected by diverse doses of foliar melatonin applications ($P<0.05$). While the highest antioxidant capacity mean was 451.96 mol TE 100 g^{-1} fw in this study, the lowest mean was 341.26 mol TE 100 g^{-1} fw (0 mmol l^{-1}). The results of a study conducted by Xu et al. (2017) revealed that the contents of 18 of the 22 detected individual phenolic compounds were enhanced by melatonin treatment; especially, the resveratrol content was largely increased concomitantly with the up-regulation of STS gene expression In the meantime, melatonin treatments enhanced the antioxidant capacity of berries. Yang et al. (2020) also declared that exogenous melatonin significantly enhanced the biosynthesis of each flavonol and flavanol component of cv. Kyoho, especially catechin, which was almost increased double by 200 µM of melatonin treatment.

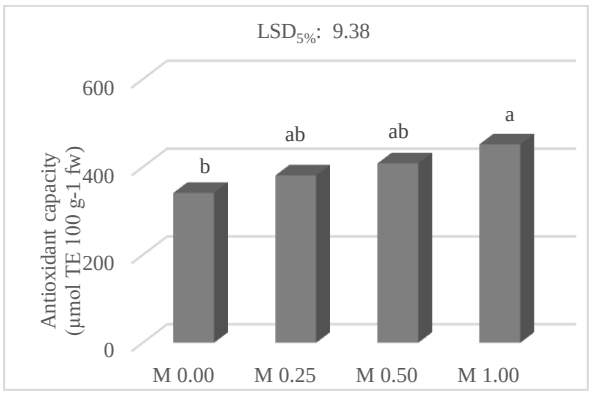


Figure 4. Influences of diverse doses of foliar melatonin applications on antioxidant capacity.

4. Conclusion

In recent times, there has been great interest in harnessing melatonin applications to boost the quality characteristics of grape varieties. Foliar melatonin applications may greatly enhance the nutritional contents and phytochemical properties of grapes. In the present study, it was found that various concentrations of foliar melatonin significantly and favorably affected the phytochemical characteristics of the table grape variety. Due to the mentioned properties of melatonin, applications of pre-harvest foliar melatonin may be useful for raising grape quality in viticulture. As a consequence of this research, 1.00 mmol l^{-1} and 0.50 mmol l^{-1} of melatonin concentrations were found to be effective for improving phenolic compounds and antioxidant properties of Crimson Seedless table grape variety.

Author Contributions

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

	D.K.	E.B.	A.İ.T.	O.E.
C	40	40	10	10
D	50	50		
S	25	25	25	25
DCP	25	25	25	25
DAI	50	50		
L	100			
W	100			
CR	50	50		
SR	50	50		
PM	30	30	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 there was no study on animals or humans.

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Research Article
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DEVELOPMENT OF BALER MACHINE FOR HUMID AREAS

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Abstract: Baler machines collect the swath straw when it reaches a sufficient level of moisture and bale it. However, the drying time of the material is prolonged in humid regions. This causes the land not to be used for the second crop and to get wet again in case of rainfall. Clogging is observed in commonly used baling machines when baling these products because they are more humid. The study aims to collect and bale the material even if the straw is not sufficiently dried in humid areas. For this purpose, dimensional improvements were made in the stubble chopper unit of a baler and compared with two widely used machines in the country. While clogging was observed in the other machine, no clogging was observed in the improved machine. In terms of direct usability, feed with particle size distribution smaller than 15 cm was 93.20% for the developed machine (DM) and 89.43% for the baler machine (BM-1). DM clogging problems have not been observed.

Keywords: Baler machine, Stubble chopper unit, Square straw bale

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1. Introduction

Feed, which is important in animal nutrition, is gaining value day by day for farmers engaged in the production of animal foodstuffs. Feed storage is important in terms of increasing feed prices and ensuring the sustainability of animal feeding. After harvesting forage crops, natural drying is impossible at the desired level due to the minimal number of sunny days, especially in regions with annual rainfall averages above 1000 mm. This can lead to storage out of the appropriate humidity conditions and partial deterioration of the product obtained. In terms of forage quality and suitable storage conditions, the moisture content of the harvested forage plants is reduced to 15-20%. However, care should be taken not to lose valuable parts such as leaves and flowers by drying the forage plants more than necessary during drying (Ulger, 1982).

Ensuring energy balance and efficient functioning of the immune system in animals is possible with adequate and balanced nutrition. This can be achieved by increasing forage consumption as early as possible with appropriate ration arrangement at the beginning of lactation. Increasing feed consumption can be achieved by using quality roughages, well-balanced quality concentrate feeds, suitable feeding areas, clean feeders, preventing stressful conditions (shower, fan, etc.) and taking timely measures to minimize imbalances in the rumen (roughage ratio, roughage particle size, etc.) (Görgülü et al., 2011). For this reason, particle size distribution of forages is important. It is reported that the chopping and grinding of grass, stalks, and straw loses flavor and digestibility. In excellent grinding, bacterial fermentation of cellulose cannot be completed because the size of the forage particles causes the feed to pass through the rumen rapidly. As a result, the degree of cellulose digestion in feeds decreases and most of the cellulose and similar substances are thrown out with manure as undigested (Akyıldız, 1986). On the other hand, insufficient particle size decreases the ruminal acetate-to-propionate ratio and the pH, which, in turn, lowers the milk fat percentage (Lammer et al., 1996). In forages with large piece sizes, problems such as difficulty in consuming these forages are encountered. Accordingly, the feeding of forages with a reduced particle size (RFPS) has been suggested earlier in the context of potentially enhancing dry matter intake (DMI) in conventional dairy cattle feeding (Haselmann et al., 2019). For example; in silage plants with low dry matter content, it is reported that it would be appropriate to chop them into pieces with an average length of 4 cm (Yalçın and Çakmak, 2005). It is recommended that the particle size ratio should be between 1.27 and 5.08 cm for feeding hay to animals as feed (Bitra et al., 2011). To prevent these problems, the most appropriate feed piece sizes are between 1-5 cm. If the hay is given directly, it is recommended to be no more than the size of the mouth. Green forage crops follow operational processes such as harvesting, mowing, crushing, barrelling, mixing, drying in the field, and baling. Balers are machines that collect many materials such as green forages, stubble, various animal feeds, cereal stalks from the field in scattered or swath form, compress them and bale them by tying them with rope or wire (Güner and Keskin, 2011). The product arriving at the stubble chopper unit is compressed and

tied in the bale room. The bale is thrown out of the channel. In this process, many factors such as the condition of the field surface, the condition of the hay, the size and uniformity of the windrows, the capacity of the picking up and feed mechanisms, the forward speed of the baler, the amount of power available affect the efficiency of the baler (Verma et al., 2019). Besides these factors product to be baled and its moisture condition is also very important. If the product moisture cannot be brought to suitable conditions according to the product and moisture condition, situations such as mold may be encountered during storage. However, the moisture condition varies regionally. Under the conditions of our country, the product to be baled can be baled at the beginning of the summer period such as June in non-humid regions, while in humid regions, this period can see the end of the summer period such as August. In humid regions, product moisture cannot be reduced below 20%. This hinders agricultural activities or causes delays. In humid regions, to continue agricultural activities in the summer months, bales may have to be collected even if they are not at the appropriate moisture level for storage. This situation may cause clogging problems in the collection and sizing units in humid areas during baling.

The extremely limited number of rain-free days required for drying and baling the barrels makes this process difficult. This study aims at the dimensional and functional development of a stubble chopper unit that can collect the product even if the product moisture is at the desired optimum levels in baling machines, as well as to solve the clogging of the collecting unit and to chop the product.

2. Materials and Methods

The experiments were conducted in Salur village (40.99349262069505, 35.90112034879492) in Ladik district of Samsun province (Figure 1) on a land that is flat and partially sloping (4%).

Figure 1. View of the terrain

The tractor was operated up and down the slope. Wheat straw was used as material. In measurements taken from different points in the field, the swath width was between 90-120 cm and the swath height was between 25-40 cm. The moisture content of the straw was determined by the standard oven method and its value was 19.2%.

The studies were carried out with three different baler machines; It was carried out with a) developed machine (DM), b) baler machine 1 (BM-1) and c) baler machine 2 (BM-2). BM-1 and BM-2 are commercially produced and are already widely used by farmers. DM, on the other hand, is a machine developed on the stubble chopper unit and produced commercially. This development was designed by changing the dimensional characteristics of the stubble chopper (straw chopping rotor) unit of a baler machine.

The pickup unit widths of the three machines were measured as 155 -170 cm. The stubble chopper unit has been redesigned with dimensional changes (Figure 2). This design has been tested on DM. While the diameter of the stubble chopper unit in BM-1 and BM-2 type balers, which are widely used in the market, is 20.8 cm, this diameter has been changed and this diameter value has been enlarged in DM. Since the patent application process for the DM machine is ongoing, this measurement value cannot be given here. In addition, the counter-beater is made of 8 separate parts with different angles and is positioned to surround the beater with a total inclination of 170°. The spacing of the stubble chopper unit and the counter beater has been increased compared to BM-1 and BM-2, allowing the material that is more voluminous due to excessive moisture to be held by the stubble chopper unit. The number of metal sheets placed on the Stubble chopper unit was increased from 6 in normal machines to 7 in the DM machine.

In the baling process with these machines, the average bale dimensions were 35×45×80 cm, while the bale weight was between 20-25 kg. For piece sizes, samples taken from the bale were divided into pieces smaller than 5 cm, between 5-15 cm and larger than 15 cm and weighed with a precision scale and determined as a percentage value.

The study was conducted according to the random parcel factorial trial design with 2 factors and 16 replications. Three different machines and 2 different tractor speeds (3.5 km h^{-1} and 5 km h^{-1}) were used as factors. Tractor speeds were determined by traveling 100m in the convenient gear.

The results were evaluated by analysis of variance in the SPSS 19.0 Program. The differences between the averages were determined according to the LSD test. However, during the work, the BM-2 type machine was constantly clogged and was found to be unsuitable for use. Therefore, the results of only two machines were evaluated.

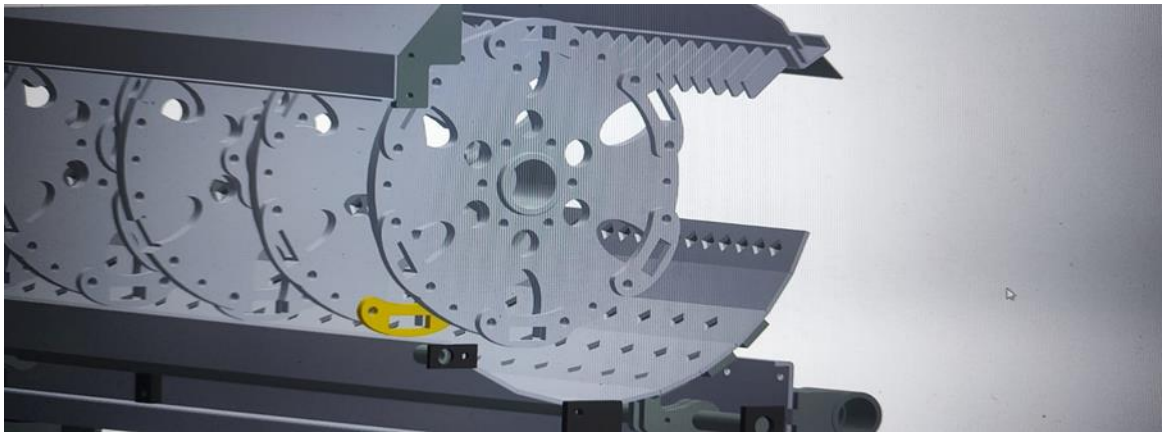

Figure 2. General view of the developed Stubble chopper unit.

3. Results and Discussions

The machine and other machines developed to solve the product collection and baling problem seen in humid regions were evaluated statistically.

Statistically, machine and speed factors were found to be significant for part sizes smaller than 5 cm and between 5-15 cm ($P<0.05$). However, it was found to be statistically insignificant for fragment sizes larger than 15 cm ($P<0.05$). Mangaraj S. And Kulkarni S.D. (2011) reported in their study that the particle size ratio larger than 15 cm in wheat straw was 58%. They found that the average length was 29.56 cm. Re-grinding is needed for this value in terms of animal nutrition. In cases where this value cannot be reduced, some difficulties may occur in terms of nutrition, especially in cattle. This causes the feed to be ground again, increasing operating and feed costs. In our study, it was determined that the feed rate larger than 15 cm varied between 1-65-12.47% for DM and 3.70-17.81% for BM-1. It was observed that the developed machine's part size value greater than 15 cm (6.80%) was lower than the other machine (10.56%) (Table 1).

Table 1. Effect of some factors on particle size

	x<5 cm	5<x<15	15<x
Machine			
DM	35.48[a]	57.72a	6.80 b
BM-1	39.92b	49.51b	10.56 a
Speed			
3.5 kmh^{-1}	42.63 a	49.18 b	8.18 a
5 kmh^{-1}	32.77 b	58.04 a	9.18 a
LSD Value			
Machine	3.33	2.81	1.98
Speed	3.33	2.81	ns
Machine × Speed	4.71	3.98	2.80
R^2	0.56	0.67	0.36
CV	0.17	0.10	0.45

ns= not significant

According to the results of the analysis of variance, the interaction between DM and BM-1 with speed was found to be significant when the fragmentation size was less than 5 cm and between 5-15 cm. For DM 3.5kmh^{-1}, the fragment sizes smaller than 5cm and between 5-15 cm varied between 30.65-45.67%, 46.17-65.63%, respectively, while for BM-1, these values varied between 26.45-63.70% and 32.59-54.14%, respectively. For DM 5kmh^{-1}, it varied between 21.54-40.61%, 51.93-72.49% for fragment sizes smaller than 5cm and between 5-15 cm, respectively, while for BM-1, it was found between 21.08-44.41%, 44.09-62.65% (Figure 3). The findings of this study differ from those reported by El-danasory and Imbabi (1998) in the context of wheat straw harvesting using a combine machine. The latter authors asserted that the feed rate increases with an increase in forward speed. However, in the present research, this pattern held for DM and BM-1 in the distribution of particle sizes between 5 and 15 cm, while the opposite was observed for particles smaller than 5 cm. It appears that the nuanced process of harvesting smaller-sized straw, coupled with structural differences between the combine and baler, accounts for this disparity in results. Another study on equipping a conventional combine available in the market with a two-stage turbine designed for wheat straw collection showed a 50% feeding rate. This change resulted in an additional collection of 0.84 tons/ha of straw (Suardi et al., 2020). However, it is noteworthy that more losses were observed compared to the baler developed in the present study; especially in particle sizes between 5-15 cm and under two different tractor speeds.

According to variance analysis, the combined effects of machine and speed were significant for part dimensions ($P<0.05$). When the Machine x speed interaction was evaluated together, part size less than 5cm was important for BM-1 (44.84%) and DM (40.41%) at 3.5kmh^{-1}. Considering the 5-15 cm piece size, it has been

determined that DM is important at 5kmh^{-1} speed. If these pieces are smaller than 5 cm in size, this may cause the bale to disintegrate. However, the baling process with piece sizes in the range of 5-15 cm has a positive effect on the bales not being easily dispersed. Statistical analysis for two methods of feeding in conventional rectangular balers shows that the ideal speed of the tractor during feeding is 4 km/h (Afify et al., 2005). Other results have been observed regarding the direct effect of tractor speed on straw harvesting efficiency. In the harvesting of rice straw, elevating the baler's forward speed from 2 to 4 kmh^{-1} and from 4 to 6 km/h resulted in a respective reduction of 30% and 26% in the overall baling costs. Additionally, a decrease in the baler's feeding rate was observed with an increase in the forward speed (Afify et al., 2002). In another study conducted by Verma et al., 2019, the highest feeding efficiency (93.47%) was achieved using a round straw baler designed for various straw types, particularly emphasizing paddy straw. This optimal performance occurred under conditions of minimal moisture (19-21%) and maximum tractor speed (0.3 kmh^{-1}). The results of the current study, demonstrating a feeding rate of 93.20% for the baler specifically designed for wheat straw harvesting, emphasize the suitability of this device for application on both national and international levels.

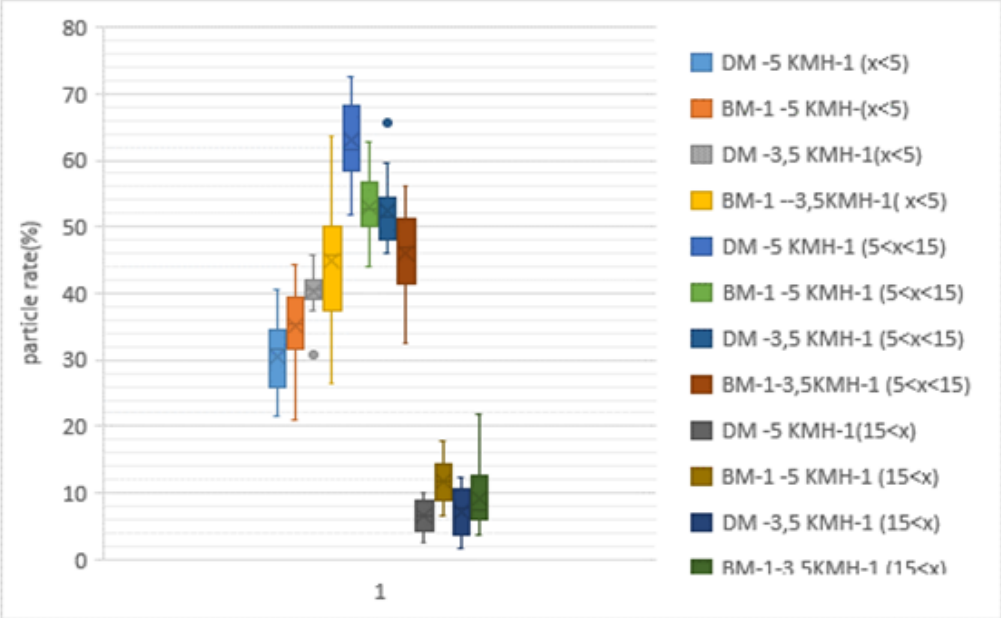

Figure 3. Part size distribution (%).

4. Conclusion

The fact that the particle size ratio larger than 15 cm does not exceed 6.58% increases the direct usability of the feed without re-grinding. Therefore, significant reductions in operating expenses and feed costs can be achieved. In terms of direct usability, while feeds with particle size distributions smaller than 15cm were 93.20% for DM, this value was 89.43% for BM-1. It was observed that the bales made between DM and BM-1 did not show any difference in size and mass. It was observed that the product loss did not exceed 1% during the machine operation, and there was no binding loss or damaged bale. In the developed machine, no clogging was observed in the collection and harvesting unit during operation. However, blockages occurred in the other machine. For the developed machine, work successes at average speeds of 3.5 km h^{-1} and 5km h^{-1} were 340 bales h^{-1} and 420 bales h^{-1}, respectively. For the other machine, the work success was 3.5 km h^{-1} and 5km h^{-1}, with an average of 276 bales h^{-1} and 332 bales h^{-1} respectively. In practice, it has been observed that the main source of this difference is the frequent blockages in BM-1. It is also important that the bale has appropriate piece sizes to ensure direct feed delivery and reduce the energy needed to break down the feed. In practice, the changes made to the stubble chopper unit, specifically aimed at improving DM during baling, have provided visible advantages over baling with BM-1. The use of such machines is important in terms of important factors such as time, cost and quality.

Author Contributions

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

	T.K.	K.Ç.S.
C	50	50
D	60	40
S	90	10
DCP	50	50
DAI	60	40
L	70	30
W	60	40
CR	50	50
SR	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.

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 there was no study on animals or humans.

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COMPARISON OF SOLUBLE FLAXSEED GUM EXTRACTS USING DIFFERENT AQUEOUS EXTRACTION METHODS

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Abstract: Gums and mucilages represent viable options for incorporation into food formulations owing to their numerous advantageous properties, including emulsification, thickening, and modulation of rheological characteristics within food products. Flaxseed is a material with its soluble gum that can be used for food fortification. Within the scope of the study, extracts were obtained using 16 flaxseed extraction methods in the literature and compared in terms of their general properties (color, flow behavior, total soluble solids). The method with 1% ratio, 80°C, 750 rpm and 15 minutes, with pH 5.3 was not suitable for fortified emulsion-based food another method with a 10% ratio, 90°C, 750 rpm and 240 minutes, with maximum Brix was suitable for fortified foods with soluble solid. The methods with 5% ratio, 100°C, 750 rpm and 30 minute parameters and 12% ratio, 90°C, 750 rpm and 240 minutes parameters, which have higher viscosity and lighter color, can be used for light color-fortified products. The A9 (8% ratio, 90°C, 750 rpm and 240 minutes) and A10 (10% ratio, 90°C, 750 rpm and 240 minutes) samples the highest viscosity with the darkest color, can be selected for fortified products where color is not important but needs to be improved in terms of consistency. The results indicate that method parameters in literature for soluble flaxseed gum (SFG) should be chosen according to the characteristics of the food to be fortified.

Keywords: Flaxseed, Aqueous extraction, Water soluble flaxseed gum, Functional foods

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1. Introduction

Flax is recommended as raw material for the textile industry (Coskuner and Karababa, 2007) and its seed can be consumed in different forms as a food ingredient or for medicinal purposes since its cultivation (Oomah and Mazza, 1998). The Latin name of it means 'very useful' because of its versatile use. In this way, flaxseed (*Linum usitatissimum L.)* can be used to describe flax when eaten by humans; on the other hand, linseed is used to describe flax when it is used for industrial purposes (Morris, 2004). In this manner, consuming of flaxseed (*Linum usitatissimum L.)* is one of the alternatives for daily nutrition. It contains high amounts of oil, protein and dietary fiber and generally, brown Canadian flaxseed composition is 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture and 3.4% ash (Morris, 2004).

Consumption of high levels of plant-derived foods can provide essential amino acids, lipids, carbohydrates, vitamins, minerals, fibers, and other phytonutrients that can be linked to lower risks of various chronic diseases (Cardosso Carraro et al., 2012; Edel et al., 2015). In literature, it is suggested that flaxseed consumption can lower the risk of cardiovascular diseases, inflammation, diabetes, cancer, kidney diseases, gastrointestinal disorders, and maintain brain health (Parikh et al., 2018; Thumann et al., 2019; Almehmadi et al., 2021; El Seedy et al., 2021; Karantonis et al., 2022). Flaxseed is one of the key sources of functional foods because of its ingredients and physical supporting properties. Ingredients isolated from flaxseed, such as oil, soluble dietary fibers, and lignans, have been incorporated into multiple food products to enhance their nutritional and functional properties (Hallund et al., 2008; Marie and Ivan, 2017; Basiri et al., 2018; Bastida et al., 2021; Kairam et al., 2021). Soluble flaxseed gum, also known as flax mucilage (SFG), is primarily found in the outermost layer of the hull. This fiber-rich hull can readily release mucilaginous material, or soluble gum, when immersed in water (Hu et al., 2020). Gums and mucilages represent viable options for incorporation into food formulations owing to their numerous advantageous properties, including emulsification, thickening, and modulation of rheological characteristics within food products (Hamdani et al., 2019; Yemencioglu et al., 2020).

In literature, the flaxseed extract was used in bread (Hao and Beta, 2012), cereal-based products (Celik and Kuzumoglu, 2020), beverages (Basiri et al., 2018), and spreads (Ghosal et al., 2022) for improving textural and rheological properties. Over the past ten years, research has predominantly concentrated on whole flaxseed-enriched food products (excluding studies on single flaxseed components in foods). Bread has been the

primary focus, accounting for 44% of the studies, followed by pasta (10%), cookies (9%), and yogurt (9%). The interplay between flaxseed ingredients and substrate components varies depending on the specific fortified food matrix (Zhang et al., 2023). This fiber-rich hull can readily release mucilaginous material (soluble gum) when soaked in water. This fiber-rich hull can readily release mucilaginous material (soluble gum) when soaked in water. Previous research analyses primarily focused on the gum extracted from the whole seed (Qian et al., 2012). The properties of the SFG depend on seed types and extraction methods. It is known that the compatibility of the mucilage produced depending on different time and ratio parameters with the food to be functionalized is important and there are studies on extraction. The main problem is that it needs to be known which extraction method is suitable for the functional food to be enriched in terms of matrix, color and brix. Color change is not desired in foods that will be given functionality, but an increase in consistency is desired. In some cases, the color change is insignificant and more consistency is required. However, it has yet to be known for which food type SFG obtained by which extraction method should be used. Comparison of the extraction methods indicates the significance of choosing the right extraction temperature to align with the SFG properties for the particular application (Kaushik, 2017). In the present study, different aqueous extraction methods reported in the literature were used to extract SFG from flaxseed samples, and the effect of extraction method on the color, rheological, pH and Brix properties of extracts was determined.

2. Materials and Methods

The flaxseeds (*Linum usitatissimum*) used in this study (Fig.1) were of commercial origin with the Arifoglu brand and obtained from a local market in Türkiye. The chemicals used for analysis were Sigma-Aldrich (Germany) and Merck (Germany) brands.

2.1. Preparation Methods of SFG Aqueous Solutions

In this study, 16 different extraction methods were used to extract (flax mucilage/ soluble flaxseed gum -SFG) from brown flaxseed (Figure 1) and the methods are listed in Table 1. In general, seed ratio (%), water temperature of used and time of mixing process are three main parameters. The parameters were ranged from 1% to 12% for seed ratio, from room temperature to 100°C for temperature, from 15 minutes to one night for mixing time and from 250 rpm to 1000 rpm for mixing rpm. Firstly, whole flaxseed weighing was carried out according to the method, then water was added according to the determined seed ratio and then mixed on a heat-controlled magnetic stirrer according to different holding times (VELP Scientifica). For the next step, whole seeds were separated with a centrifuge of 5500 g and 15 minutes to get a clear extract (Hettich Universal 320R). This last process, including separating the seed from SFG, applied all the methods as a last step. The SFG samples obtained according to the methods was used in the analysis.

2.2. Proximate Analysis

In summary, 2 grams of ground flaxseed were subjected to reflux extraction with 250 mL of petroleum ether for 6 hours. The solvent was then removed using a rotary evaporation system at 50°C, and the extracted oil content of the flaxseed was determined by measuring the weight difference before and after the extraction process (Bouaziz et al., 2016). The total nitrogen content of the sample was determined according to Kjeldahl's method by determining protein content (multiplying the nitrogen content by 6.25) (Qian et al., 2012).

Table 1. Details of the methods for flaxseed aqueous extraction

Samples	Seed ratio (%)	Temperature of water (°C)	Mixing Process	References
A1	1	80	750 rpm and 15 minutes	Yesil (2020)
A2	5	100	750 rpm and 30 minutes	Erkoc et al.(2021)
A3	10	50	300 rpm and 120 minutes	Hellebois et al. (2021)
A4	5	55	250 rpm and 180 minutes	Alhssan (2021)
A5	3	80	1000 rpm and 180 minutes	Tee et al. (2016)
A6	3	100	1000 rpm and 180 minutes	Tee et al. (2016)
A7	10	85	750 rpm and 180 minutes	Kaur et al. (2018)
A8	10	25	750 rpm and 180 minutes	Wu et al. (2010)
A9	8	90	750 rpm and 240 minutes	Bostanoglu (2015)
A10	10	90	750 rpm and 240 minutes	Bostanoglu (2015)
A11	10	60	400 rpm and 300 minutes	Vieira et al. (2019) Wang et al. (2009)
A12	12	90	750 rpm and 240 minutes	Bostanoglu (2015)
A13	12	100	750 rpm and 360 minutes	Bostanoglu (2015)
A14	10	25	400 rpm and 300 minutes	Vieira et al. (2019)
A15	10	40	400 rpm and 300 minutes	Vieira et al. (2019)
A16	12	25	750 rpm and one night	Qian et al. (2012)

Figure 1. Brown flaxseed sample.

Dry matter was determined by drying the sample at 105°C in an oven (NUVE FN 500) until getting constant weight and total ash was determined after combustion of 5 g sample for four hours in a muffle furnace (Protherm Laboratory Furnace, PLF100-3, Türkiye) maintained at 550 ˝C (Qian et al., 2012).

2.4. Total Soluble Solids (TSS-°Brix)

A portable handheld refractometer (ATAGO 0-32°Brix, Japan) was used to measure the brix values of the SFG samples. Before taking measurements, the refractometer surface was cleaned with cotton and calibrated using distilled water.

2.5. pH Measurement

Before the measurements, the pH meter (EUtech 700, Singapore) was calibrated with buffer solutions (pH=4.0 and pH=7.0). After calibration, the pH measurements for all extracts were carried out in triplicated SFG samples.

2.6. Color Measurements

The color parameters L * (darkness-lightness), a * (green-redness), and b * (blue-yellowness) of the SFG samples were measured with a color measurement device (Minolta Chroma Meter, CR-400, Osaka, Japan).

Hue angel was calculated from the a* and b* parameters according to Equation 1:

$$\text{Hue angel}=180/\pi + \tan^{-1}(b^*/a^*) \quad (1)$$

Chroma (C*) values represent colorfulness and are defined as the strength or dominance of the hue. It was calculated as Equation 2:

$$C*=\sqrt{(a*)^2+(b*)^2} \quad (2)$$

2.7. Rheological Measurement

Rheological measurements of the SFG samples were carried out according to Kaur's method (Kaur et al., 2018) with HAAKE MARS III device (Thermo Scientific, Germany).

2.8. Statistical Analysis

The data multiple comparison tests used the Duncan test with a 95% confidence interval, and these analyses were conducted in triplicate for each replicate. The SPSS package program (IBM SPSS Statistics 22, USA) was utilized for the statistical analysis.

3. Results

The whole flaxseed was used for the analysis and its general chemical composition of the flaxseed was determined as 7.5% moisture, 21.3% protein, 34.9% fat and 3.7% ash. In this study, it was revealed how mixing time, mixing power and temperature change the pH of the same product by applying different methods. The pH of the final extract is important for emulsion stability. All the samples were prepared with the same water pH of 7.4 but the samples had different pH values after process conditions were applied. The pH values of the samples are shown in Table 2. Although pH values of the extract samples were measured around 5-6 pH, the highest (7.4) and lowest (5.3) values belong to A1 (1% ratio, 80°C, 750 rpm and 15 minutes) and A11 (10% ratio, 60°C, 400 rpm and 300 minutes), respectively. Only three samples (A1, A2 and A3) have 7 pH values and eight samples have pH values under 6. It was criticized for emulsions in terms of emulsion stability. The pH of flaxseed gum solutions significantly impacts their physicochemical properties, encompassing flow behavior and viscosity. Total soluble solid values (Brix) of the samples were shown in the Table 2. The highest value was measured at A10 (10% ratio, 90°C, 750 rpm and 240 minutes) and A9 samples (8% ratio, 90°C, 750 rpm and 240 minutes). High ratio with high process temperature effect on getting more soluble solids from the seed. This situation is natural when general extraction parameters are considered.

Table 2. Physical properties of the samples

Samples	pH	Brix
A1	7.4±0.1^{g}	0.20±0.00^{a}
A2	7.3±0.0^{fg}	0.46±0.05^{b}
A3	6.1±0.2^{d}	0.30±0.00^{a}
A4	5.6±0.1^{b}	0.20±0.00^{a}
A5	5.7±0.0^{bc}	0.20±0.00^{a}
A6	6.5±0.2^{e}	0.66±0.05^{c}
A7	6.7±0.0^{e}	0.90±0.00^{e}
A8	6.5±0.1^{e}	0.20±0.00^{a}
A9	7.1±0.1^{f}	1.43±0.11^{h}
A10	6.8±0.0^{e}	1.70±0.10^{i}
A11	5.3±0.1^{a}	0.76±0.05^{d}
A12	5.6±0.1^{ab}	1.20±0.10^{f}
A13	5.6±0.0^{ab}	0.20±0.00^{f}
A14	5.6±0.0^{ab}	0.23±0.05^{a}
A15	5.8±0.1^{bc}	0.43±0.11^{a}
A16	5.9±0.0^{cd}	0.64±0.04^{b}

a,b= Different letters in the same column correspond to statistically different samples (P<0.05).

In this study, color values (L*, a*, b*, Chroma and Hue) of the samples (Figure 2) are given in Table 3. The highest L* value was measured in the A9 (8% ratio, 90 °C, 750 rpm and 240 minutes) sample, while the lowest L* value was measured in the A1 (1% ratio, 80 °C, 750 rpm and 15 minutes) sample. In addition, the highest a* and b* values of the samples were measured in A5 (3% ratio, 80 °C,

1000 rpm and 180 minutes) and A2 (5% ratio, 100 °C, 750 rpm and 30 minutes) samples, the lowest ones belong to A2 and (5% ratio, 100 °C, 750 rpm and 30 minutes) A14 (10% ratio, 25 °C, 400 rpm and 25 minutes), respectively. In the extractions at different temperatures on the samples with the same concentration percentages, it is seen that there is a statistically significant increase in the L* value as the temperature rises. The A7 and A8 samples were compared; although they have the same ratio and same mixing process with temperature differences, A7 with high-temperature water application (85°C) has a higher L* value. Table 3 indicates that the L* value increased significantly with increasing temperature (P<0.05). The a* and b* parameters represent red/green and blue/yellow parts of color. For a* value, the highest and lowest values were found at the A10 sample (10% ratio, 90°C, 750 rpm and 240 minutes) and A2 (5% ratio, 100 °C, 750 rpm and 30 minutes) sample. In this way, b* values were measured as 7.25 and 0.35 for the highest and lowest values at the samples shown at Table 3. The H* (hue angel) was calculated from the a* and b* parameters and the highest H* value was calculated for A16 sample (12% ratio, 25 °C, 750 rpm and one night). In addition to that the lowest one was calculated for the A1 sample (1% ratio, 80 °C, 750 rpm and 15 minutes). Generally, all the sample values were found to be different statistically (P<0.05). The highest Chroma value was detected in the A2 sample with a value of 11.03, and the lowest was detected in the A14 sample with a value of 0.91. The values of all samples showed statistical differences (P<0.05).

The samples A9 and A10 exhibited the highest viscosity values, surpassing those of A3, A7, and A8 despite having identical concentration levels. A closer examination reveals that, while these samples share the same concentration, prolonged mixing time and increased rpm led to heightened viscosity. Consequently, noticeable changes in color values were observed. Upon analyzing the color values, it was determined that the L* values of A9 and A10, characterized by the highest viscosity, were the most elevated with 26.53 and 24.89 (Pa.s) values, respectively. Properties of the extract samples according to G' and G'' were given in Table 4. The G′ and G″ values were parallel with viscosity results. The lowest values of G′ and G″ were 0.0358 Pa and 0.0461 Pa for A8 and A1, respectively. The values of tan δ are presented in Table 4, with the highest value observed in sample A8, and the lowest in sample A15.

Table 3. Color values of the samples

Samples	L*	a*	b*	C*	Hue (°)
A1	7.25±0.91^{a}	-0.60±0.15^{f}	3.29±0.39^{g}	3.35±0.07^{g}	-81.98°±0.13^{a}
A2	23.39±0.25^{h}	-2.05±0.07^{a}	10.98±0.27^{l}	11.03±0.20^{m}	-78.06°±0.06^{b}
A3	22.37±0.47^{gh}	-0.64±0.03^{ef}	1.71±0.05^{ef}	1.88±0.03^{f}	-74.27°±0.07^{d}
A4	16.45±0.05^{c}	-0.74±0.02^{de}	1.19±0.02^{de}	1.40±0.06^{d}	-74.76°±0.01^{c}
A5	17.98±0.16^{de}	-0.55±0.11^{f}	1.57±0.21^{def}	1.70±0.02^{e}	-73.09°±0.05^{k}
A6	19.63±0.22^{f}	1.91±0.06^{j}	4.97±0.08^{i}	5.17±0.03^{j}	69.51°±0.64^{f}
A7	19.59±0.28^{f}	1.33±0.04^{i}	4.36±0.09^{h}	4.54±0.02^{i}	69.84°±0.07^{g}
A8	15.55±0.30^{bc}	-0.56±0.07^{f}	0.40±0.04^{a}	0.63±0.00^{a}	73.15°±0.02^{j}
A9	26.53±0.88^{j}	2.30±0.12^{k}	6.74±0.40^{j}	7.43±0.01^{k}	70.22°±0.04^{i}
A10	24.89±0.78^{i}	2.86±0.14^{l}	7.25±0.30^{k}	7.93±0.05^{l}	69.95°±0.08^{h}
A11	19.31±0.07^{ef}	-0.89±0.03^{c}	1.80±0.05^{f}	1.93±0.08^{f}	-66.27°±0.05^{e}
A12	25.36±0.44^{ij}	0.55±0.04^{h}	4.10±0.21^{h}	4.23±0.06^{h}	83.23°±0.05^{l}
A13	21.28±0.19^{g}	0.31±0.01^{g}	3.36±0.04^{g}	3.40±0.10^{g}	83.74°±0.05^{m}
A14	14.73±0.30^{b}	-0.87±0.03 ^{cd}	0.35±0.14^{ab}	0.91±0.02^{b}	106.37°±0.02^{n}
A15	15.78±0.16^{bc}	-1.06±0.03 ^{b}	1.08±0.13^{cd}	1.34±0.06^{c}	125.98°±0.06^{o}
A16	17.76±0.12^{cd}	-0.60±0.00^{f}	0.64±0.03^{bc}	0.94±0.00^{b}	145.19°±0.02^{p}

a,b= Different letters in the same column correspond to statistically different samples (P<0.05).

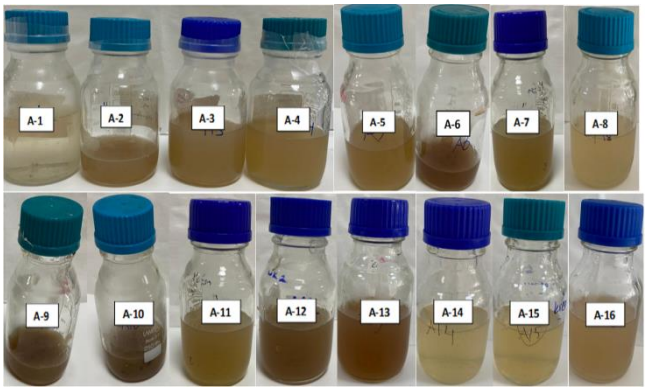

Figure 2. Samples produced by applying different methods.

Table 4. Rheological properties of the samples

Samples	μ(Pa.s)	G' (Pa)	G'' (Pa)	tan δ (G″/G′)
A1	0.0170±0.0014^{a}	0.0920±0.0005^{a}	0.0461±0.0045^{a}	0.5011±0.0009^{f}
A2	0.7849±0.0088^{h}	2.3154±0.0626^{c}	0.6843±0.0351^{d}	0.2957±0.0028^{cd}
A3	0.0586±0.0010^{d}	0.2600±0.0030^{a}	0.1322±0.0088^{ab}	0.5085±0.0015^{f}
A4	0.0308±0.0007^{c}	0.0482±0.0059^{a}	0.0946±0.0011^{ab}	1.9618±0.0008^{k}
A5	0.0357±0.0023^{d}	0.0556±0.0007^{a}	0.0583±0.0069^{a}	1.0496±0.0230^{h}
A6	0.4550±0.0345^{l}	1.6332±0.0133^{b}	0.4665±0.0857^{c}	0.2857±0.0005^{d}
A7	0.2726±0.0127^{k}	2.8915±0.0827^{d}	0.7792±0.0222^{d}	0.2695±0.0004^{c}
A8	0.0385±0.0004^{d}	0.0358±0.0041^{a}	0.1240±0.0152^{ab}	3.4665±0.0010^{l}
A9	1.1645±0.0163i	8.7954±0.1601^{g}	2.3763±0.1517^{e}	0.2702±0.0090^{c}
A10	1.2560±0.0290^{j}	6.7006±0.0475^{f}	2.3002±0.0778^{e}	0.3433±0.0004^{e}
A11	0.0757±0.0009^{f}	0.0986±0.0070^{a}	0.1405±0.0060^{ab}	1.4241±0.0010^{i}
A12	0.6949±0.0204^{h}	3.9895±0.1689^{e}	0.8378±0.0125^{d}	0.2098±0.0503^{b}
A13	0.4733±0.0309^{g}	2.5136±0.3304^{c}	0.7584±0.0043^{d}	0.3018±0.0006^{cd}
A14	0.0289±0.0019^{b}	0.1219±0.0132^{a}	0.2363±0.0030^{b}	1.9374±0.0007^{j}
A15	0.0278±0.0011^{b}	0.3514±0.4770^{a}	0.0638±0.0030^{a}	0.1816±0.0037^{a}
A16	0.0660±0.0023^{e}	0.2407±0.0311^{a}	0.2363±0.0021^{b}	0.9816±0.0004^{g}

a,b= Different letters in the same column correspond to statistically different samples (P<0.05).

4. Discussion

Flaxseed emerges as a crucial functional food, offering a range of potential health benefits. Key nutritional components in flaxseed encompass alpha-linolenic acid, protein, lignin, and dietary fiber (Jenkins et al., 1999). The average composition of flaxseed comprises 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash (Shim et al., 2014). Similarly, the compositions of whole flaxseed were found as 30–41% fat, 20–35% dietary fiber, 20–30% protein, 4–8% moisture, and 3–4% ash (Carter, 1993; Coskuner and Karababa, 2007). Flaxseed boasts a wealth of fat, protein, and dietary fiber. However, it is essential to note that the composition of flaxseed can fluctuate based on genetics, growing conditions, and the method used for seed processing (Daun et al., 2003). Flaxseed's dietary fiber consists of 9% soluble dietary fiber and 20% insoluble dietary fiber (Cui, 2000). The soluble fiber, a viscous seed coat gum, is comprised of neutral (75%) and acidic (25%) monosaccharides (Warrand et al., 2005). This soluble fraction of SFG stands out for its nutritional and functional properties. Various studies suggest that incorporating SFG into the diet can potentially lower the risk of diabetes and coronary heart diseases, as well as contribute to the prevention of colon and rectal cancer (Thakur et al., 2009).

In acidic conditions, the gel strength of SFG diminishes with decreasing pH values, whereas in alkaline conditions, the gel strength declines with increasing pH values (Cui et al., 2006; Chen et al., 2006). The pH of flaxseed gum solutions significantly impacts on their physicochemical properties, encompassing flow behavior and viscosity. In acidic conditions, the gel strength of FG diminishes with a decrease in pH, while under alkaline conditions, the gel strength decreases with an increase in pH (Cui et al., 2006; Chen et al., 2006). Analyzing alterations in SFG solution properties during storage due to pH variations is important, as such changes may be undesirable. The optimal behavior of FG is observed within a pH range of 6.0–8.0 (Mazza and Biliaderis, 1989). On the other hand, eight samples have pH values under six value and it was critical values for emulsion stabilities.

The pH values effect the viscosity of the samples. In this way, the rheological parameters of the samples have been measured because the SFG has an important role in functional food viscosity. The SFG emulsions exhibited shear-thinning behavior, signifying a decrease in apparent viscosity with an increase in shear rate. This observation indicates that the samples displayed non-Newtonian behavior (Wang et al., 2017). Additionally, flaxseed gum exhibits notable gelling, foaming, and emulsifying capacities, offering the potential to replace gum Arabic in food emulsions (Chen et al., 2006; Wang et al., 2010).

Since SFG is expected to serve as a potential food or beverage additive or component, its color influences the visual appeal of products, particularly beverages (Hu et al., 2020). Adding SFG can affect the physical properties of the functional foods enriched with SFG. For this reason, SFG with matching colors should be added to the foods. Considering SFG's composition, which includes protein and carbohydrate, the observed darkening may be attributed to a plausible Maillard reaction between these components (Hu et al., 2020). It has been observed that the main difference between the two existing samples is the temperature difference of 75°C. It has been observed that color differences in the extraction samples occur as the temperature increases (Hao and Beta, 2012). While concentration and seed ratio were effective on the consistency differences between the samples, the temperature parameter was effective on the color differences. The reason for the darker color was the presence of tannin and the occurring Maillard reaction. In addition, soluble flaxseed gum has some

monosaccharides as rhamnose (21.2–27.2%), fructose (5–7.1%), arabinose (9.2–13.5%), xylose (21.1–37.4%), galactose (20–28.4%), glucose (2.1–8.2%) (Barbary et al., 2009).

Xing et al. (2015) stated the impact of extraction temperature on the rheological properties of FG. They observed that the polysaccharide and protein content in the gel increased with temperature, peaking at 70°C. This discovery aligned with Cui et al. (1994)'s findings from a response surface analysis, which identified 70°C as the temperature for maximum gum extraction. Interestingly, there was no significant difference in viscosity between gums extracted at 70°C and 80°C (Xing et al., 2015). On the other hand, recognizing that 70°C might not sufficiently eliminate all microorganisms and inactivate all enzymes in the gum, a higher temperature (98 °C) was considered for further exploration of gum appearance and other properties (Hu et al., 2020).

The viscosity of the samples increased rapidly with an elevated concentration of SFG (Zhao, 2015; Wang et al., 2017). A similar situation was observed in the study in question, but not only the increase in concentration but also the mixing time and temperature increased the viscosity. This finding suggests that if avoiding color alterations in food products intended for use as thickeners is a priority, it is advisable to refrain from using the A9 and A10 samples. It would be more appropriate to choose a method that enables the production of a sample with higher viscosity and more transparent color, in other words, a combination that provides the production of a lighter-colored added-value product. Accordingly, the A2 and A12 samples, which have higher viscosity and lighter color, can be used and the methods in which the samples are produced can be selected. The neutural polysaccharide has a larger molecular size and shows shear thinning flow behavior in aqueous solution above 1% (w/w) flaxseed ratio (Barbary et al., 2009).

At frequencies spanning from 0.1 to 10 Hz, the storage modulus (G′) and loss modulus (G′′) were determined. G′ indicates the capacity of a viscoelastic material to store energy in one cycle during alternating stress, offering insights into the elasticity changes in the sample. Conversely, G′′ represents the energy lost as a material undergoes irreversible deformation due to viscosity, providing information about the material's viscosity magnitude (Ingrassia et al., 2019). In addition to the changes in the elastic component (G'), data for tan δ (G″/G′) can also be presented as a function of stress to interpret the contribution of the viscous component (G″) to the viscoelastic behavior (Ross-Murphy, 1994). The viscoelastic properties of polymer solutions are classified into four different categories according to the change in modules depending on the frequency: (i) “a dilute solution” if G″>G′ during frequency scanning, (ii) G″>G′ at low-frequency values, but G″< at high-frequency G′ is “high-frequency elastic character”, (iii) G″<G′ throughout the whole frequency and the modules are parallel to each other at a certain angle, “weak gel” (iv) G″<G′ for “strong gel” (Sun et al., 2014). Samples produced with high temperature and high ratio have high-frequency elastic character. A tan δ value less than 0.1 indicates strong or true gel-like characteristics, while a higher value suggests weak gel properties (Ross-Murphy, 1994). For all the extraction temperatures, SFG aqueous solutions showed a shear time-independent and shear-thinning behavior. Furthermore, oscillatory measurements showed a prevailing viscous character. However, the decrease of the extraction temperature resulted in an increase of both G' and G". Therefore, SFG extracted at low extraction temperatures showed higher viscous and elastic properties. In contrast, high extraction temperatures increased the antioxidant activity.

5. Conclusion

Flax seed has been successfully used reparation of various value-added products in direct or extracted form as SFG. Flaxseed gum-containing fractions are hydrocolloid polysaccharides utilized in the food industry due to their valuable properties, which enhance both foods and solutions. These fractions are becoming increasingly popular for such applications. In this study, comparing methods in literature related to extraction of SFG was investigated. Choosing of suitable method is important for utilization of SFG preparation of various value-added products in terms of nutritional, physicochemical, phytochemical and sensory properties.

The A2 (5% ratio, 100 °C, 750 rpm and 30 minutes) and A12 (12% ratio, 90 °C, 750 rpm and 240 minutes) samples, which have higher viscosity and lighter color, can be used for light color fortified products. The A9 (8% ratio, 90 °C, 750 rpm and 240 minutes) and A10 (10% ratio, 90 °C, 750 rpm and 240 minutes) samples with the highest viscosity with the darkest color, can be selected for fortified products where color is not important but needs to be improved in terms of consistency. Another parameter to consider is pH and it is important for emulsion or emulsion-based products. A total of eight samples have lower pH value than six. The extract to be used as an additive to food should not have a negative sensory impact and should support the consistency and textural structure of the product while also contributing to its nutritional value. Therefore, this study can be useful for the further studies and sectors related to SFG-added food products.

Author Contributions

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

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

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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THE USE OF RHIZOBACTERIA ON WHITE ROT DISEASE AND GROWTH OF LETTUCE

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Abstract: White rot caused by *Sclerotinia sclerotiorum* [(Lib.) de Bary] is one of the most important diseases negatively affecting lettuce production. In this study, the effects of rhizobacteria containing different species on *S. sclerotiorum* were investigated. Also effect of rhizobacteria were determined on the growth of lettuce. Eight rhizobacteria strains (*Enterobacter cloacae*, *E. aerogenes*, *Bacillus cereus*, *Microbacterium testaceum*, *Pseudomonas putida*, *P. chlororaphis, Acinetobacter calcoaceticus,* and *Burkholderia cepacia*) were used in the study. Firstly, the *in vitro* effects of rhizobacteria strains were investigated on the mycelial growth and sclerotia viability of *S. sclerotiorum*. Then, pot experiments were carried out under controlled greenhouse conditions to determine the effect of selected strains on white rot disease and the growth of lettuce. The effect of tested bacteria on the mycelial growth of *S. sclerotiorum* ranged between 38.09-79.84%, and the *P. putida* strain had the highest impact. The bacterial strains were also effective on the sclerotia viability of *S. sclerotiorum*. The efficiency in the pot experiment was between 50-90% on white rot, and the highest effect was recorded in *A. calcoaceticus* strain. In the pot experiment rhizobacteria also increased plant growth. In particular, *E. aerogenes* was the most successful strain in plant growth. The results revealed that bacterial strains have different inhibitory effects in *in vitro* and *in vivo* experiments, while having the potential in the biological control of white rot disease and positive results on lettuce growth.

Keywords: Biological control, Lettuce, Plant growth promoting bacteria, *Sclerotinia sclerotiorum*

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1. Introduction

Lettuce (*Lactuca sativa* L.) is a temperate climate vegetable and contains a wide variety of minerals such as vitamins A and C, potassium, calcium and iron. In addition to its rich nutrient content, lettuce also has a large commercial market share among vegetables. Annual lettuce production is 27011747 tons globally, and China produces 10.8 million tons, followed by the USA, India and Spain (FAO, 2021). Turkey ranks 8th in the world with 561990 tons production of 3 prominent commercial lettuce varieties (Iceberg, Kıvırcık and Göbekli) (TUIK, 2022).

Diseases and pest control are highly important in lettuce cultivation as well as practices such as planting, irrigation and fertilization. Many diseases and pests hamper lettuce growth and cause great losses. White Rot Disease, caused by the soil-borne fungal pathogen, *Sclerotinia sclerotiorum* [(Lib.) de Bary], affects both field crops and vegetables and is prevalent all the over world, especially in temperate regions (Ferreira and Boley, 2002; Albert et al., 2022). The *S. sclerotiorum* can form sclerotia by the combination of mycelium and is easily recognized with the identification of sclerots. The white rot disease causes great economic losses every year in lettuce production (Bardin and Huang, 2001; Chitrampalam et al., 2008; Clarkson et al., 2014). The pathogen attacks the root and root collar of the lettuce plant and grows in the plant body causing softening and rotting. A mold layer similar to white cotton forms appears on the root as lettuce withers and dies (Anonymous, 2008). On diseased plants, *S. sclerotiorum* forms white mold and the sclerotia (Smolinska and Kowalska, 2018; Ficker, 2019). *S. sclerotiorum* overwinters on plant residues in soil as sclerotium or mycelium, germinates in the spring, and produces ascospores from the apothecium. Ascospores initiate infection following the penetration and germination in host plants (Agrios, 1997; Bolton et al., 2006).

Cultural measures such as the use of disease-free seedlings/seeds, resistant varieties, seed dressing, removal of plant residues, and chemical control are commonly used to control white rot disease; however, expected efficient results cannot be obtained after the development of resistant fungal strains. Therefore, different methods have been tested as the alternative to cultural and chemical control of the disease. The use of plant growth promoting rhizobacteria (PGPR) is one of such alternatives. The rhizobacteria are densely located in the rhizosphere and are in close contact with the plant roots. The population of these bacteria, composed of different bacterial genera, increases rapidly and spreads in the rhizosphere. The rhizobacteria increase root

growth and plant height and protect plants from diseases caused by soil-borne pathogens (El-Kafrawy, 2008; Soylu, 2011; Imriz et al., 2014; Abdeljalil et al., 2016; Sen et al., 2016).

Many studies have been conducted on the biological control of *S. sclerotiorum* or *S. minor*, and the causative agent of white rot on lettuce (Budge and Whipps, 2001; Fiume and Fiume, 2005; Rabeendran et al., 2006; Chitrampalam et al., 2008; Villalta et al., 2012; Baniasadipour and Shahidi Bonjar, 2014; Elias et al., 2016; Yıldız and Cenberci-Coskun, 2017; Soylu et al., 2021; Ramona et al., 2022). The studies reported that *Coniothyrium minitans*, *Trichoderma harzianum*, and *Gliocladium* spp. species are particularly effective on *S. sclerotiorum*. The *Bacillus* and *Pseudomonas*, which are bacterial species, have been reported as highly effective against soil-borne pathogens such as *Sclerotinia* (Hwang et al., 2006; Soylu et al., 2006; Monteiro et al., 2013; Kara et al., 2020).

Accordingly, this study aimed to determine the effects of rhizobacteria on the mycelial growth and germination of *S. sclerotiorum in vitro*, and biological control of white rot disease, and on growth of lettuce.

2. Materials and Methods

2.1. Materials

Fungal pathogen, *Sclerotinia sclerotiorum*, was isolated from the infected parts of lettuce plants and grown on Potato Dextrose Agar (PDA; Merck) medium. *Enterobacter cloacae* (ZE-2), *E. aerogenes* (ZE-5), *Bacillus cereus* (ZE-7), *Microbacterium testaceum* (ZE-8), *Pseudomonas putida* (ZE-12), and *Acinetobacter calcoaceticus* (ZE-13) bacterial strains, isolated from pepper production areas (Kayaaslan, 2021), and *Burkholderia cepacia* (7-a-2) and *Pseudomonas chlororaphis* (11b) strains, obtained from clove plants and identified using hypersensitivity reaction tests in tobacco (HR) and pectolytic activity test on potato and Matriks assisted laser desorption ionization-time of flight mass spectrometry-MALDI-TOF MS technique; bacteria developed for 24 hours were placed in the device after being treated with ethanol/formic acid and their diagnosis was made by comparing them with the microorganisms in the device's library with BiotyperTM 1.1 software (Bruker Daltonics), were used as the test organisms.

2.2. Methods

2.2.1. Effects of rhizobacteria on mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum*

The bacterial strains were grown in Nutrient Agar (NA; Merck) medium from stock cultures, and *S. sclerotiorum* was grown in PDA medium. Tryptic Soy Agar (TSA; Merck) medium was used in the *in vitro* studies to determine the effect of bacterial strains on mycelial growth of *S. sclerotiorum*. Bacterial strains grown in NA medium for 24 hours were streaked in rings on the end of TSA medium in 90 mm diameter Petri dishes. Following the inoculation of the bacteria at 25±2 °C for 24 hours, a 5 mm diameter mycelium disc taken from the edges of a *S. sclerotiorum* culture was placed in the center of the medium. In the control, no bacteria was added and only a *S. sclerotiorum* mycelial disc was placed on the Petri dishes. All treated petri dishes were sealed with parafilm, and incubated at 25±2 ºC. The diameters of mycelial growth were measured when the fungus completely covered the medium (Tozlu et al., 2016), and the inhibition rate was calculated using the diameters measured (Mari et al., 1996). Five petri dishes were used for each application, and the experiment was repeated twice.

The surface of sclerotia grown in PDA for 10 days was sterilized three times with 2% sodium hypochlorite, and rinsed with sterile distilled water to determine the effect of the bacteria on sclerotial germination of *S. sclerotiorum*. The surface sterilized sclerotia were placed in glass tubes containing 10 ml of Luria Bertani Broth (LB Miller; Merck) and bacterial suspensions at a density of $1x10^8$ cell/ml. Five sclerotia were placed in each glass tube, and 3 glass tubes were used for each bacterial isolate. The tubes were shaken at 175 rpm for 24 hours. In the control treatment, sclerotia was placed in the LB medium without bacterial isolates. Following the incubation period, the bacteria-treated sclerotia were cut in the middle with a sterile scalpel and transferred to the PDA medium, and the petri dishes were incubated at 25±2 °C. The effect of bacterial isolates on the viability of sclerotia was determined according to mycelium growth in the media (Abdeljalil et al., 2016). Each treatment was repeated 2 times.

2.2.2. Biological control of white rot disease using rhizobacteria on lettuce

After the *in vitro* tests, a pot experiment was carried out to determine the effect of bacteria on white rot disease in lettuce under controlled conditions. The pot experiment was carried out in the greenhouse at Tokat Gaziosmanpasa University, Research and Application Center between March and June 2021. A bacterial suspension was prepared by culturing bacteria in NA medium at 25±2 °C for 24 hours, and then adjusting the suspensions to $1x10^8$ cells/ml in saline suspension (0.85% NaCl; Merck). Lettuce seedlings, (Maritima variety) planted in pots filled with sterile soil, peat and perlite (1:1:0.5), were treated with prepared bacterial suspensions for 1 hour. Another 100 ml bacterial suspension was applied to each plant after 10 days. After 10 days, the suspension of bacteria was sprayed on the plant leaves.

Following the bacterial spray, the scar tissue was opened with a scalpel at the root neck of the plant body and a bacterial suspension was applied to the opened part. Immediately after the application, a 5 mm diameter mycelial disc of S. *sclerotiorum* was placed on the opened wound. This inoculated part was wrapped with moist cotton and the plants were placed in a humidity chamber for 1 day. For positive control, plants were inoculated only with *S. sclerotiorum* (Tozlu et al., 2016), while distilled water was applied for negative control. The

experimental layout was randomized plots with 10 replicates, and the treatments were repeated 3 times. After the pathogen application, dead and viable plant counts were carried out according to the growths in the positive control plants (Bayram and Belguzar, 2021).

2.2.3. Effects of rhizobacteria on the growth of lettuce

Under controlled conditions, a pot experiment was carried out to determine the effect of rhizobacteria on the growth of lettuce. The pot experiment was carried out in the greenhouse between March and June 2021. Pathogen wasn't applied to the plants in this part of the study. As in the biological control experiment, bacterial suspensions were prepared, then lettuce seedlings were treated with prepared bacterial suspensions for 1 hour. Another 100 ml bacterial suspension was applied to each plant after 10 days. Then, 10 days after this application, the bacterial suspensions were sprayed onto the plant leaves. Distilled water was applied for negative control. The experimental layout was randomized plots with 10 replicates, and the treatments were repeated 3 times. At the end of approximately 45 days, head diameter, head height, head fresh and head dry weight, stem diameter, root length, root fresh and root dry weight were measured in plants that were daily maintained and irrigated (Bayram and Belguzar, 2021).

2.3. Statistical Analysis

The data obtained were subjected to variance analysis (ANOVA) using SPSS, version 25 statistics software (SPSS Inc. USA), and the differences in the means between the treatments were determined with Duncan multiple comparison test ($p \leq 0.05$).

3. Results

3.1. Effects of rhizobacteria strains on mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum*

The effects of eight rhizobacteria strains on the mycelial growth of *S. sclerotiorum* in the Petri study ranged between 38.09-79.84%. *Pseudomonas putida* (ZE-12) (79.84%) was the most effective isolate. Bacterial strains ZE-5 (78.77%) (Figure 1), ZE-2 (77.43%), ZE-7 (76.37%), ZE-13 (72.24%) were also highly effective on pathogenic fungi. The differences on the mycelial growth of *S. sclerotiorum* among the isolates and control were significantly different (Table 1). The effect of bacterial strains on sclerotial germination of *S. sclerotiorum* was also statistically significant ($p<0.05$). Germination was recorded in sclerotia immersed in bacterial suspensions of ZE-2, ZE-5, ZE-7, ZE-8 and ZE-12, while sclerotia treated ZE-13, 7-a-2 and 11-b strains died. The ZE-5, ZE-8 and ZE-12 strains had a significant effect on the mycelium growth of the pathogen; on the contrary, sclerotia were still viable after exposure. The mycelium growth of sclerotia treated with ZE-2 and ZE-7 bacteria also was significantly suppressed (Table 1). The ZE-13 strain was particularly effective on both mycelium growth and sclerotia germination in *in vitro* study, and thus it was determined as the most successful strain.

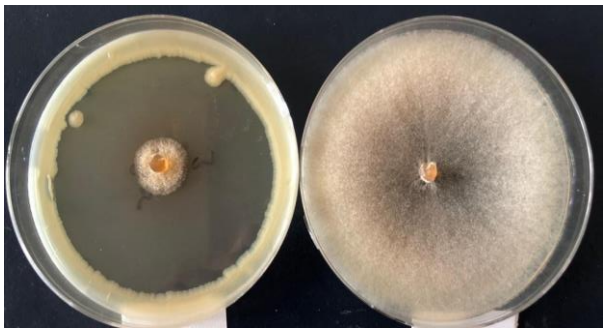

Figure 1. Effect of *Enterobacter aerogenes* (ZE-5) on the mycelial development of *S. sclerotiorum.*

Table 1. The effects of bacterial strains on the mycelial growth and sclerotia viability of *Sclerotinia sclerotiorum*

Codes	Treatments	Diameter of mycelium (mm)	Mean effect (%)	Sclerotia viability	Diameter of mycelium (mm)
C	Control	77.27±0.60 a*	0	Viable	81.32±0.70 a
11b	*Pseudomonas chlororaphis*	48.06±5.10 b	38.09	Dead	0.00±0.00 d
7-a-2	*Burkholderia cepacia*	44.85±7.69 b	42.22	Dead	0.00±0.00 d
ZE-8	*Microbacterium testaceum*	38.49±7.75 c	50.41	Viable	54.64±4.72 b
ZE-13	*Acinetobacter calcoaceticus*	21.55±5.74 d	72.24	Dead	0.00±0.00 d
ZE-7	*Bacillus cereus*	18.34±7.52 de	76.37	Viable	2.59±4.99 d
ZE-2	*Enterobacter cloacae*	17.52±8.89 de	77.43	Viable	1.73±3.97 d
ZE-5	*Enterobacter aerogenes*	16.48±2.99 de	78.77	Viable	42.47±11.26 c
ZE-12	*Pseudomonas putida*	15.66±5.67 e	79.84	Viable	44.64±8.24 c

*The same letters in the same column indicate that differences between applications are not significant ($p \leq 0.05$).

3.2. Biological control of white rot disease using rhizobacteria on lettuce

The plants started to die 6 days after the pathogen application in the pot experiment. Dead/viable plants were counted in the treated groups according to the developments in the positive control plants. All plants in positive control in which only *S. sclerotiorum* was applied, died (100% mortality). No signs of white rot disease were observed in plants of the negative control group. The inhibitory effect of bacteria in bacterial treated plants varied between 50-90%. The highest effect was recorded in strain ZE-13 (90% effect) (Figure 2), followed by strains ZE-5 and ZE-12 (80% effect). The ratio of effect in strains ZE-2 and ZE-7 applications was 70%, and followed by strain 7-a-2 (60% effect). The lowest effect of rhizobacteria was observed in strain 11-b application (Table 2).

Figure 2. Disease development in plants with PC, NC and ZE-13.

Table 2. The effect of rhizobacteria on white rot disease in lettuce

Codes	Treatments	Rate of effect (%)
PC	Treatment with *Sclerotinia sclerotiorum*	0
NC	Treatment with pure water	100
ZE-13	*Acinetobacter calcoaceticus*	90
ZE-5	*Enterobacter aerogenes*	80
ZE-12	*Pseudomonas putida*	80
ZE-7	*Bacillus cereus*	70
ZE-2	*Enterobacter cloacae*	70
7-a-2	*Burkholderia cepacia*	60
11b	*Pseudomonas chlororaphis*	50

3.3. Effects of rhizobacteria on the growth of lettuce

In this experiment, only rhizobacteria were applied to lettuce that were not infected with *S. sclerotiorum* and plant vegetative growth was examined. It is seen that rhizobacteria significantly increase the development of lettuce plants (Table 3). In NC plants, lettuce head diameter was measured as 27.65 cm, and in rhizobacteria-applied plants head diameter was measured in the range of 29.02-36.0 cm. Compared with NC, the highest effect was seen in plants treated *E. cloacae* (ZE-2). Head fresh (93.4-149.8 g) and dry weight (3.27-23.5 g) were also higher in rhizobacteria treated plants compared to the control. Besides, rhizobacteria were also effective on stem and root structure. *E. aerogenes* (ZE-5) significantly increased the stem diameter (6.75 cm) (Figure 3). Compared to the control (17.9 cm), significant increases were observed in the plants to which the rhizobacteria were applied (15.65-28.32 cm). Strain ZE-5 had a high impact on the root length parameter. Also, root wet weight was measured in the range of 8.48-16.08 g in bacteria applied plants. It was observed that strain ZE-2 (16.08 g) and strain 7-a-2 (15.39 g) showed a remarkable effect when compared to the control (8.03 g).

Table 3. Growth parameters in plants treated with rhizobacteria

	Head diameter (cm)	Head height (cm)	Head fresh weight (g)	Head dry weight (g)	Stem diameter (cm)	Root lenght (g)	Root fresh weight (g)	Root dry weight (g)
NC	27.65±2.8c*	19.5±1.21a	91.3±14.03b	3.24±0.94a	5.52±0.65bc	17.9±6.24b	8.03±3.94d	1.14±0.56c
ZE-2	36.0±2.46a	20.7±2.56a	140.3±23.75a	10.02±1.93a	4.5±0.79d	19.8±4.42b	16.08±3.13a	2.06±0.76a
ZE-5	29.02±2.74c	19.75±1.64a	103.1±14.57b	3.5±1.07a	6.75±0.67a	28.32±7.0a	11.89±3.97bc	1.87±0.88abc
ZE-7	30.67±1.67bc	21.17±1.2a	103.4±10.84b	3.9±1.06a	6.05±0.35ab	25.6±5.83a	8.48±2.13cd	1.26±0.58bc
ZE-12	29.57±3.15c	19.85±1.82a	93.4±11.08b	3.27±0.87a	6.02±0.54ab	24.8±4.71a	8.68±2.53cd	1.09±0.32c
ZE-13	33.67±8.24ab	19.75±5.16a	143.1±38.0a	9.8±2.97a	4.6±1.25d	15.65±4.5b	14.6±4.89ab	2.18±1.24a
7-a-2	35.32±2.81a	20.02±2.2a	148.3±30.2a	9.86±2.31a	5.1±0.94cd	17.75±4.0b	15.39±4.73ab	2.03±0.98ab
11b	35.67±2.71a	21.0±3.34a	149.8±32.85a	9.69±3.09a	5.05±0.91cd	18.02±3.2b	14.58±4.08ab	1.83±0.76abc

*The same letters in the same column indicate that differences between applications are not significant (p≤0.05).

Figure3. Development in NC and ZE-5 applied plants.

4. Discussion

In this study, the effects of rhizobacteria on *S. sclerotiorum* were investigated *in vitro* and *in vivo* conditions. The effects of antagonistic bacterial isolates on *S. sclerotiorum* were significantly different in *in vitro* tests. Similar to our study, Fatouros et al. (2018) reported that *Paenibacillus alvei* K165 inhibited the growth of *S. sclerotiorum* in lettuce. The inhibitory effect of *Pseudomonas* spp. AFP104 isolate on mycelial growth of *S. sclerotiorum* was reported as 83.3% by Soylu et al. (2005), while the inhibition ratio of pathogenic fungus by *Streptomyces* sp., *Pseudomonas fluorescens* and *Bacillus subtilis* (Bs1) bacteria was 72.2, 68.0, 62.22%, respectively (Helmy, 2016). El-Tarabily et al. (2000) reported the inhibitory effect of *Serratia marcescens, Streptomyces viridodiasticus* and *Micromonospora carbonacea*, on the growth of *S. minor in vitro.* The inhibitory effect of rhizobacteria can be attributed to the various antibiotics, antimicrobial compounds, enzymes, and organic volatile compounds secreted by the bacteria, that suppress the growth of pathogens and affect systemic resistance in the host plant. For example, *Pseudomonas* species produce numerous antimicrobial compounds such as pyoluteorin, pyrrolnitrin, phenazines, siderophores, cyanide, 2,4-diacetylphloroglucinol (Compant et al., 2005) and enzymes such as cellulose, chitinase, proteases and beta-glucanase (Hernandez-Leon et al., 2015). Antibiotics such as phenazine-1-carboxylic acid, 2-hydroxyphenazine and pyrrolnitrin produced by bacteria are effective on sclerotium and spore germination (Savchuck, 2002; Fernando et al., 2007; Zhang et al., 2004; Selin et al., 2010).

Also in our study, the bacterial strains tested exhibited promising results for sclerotia viability. A significant suppressive effect of *Pseudomonas fluorescens* isolates on sclerotia formation has been indicated by El-Kafrawy (2008). In another study, Onaran and Yanar (2011) stated that 12 of the 23 isolates tested had an inhibitory impact on sclerotia, and the bacterial strains of *Pseudomonas putida, P. fluorescens, Paenibacillus macerans* and *Bacillus pumilis* killed the sclerotia. The mycelial growth of S. *sclerotiorum* by *Bacillus megaterium, B. cereus, B. subtilis, Arthrobacter nicotianae, A. ramosus, Pseudomonas filiscindens, Stenotrophomonas maltophilia, Brevibacterium frigoritolerans* and *Sphingobacterium faecium* was inhibited up to 80% and the sclerotial germination was between 0-100%. Similarly, *Pseudomonas brassicacearum, P. thivervalensis,* and *P. chlororaphis* species were reported to have a significant inhibitor effect on sclerotia viability (Bayram and Belgüzar, 2021).

The potential of bacterial strains, belonging to different genera, in the biological control of white rot disease in lettuce has been investigated in this study. Similarly, Soylu (2011) investigated the effect of antagonistic root bacterial strains (*Lysobacter enzymogenes* C3R5 and N4-7) and PGPR strains (*Bacillus pumilus* T4, *B. amyloliquefaciens* IN937a, *Pseudomonas fluorescens* WCS417r and *P. putida* 89B-61) on *S. sclerotiorum*. In *in vitro* studies, *L. enzymogenes* strain C3R5 and N4-7 significantly inhibited the pathogen whereas in *in vivo*, all strains showed a suppressive effect on disease development compared to the positive control. Chon et al. (2013) reported that the efficiency of *B. megaterium* (DK6) and *B. cereus* (C210) on sclerotia viability of *S. sclerotiorum* in the greenhouse was 20 and 35%, respectively. The disease incidence of 9 out of 26 strains was 20% in a pot experiment carried out in winter. Therefore, 9 strains were considered as potentially antagonistic bacteria for biological control of Sclerotinia rot of lettuce caused by *S. sclerotiorum.* Chen et al. (2016) indicated that two *Streptomyces* species (*S. exfoliatus* FT05W and *S. cyaneus* ZEA17I) inhibited fungal mycelium growth of *S. sclerotiorum* more than 75%. *S. exfoliatus* FT05W inhibited the disease by 40% in lettuce plants under field conditions. In another study, *Bacillus subtilis* GG95 was reported to suppress white rot disease in lettuce by 88% (Lee et al. 2015). *Arthrobacter* FP15 and *Blastobotrys* FP12 isolates also significantly reduced the severity of white rot disease in lettuce (Aggeli et al., 2020). A pot experiment carried out under greenhouse conditions for the biological control of white rot disease in cucumber plants showed that *Pseudomonas chlororaphis* alone and the co-application of *P. brassicacearum* and *P. chlororaphis* had the highest impact on white rot disease. In both applications, 8 out of 10 plants remained viable (80% effect), and had no signs of white rot (Bayram and Belguzar, 2021).

Acinetobacter calcoaceticus exhibited the highest effect on *S. sclerotiorum* in both *in vitro* and *in vivo* experiments. In this study, studies on the action mechanism of antagonistic bacteria have not been conducted. But, in the literature review, *A. calcoaceticus was* also highly effective against *Fusarium* disease caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The bacteria uses siderophore, biofilm, proteases, endoglucanases and

indole acetic acid, and phosphate solubilization mechanisms, thus promotes plant growth and biocontrol pathogen (Khalil et al. 2021). The *A. calcoaceticus* also increases plant growth by producing malic, succinic, citric and other organic acids (Kang et al., 2012). In our study, besides *A. calcoaceticus, Pseudomonas putida* was successful against the pathogenic fungus. Similarly, the results of a study using cucumber plants under greenhouse conditions, demonstrated that *P. putida* inhibited white rot disease by 83.64% (Onaran and Yanar, 2011). Likewise, *Enterobacter cloacae* strain had a significant effect on the mycelial growth and sclerotia viability of *S. sclerotiorum*. The findings are in agreement with the findings of Mohamed et al. (2020) who also tested the same bacteria against a different pathogen (*Ralstonia solanacearum*). The researchers showed that siderophore, indole acetic acid, hydrogen cyanide, salicylic acid production and *E. clocae* strain PS14 had the highest effect on controlling the agent both *in vitro* and *in vivo*. Similarly, as in this study, *Burkholderia cepecia* had biocontrol activity against various lettuce pathogens including *S. sclerotiorum* and *Burkholderia* strains harbor for the biosynthesis of pyrrolnitrin, burkholdins and cepacin (Biessy et al., 2022).

In this study, rhizobacteria also increased plant growth. There are also studies conducted by Celik (2022) and Karagöz and Kotan (2010) similar to this study. Celik (2022) stated that *Bacillus cereus* provided a yield increase of up to 57.4% compared to the lettuce plants in the control. Rhizobacteria increase plant growth due to the plant hormones and various vitamins, they have produced from the moment they are in the plant, and in this way the root length of the plants is elongated, thus increasing yield. In addition, rhizobacteria provide the transformation of minerals into suitable, degradable forms in the translocation process and incorporation. A large of microorganisms produce Indol-3-acetic acid, giberalic acid and phytohormone, which provide a significant increase in shoot, root, and yield in plants (Çakmakçı, 2005; Chakraborthy et al., 2006).

5. Conclusion

Intense pesticide use in agricultural production areas causes serious environmental as well as health problems. The use of antagonistic bacteria, rhizobacteria that promote plant growth, and beneficial microorganisms are important to reduce pesticide use to sustain agricultural production and increase the yield. The results of this study revealed that microorganisms such as antagonistic bacteria can be widely used as biopreparations or biofertilizers in agricultural areas. White rot disease has a wide host range; therefore, can be a problem in agricultural areas and is very difficult to control. Cultural measures are insufficient to control the disease. Resistance problems and sclerotia formation of the pathogen cause serious difficulties in chemical control. In this study, the effects of 8 antagonistic bacteria against *S. sclerotiorum* were tested both *in vitro* and *in vivo* conditions. The results demonstrated that bacterial isolates with different inhibitory effects have the potential to be used in the biological control of white rot disease. Further studies with these bacteria should be conducted under field conditions, and the effect of antagonistic bacteria tested in this study should also be investigated for other soil-borne pathogens.

Author Contributions

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

	A.C.A.	S.B.
C	50	50
D	50	50
S		100
DCP	50	50
DAI		100
L	50	50
W		100
CR	50	50
SR		100
PM		100
FA		100

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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EFFECT OF DIFFERENT ORGANIC REGULATOR APPLICATION TO PROBLEMATIC AREAS ON SOIL ERODIBILITY PARAMETERS (SERPENTINE SOIL SAMPLE)

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Abstract: In the study, the changes in the structural stability and erodibility properties of the organic regulators (TG, TAGG and SG) applications to serpentine soils were investigated. In line with the study's objective, organic amendments based on oven dry weight were applied to the soils in different dose combinations. The study, designed according to a completely randomized design (CRD) was conducted in plastic pots maintained under greenhouse conditions. Six months after the experiment was established, the pots deteriorate pots were and the necessary measurements were made. WAS, DO, EO, SSI, OM values were measured to evaluate the change in the erosive and structural stability of the soils. As a result of the study, the OM values of the soils increased with the organic regulator applications (TAGG, TG, SG). The highest increase was observed in pots where TG and TAGG were applied. The erodibility parameters of the soils, DO and EO, showed a decrease with increasing dosage applications. WAS and SSI parameters, which are soil erosive variables, increased with increasing application dose. These increases (WAS, OM, SSI) and decreases (DO and EO) depending on the applications were statistically significant ($P<0.05$). This positive improvement in soil variables (WAS, SSI, DO and EO) was attributed to the increase in soil organic matter.

Keywords: Serpentine soil, Organic regulators, Erosion, Improvement

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1. Introduction

Serpentine soils are formed as a result of the weathering and disintegration of a series of ultramafic rocks (peridotite: dunite, lherzolite, harzburgite) consisting of ferromagnesian (Fe, Mg or Fe-Mg) silicates. These soils are generally less fertile compared to other normal soils (low in N, P, K, and Ca) and are rich in heavy metals (Ni, Co, Cr) (Kara, 2019). Moreover, they contain asbestos minerals (chrysotile, lizardite, antigorite) that pose a threat to human health. In addition to these unfavorable conditions, the susceptibility of serpentine soils to erosion has gained global significance (Kara et al., 2023). Soil erosion is a significant problem environmentally, economically, and socially. It leads to severe land degradation and soil loss (Jing et al., 2005), and the transport of problematic areas like serpentine soils from one point to another threatens public health (Kara et al., 2018a). Many studies have reported abnormal concentrations of heavy metals (Ni, Cr, Fe, Co, Mn) in serpentine soils (Kara et al., 2018b; Kara, 2019; Altunbaş, 2023).

To prevent the entry of heavy metals into terrestrial, atmospheric, and aquatic environments, and to mitigate contaminated land, improvement measures need to be taken (Hasan et al., 2019). The importance of adding organic matter to soils has been mentioned to prevent heavy metals from being transported to other places through erosion and mixing with drinking water (Solak, 2020; Kara, 2023; Saltalı et al., 2023). Organic matter positively improves soil aggregate stability. It has been reported in many studies that organic matter (organic regulators: worm manure, leonardite, gyttja, olive pomace, cattle and sheep waste) reduces the resistance of soils to erosion (Spaccini et al., 2004; Kavdır and Killi, 2008; Turgut and Aksakal, 2010; Kara et al., 2022).

In this study, different organic amendments (poultry litter used as poultry litter ash (TAGG), cattle manure (SG), and chicken manure (TG)) were applied to serpentinite soils that have the potential to negatively impact human health, the environment, and ecology. The study investigated their effects on soil erodibility (dispersion ratio, erosion rate, aggregate stability, structural stability index).

2. Materials and Methods

Serpentine soils were used in the experiment. The map representation of the serpentine soil taken (37S X: 322068 Y: 4153161) is given in Figure 1.

Soil samples taken from the field were dried under appropriate conditions and passed through 2 mm sieves. 1 kg of soil sample was weighed for each pot. Except for the pots designated as control treatments, different organic regulators (TAGG, TG and SG) were applied to the other pots in different amounts (1%, 2%, 4%, 6% and 8%) and mixed homogeneously. The homogenized soil + organic regulator (TAGG, TG and SG) mixture was brought to field capacity with tap water. The prepared soil + organic regulator mixtures were incubated under greenhouse conditions for 6 months, and as the soil + TAGG, soil + TG and soil + SG mixtures in the pot dried, they were watered with tap water until they reached field capacity. When the treatment period (6 months) was completed, the samples in the pots were disrupted and made ready for analysis. Soil samples prepared for analysis were determined according to appropriate methods. Some properties of the organic fertilizers used as materials in the study are given in Table 1, and some properties of the tap water applied to bring the soil in the pots to field capacity are given in Table 2.

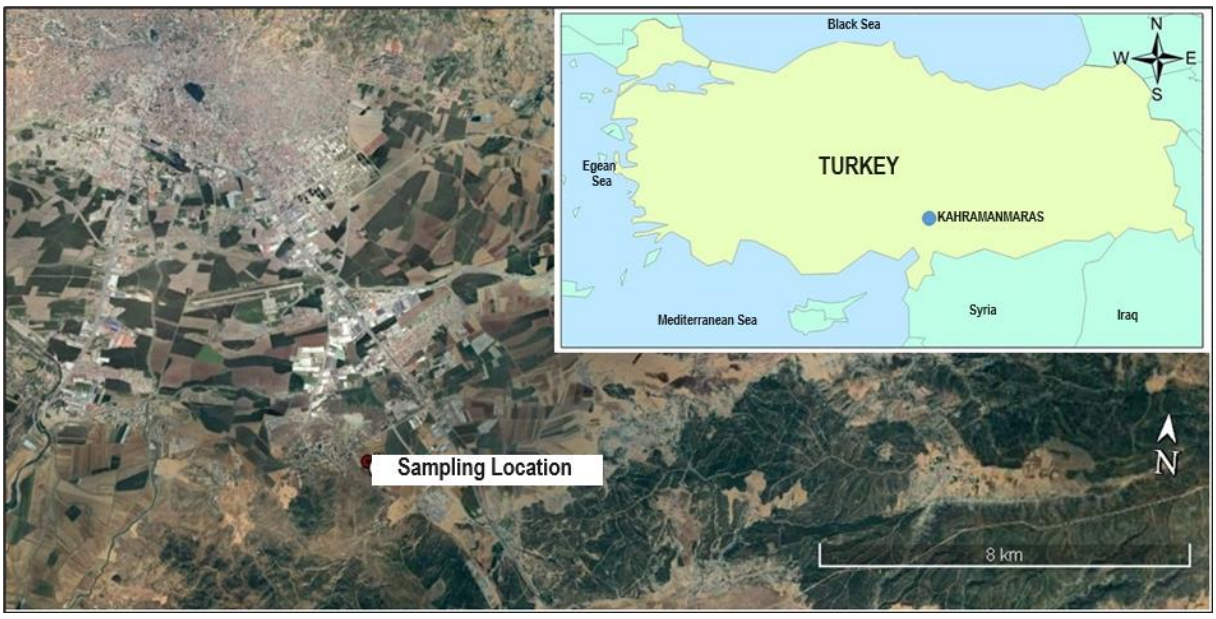

Figure 1. Display of the soil sample taken on Google Earth.

Table 1. Some properties of organic fertilizers used as materials in the study

AF	N (%)	Ca (ppm)	Mg (ppm)	K (ppm)	P (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
TAGG	1.86	110500	3401	14610	2647	1393	4,03	103.1	132
SG	1.31	42940	6463	10510	3357	2691	14.2	80.13	207.7
TG	2.81	5425	2173	12280	2669	173.3	19.18	142.9	198

AF= application fertilizer, TAGG= gyttja manure used as chicken bedding, SG= cattle manure, TG= chicken manure

Table 2. Some characteristics of tap water applied to bring the soil in pots to field capacity

pH	Ec	Ca	Mg	Na	Klorür	Sülfat
	mikroS/cm	ppm	ppm	ppm	ppm	ppm
7.9	490	47.28	17.82	2.32	6.86	11.34

2.1. Chemical Analysis

Soil organic matter content was determined according to the wet combustion method (Nelson and Sommers, 1996). Total N content of organic regulators (TAGG, TG and SG) was determined according to the Kjeldahl method (Bremner, 1996). Total macro (Ca, Mg, P, K) and micro (Fe, Zn, Mn, Cu) element contents were determined based on the Hossner (1996) method.

2.2. Physical Analysis

Soil structure was determined according to the Bouyoucus hydrometer method (Gee and Bauder, 1986). Aggregate stability was determined with a wet sieving device (Kemper and Rosenau, 1986). The dispersion rate of soil wear parameters was determined based on the Bryan (1968) method. Structure stability index (Leo, 1963) and erosion rate (Lal, 1988) were determined according to generally accepted methods.

2.3. Statistical Analysis

Multiple comparison analysis (Tukey Test) of the findings was determined with the JMP 7.0 package program (JMP, 2007).

3. Results and Discussion

According to the findings, the effects of different organic regulators and applications on the amount of soil organic matter are given in Figure 2. Accordingly, compared to the control treatment (1.61%), the application of TAGG, SG and TG organic regulators increased the amount of soil organic matter due to the increase in dose (Figure 2). While the highest organic matter increase was achieved in the TG-8% (2.89%) application, they were listed as TAGG-8% (2.81%) and SG-8% (2.65%), respectively. It has been reported in many studies that organic regulators (such as leonardite, gyttja, worm manure, pomace, cattle and sheep waste, pigeon and quail waste) increase the amount of soil organic matter (Ece et al., 2007; Durmuş and Özdemir, 2020; İlay et al., 2021; Kara et al., 2022; Kara, 2023). The change of soil organic matter depending on the applications was found to be statistically significant (P<0.05) (Table 3).

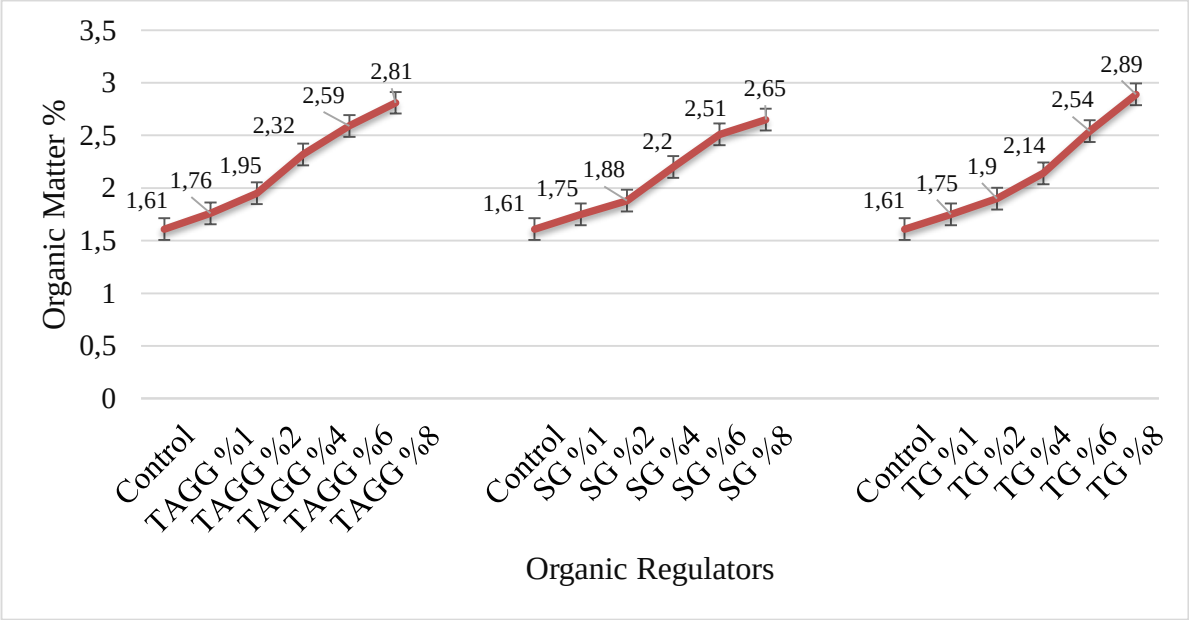

Figure 2. Effect of different organic regulators and applications on soil organic matter

Table 3. Effect of organic regulators (TAGG, SG, TG) applied to soils on soil properties (TUKEY Analysis)

Organic Regulators	AD	OM	SSI	DO	WAS	EO
Gyttja manure used as chicken bedding	TAGG %8	2.81a	34.4a	30.9d	43.96a	34.02d
	TAGG %6	2.59b	31.7a	36.03d	33.29b	40.81cd
	TAGG %4	2.32c	27.9b	43.66c	27.11c	47.55bc
	TAGG %2	1.95d	25.6bc	48.5bc	25.08c	53.77ab
	TAGG %1	1.76e	23.1c	53.64b	19.52d	55.25a
	Control	1.61f	16.4d	66.84a	18.31d	58.18a
Cattle manure	SG %8	2.65a	34.83a	34.75d	40.70a	38.10b
	SG %6	2.51a	32.05a	40.18c	35.50b	43.65b
	SG %4	2.20b	25.09b	50.82b	29.43c	54.63a
	SG %2	1.88c	23.01bc	53.64b	21.66d	57.24a
	SG %1	1.75cd	21.56c	55.57b	19.16de	59.51a
	Control	1.61d	16.45d	66.84a	18.31e	58.18a
Chicken manure	TG %8	2.89a	33.47a	32.37d	43.72a	35.74b
	TG %6	2.54b	31.75ab	36.03cd	26.87b	38.18b
	TG %4	2.14c	29.94ab	39.70cd	25.56b	41.33b
	TG %2	1.90d	28.91b	41.90c	23.06bc	41.56b
	TG %1	1.75e	23.11c	53.64b	21.63bc	55.18a
	Control	1.61e	16.45d	68.84a	18.31c	58.18a

AA= application dosage, TAGG= gyttja manure used as chicken bedding, SG= cattle manure, TG= chicken manure

The changes in soil variables such as structure stability index (SSI), dispersion rate (DO), aggregate stability (WAS) and erosion rate (EO) depending on organic regulator applications are given in Figure 3. Looking at Figure 3, SSI and WAS variables increased depending on the application dose increase of TAGG, SG and TG organic regulators (Figure 3).The highest increase in the SSI variable was at a similar level in the three organic regulators (TAGG-8%, SG-8%, TG-8%). While the lowest SSI value was seen in control pots (16.4%), the highest values were detected in SG-8% (34.83%), TAGG-8% (34.4%) and TG-8% (33.47%) applications, respectively. It is accepted that soils with SSI values below 40% are highly susceptible to erosion (Leo, 1963). Although the soil in the study showed a tendency to exhibit a more stable structure, the SSI values were determined to be below 40%.

The highest increase in the WAS parameter was provided by the TAGG-8% organic regulator (43.96%), followed by TG-8% (43.72%) and SG-8% (40.70%), respectively. The changes in SSI and WAS variables depending on the applications compared to the control treatment were found to be statistically significant (P<0.05) (Table 3). They stated that leonardite fertilizer, one of the organic regulators, increased soil aggregate stability and this increase was statistically significant (İlay et al., 2021). Other researchers reported similar results (Chaney and Swift, 1984; İlay and Kavdır, 2008; Herath et al., 2013; Kara et al., 2022). They reported that there is a positive relationship between the structure stability index and soil organic matter, which are among the soil variables (Mbagwu and Bazzoffi, 1989; Tejada et al., 2008; Erdal et al., 2010; Turgut and Aksakal, 2010). The positive improvement of SSI and WAS variables compared to the control treatment (SSI: %16.4; WAS: %18.31) was attributed to the increase in soil organic matter.

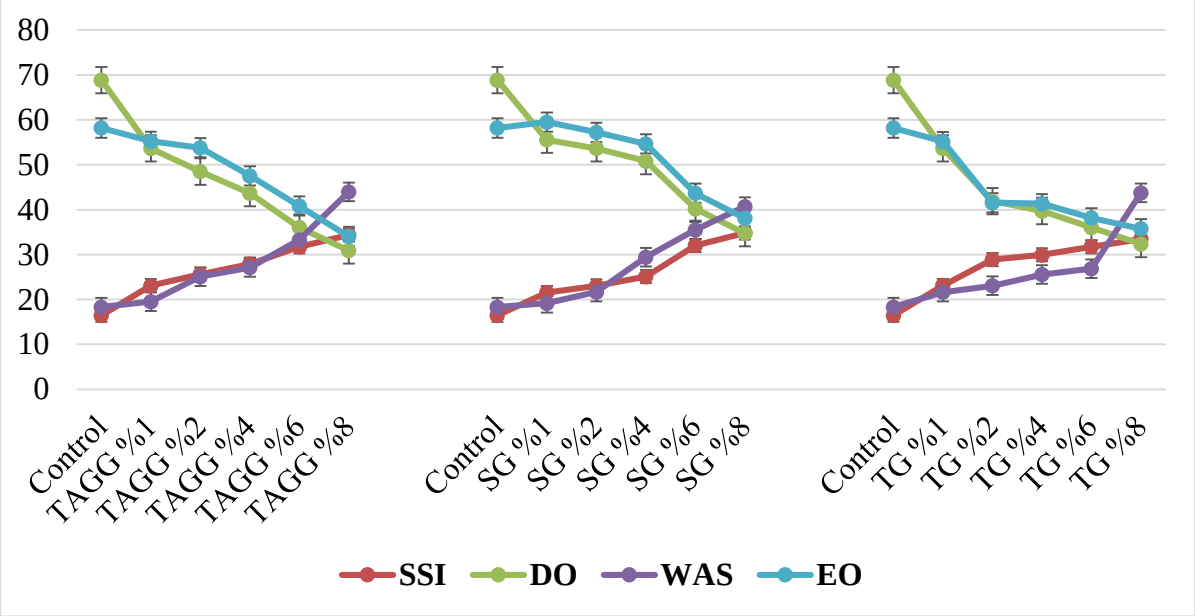

Figure 3. Effect of organic regulators on soil variables SSI, DO, WAS and EO

The changes of DO and EO, which are soil variables, depending on the applications are given in Figure 3 and Table 3. Accordingly, while the DO parameter was highest at the control treatment (66.84%), its lowest value was obtained in the pots where TAGG-8% (30.9%) was applied (Table 3). This was followed by TG-8% (32.37%) and SG-8% (34.75%), respectively. It has been reported that soils with a dispersion ratio greater than 15% are susceptible to erosion (Bryan, 1968; Lal, 1988).Accordingly, although different organic amendments and application doses reduced the sensitivity of soils to erosion compared to the control treatment, they were all greater than 15%. According to the Tukey analysis result, the dispersion rate change was found to be significant (P<0.05) (Table 3). There is an inverse relationship between soil organic matter and dispersion ratio (Saygın et al., 2019). Similar findings have been reported by other researchers (Turgut and Aksakal, 2010; Kara et al., 2022).

The erosion ratio, like the dispersion ratio, showed its highest value in the control treatment (58.18%) pots (Table 3). The erosion ratio tended to decrease depending on the applications, and the lowest values were observed in TAGG-8% (34.02%) and TG-8% (35.74%) pots. Organic regulators and application doses reduced the soil erosion ratio compared to the control treatment. It was reported that soils with a soil erosion ratio greater than 10% are erodible (Lal, 1988). Different organic practices reduced the risk of soil erosion. But it appears to be above the limit stated by Lal (1988). The change in erosion ratio depending on the applications was found to be statistically significant (P<0.05) (Table 3).

4. Conclusion

In this study, the application of TAGG, TG, and SG to the soil reduced the erodibility parameters EO and DO while increasing WAS and SSI. The organic amendments applied to the soils (TAGG, TG, and SG) significantly increased the soil organic matter content. The increase in soil organic matter positively influenced the DO, EO, SSI, and WAS parameters. The improvement effect of organic matter on soil parameters was found to be statistically significant. When comparing organic amendments to each other, the positive impact of TAGG and TG on soil structure was slightly more pronounced than that of SG. Overall, all three organic amendments restored the stability of the soil. On the other hand, the addition of organic matter to the soil improved its structural composition and reduced its susceptibility to erosion. In conclusion, considering the low Ca/Mg ratio and lime content (Kara, 2019) of serpentine soils, TAGG from organic amendments can be recommended for these areas. Additionally, for areas sensitive to erosion but with a suitable Ca/Mg ratio and lime content, the TG organic amendment may be suggested..

Author Contributions

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

	Z.K	F.K	M.Ç	A.Ç
C	40	20	10	30
D	80			20
S	50	10		40
DCP	70	15	5	10
DAI	65	10		25
L	70	10	10	10
W	85			15
CR	40	25		35
SR	60			40
PM	60	10		30
FA	25	25	25	25

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

Conflict of Interest

The authors 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 FORM AND DOSES OF NITROGEN FERTILIZER ON GRASS QUALITY PERFORMANCE AT THE PLANT OF FINE-TEXTURED LAWNS

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Abstract: This study was carried out in Igdir University Sehit Bulent Yurtseven Campus in the vegetation period of 2020 to determine the effects of nitrogenous fertilizer sources and doses on the quality of lawn plants in turfgrass areas. 20-10-10 7 SO_3, 15-5-20 + 2 CaO + 2 MgO, ammonium sulfate (21% N), urea (46% N) fertilizers with nitrogenous fertilizer sources were applied on the parcels in doses of 0-2-4-6-8 g/m^2/month. The effects of fertilizers used after structuring in lawns on plant height, leaf green tone, fresh grass quantity, quality and coating ratios were examined. *Festuca rubra* 40%, *Festuca rubra commutata* 30%, *Festuca rubra trichopylla* 25%, *Poa pratensis* 5%, which are thin textured from cool climate grass species, were used in the mixture. The trial was established according to the factorial trial pattern in coincidence blocks with three repetitions. As a result of the research, ammonium sulfate and 8 g m^{-2} doses were found to be more effective when evaluated for grass quality and performance after structuring in thin-textured grass species.

Keywords: Cool climate, *Festuca rubra*, Urea, Structuring

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1. Introduction

Due to industrialization and urbanization, people need spaces where they can relax, spend time comfortably, get away from city stress and find morale. Nowadays, lawns not only provide people relaxing mentally, but also, they are indispensable for recreational areas, landscaping areas, football, and golf courses where they have a pleasant time. Mixtures to be created by arranging grass species such as *Poa pratensis*, *Festuca rubra* in various proportions can be used in grass areas to be installed for different purposes (Vengris and Torello, 1982). Therefore, the presence of nitrogen is important for vegetative green leaf development, and it is needed more than other nutritional elements (Orçun, 1979). Nitrogen has a positive effect on development by accelerating development and increasing the growth rate, especially in wheat crops (Kaçar, 1977). The fact that the emerald green colors of the grass always remain vivid, and the quality performance of the grass can remain consistently high depends on the regular intake of the elements it needs. Areas whose maintenance is disrupted lose their desired appearance by surrendering to weeds in a short time. This study aimed to determine the most efficient fertilizer type and dose that can be beneficial to the soil and plants for the grass areas created with great dedication to maintain their appearance for a long time.

2. Materials and Methods

2.1. Description of the Research Area

A total of five mixtures of two species were prepared in the study of adaptation of cool climate and fine textured grass species for grass fields under Igdir conditions. In this mixture, *Festuca rubra* 40%, *Festuca rubra commutata* 30%, *Festuca rubra trichopylla* 25%, *Poa pratensis* 5% were used. The effect of nitrogen form fertilizer and doses on grass quality and performance on the created grass composition was studied. The trial was established on the field at the back of the rectorate building and next to the Faculty of Agriculture of Igdir University between the dates from May 2020 to August 2020. Igdir province is in the south of the Igdir Plain, at an altitude of 850 m above sea level and located northwest of Mount Agri. The research location is 15 km away from the city center of Igdir and has a latitude of 39^0 82” and longitude of 44^0 08” degrees.

In the mixtures used in the grass field facility, *Festuca rubra* and *Poa pratensis* species are provided with irrigation water in general, as precipitation, which is one of the most important climatic features for their development, is not in sufficient quantity at the Igdir location, the water needs of the grass fields during the vegetation period are provided. The relative amount of humidity and temperature are sufficient for plant development during the year (Table 1).

2.2. Soil Properties of the Experiment Area

When the soil sample taken from a depth of 0-30 cm in the test area was examined, the lime ratio was found to be 10.57 (medium), pH 8.38 (alkaline), potassium 73.27 kg da^{-1} (high), phosphorus 13.76 kg da^{-1} (very high), organic matter 2.04% (medium), total salt 0.01% (unsalted), soil structure (clay loam).

Table 1. Igdir province climate data (Igdir Provincial Directorate of Meteorology, 2020)

Year/month	1	2	3	4	5	6	7	8	9	10	11	12	Top.
Total Monthly Precipitation (mm: kg m^{-2})													
2020	7.3	14.1	18.1	83.6	76.1	15.7	30.2	15.3	1.4	7.3	7.3	7.8	284,2
Average Monthly Temperature (^{0}C)													
2020	0.0	1.9	10.6	11.7	18.6	23.9	26.7	24.2	23.5	14.5	7.2	3.2	
Average Monthly Relative Humidity (%)													
2020	65.2	64.5	56.5	64.8	55.0	44.7	48.4	47.6	47.7	62.9	67.0	83.6	

2.3. Material

2.3.1. The types and proportions of grass used in the experiment

Festuca rubra (40%), *Festuca rubra commutata* (30%), *Festuca rubra trichopylla* (25%) and *Poa pratensis* (5%), which are cool climate grass species and selected according to their fine textured characteristics, were used in the mixture. To determine the pure and viable seediness, purity and germination rates were determined in the laboratory before planting, and the planting rates of grass species were determined (Oral, 1998).

2.3.2. Nitrogen-form fertilizers applied in the experiment and their doses

From pure and compositely arranged commercial fertilizers containing nitrogen in different proportions in their content; 20-10-10 + 7 SO3 (20% N), 15-5-20 + 2 CaO + 2 MgO (15% N), ammonium sulfate (21% N) and urea (46% N) varieties were used. According to the increasing nitrogen doses, 0-2-4-6-8 g m^{-2} was applied monthly during the vegetation period.

2.4. Method

2.4.1. The experiment plan and the planting operations

The experiment was set up according to the factorial trial pattern in coincidence blocks, with three repetitions. Before the experiment area was prepared, foreign substances were removed from the area and rough leveling was carried out with agricultural machines. After the fine leveling was made using a rake, the parcels were formed by determining the lines with ropes and piles. The parcel area was established as 2 m x 1 m = 2 m^2 (Misia, 1991; Hunt and Dunn, 1993). The distance between the parcels is set to 0.5 m. Four types of commercial fertilizers containing nitrogen in different proportions were applied to all parcels in 5 different doses consisting of 2, 4, 6 and 8 g to m^{-2}, one of which was a control dose (0 g m^{-2}), with 3 repetitions (3 x 5 x 4 = 60) after the form every month (May, June, July)

The planting process was carried out on May 15, 2020. Pure phosphorus fertilizer is given to the seedbed in the form of TSP granular fertilizer to be 8 g m^{-2} before planting, and it is aimed the grass roots to develop better and start to build up quickly. Planting of seeds was made in a parcel area of 2 m^2 (size: 2 m x 1 m) with 40 g per m^2. For the cover soil, peat was used, which was available as economic conditions permitted and would allow the grass to grow easily. After preparing the cover soil, it was applied to the seed to be 1 cm and pressed with a cylinder. After planting, regular irrigation was carried out with a sprinkler irrigation system until the grass output was ensured to be homogeneous.

Although it depends on the period, climate and daily weather, irrigation was performed every two days afterwards. The weeds formed in the parcels have been purified by physical intervention, and the grass has been completely spread in the area. It is aimed to increase fraternization and structuring by making regular forms. During the 3-month devoted maintenance and control process of the grass, the parcels were cleaned, especially 3 weeks (21 days) before the measurement and weighing process.

2.4.2. The characteristics examined in the research

After the cleaning procedure was carried out in the experiment area on July 4, all observations, measurements, and weighing were carried out 1 time on July 25. The characteristics examined at this stage are the amount of fresh grass, plant height, color tone, grass quality and coverage rate. The variance analyses of the obtained numerical data and LSD multiple comparison tests were performed according to JMP 5.0.1 statistical package program (Steel and Torrie, 1980).

2.4.3. The amount of fresh grass

The fresh grass obtained from the research were weighed with sensitive measuring instruments and the amount was indicated as g.

2.4.4. Plant height

Measurements were made with the help of a ruler from the places determined at 10 separate points of each research parcel, and the plant height was determined as cm (Mulvalı, 1999).

2.4.5. Color tone

The leaf color shades of the plants in the parcel were determined by visual evaluation for the summer season using a scale of 1-9. Accordingly, 1 represented yellow and 9 represented dark emerald green tone (Spangenberk et al., 1986).

2.4.6. Grass quality

The homogeneous image formed by the lawns in the parcels, the evaluation created because of visual examinations such as frequent structuring and weeding was determined by scoring according to the 1-9 scale. On the scale, 1 represented the worst and 9 the best turf quality values (Sills and Carrow, 1983).

2.4.7. Coverage rate

In the experiment area, the plant coverage rate was determined with the help of a frame measuring 50 x 50 cm, the inner area of which was divided into 100 equal parts by a rope. The degree of coverage the area of the plants was calculated with the help of this frame in each parcel by counting the squares of 25 cm^2 and proportioning them to the total (Avcioglu, 1983).

3. Results and Discussion

3.1. Lawn Quality and Performance Values

The numerical data obtained from all parcels are processed as fresh grass quantity, plant height, color tone, grass quality and coverage rate, and it has been made comparisons with similar studies conducted previously.

3.2. The Amount of Fresh Grass

The effects of fertilizer type, fertilizer dose and the interactions of these factors on the amount of fresh grass were found to be significant at the level of 1% (Table 2). The amounts of fresh grass obtained in nitrogen fertilizer forms and doses are given in Table 3.

According to Table 3, in terms of grass yields, ammonium sulfate and urea fertilizer varieties were found in the highest amounts with 800.7 g and 724.7 g values in the average fertilizer varieties, while 20-10-10 and 15-5-20 fertilizer varieties remained in low amounts with 713.3 g and 523.3 g values. In terms of the average fertilizer dose, the 8 g m^{-2} fertilizer dose remained the highest with a value of 1004.2 g, and the control fertilizer dose remained the lowest with a value of 310.8 g. In the interaction of fertilizer dose with fertilizer type, urea fertilizer 8 g m^{-2} fertilizer dose remained the highest with a value of 1200 g, and the control fertilizer dose remained the lowest with values of 273.3 g, 300 g, 303.3 g, 366.6 g, respectively, in all fertilizer types.

3.3. Plant Height

Nitrogen fertilizer forms and fertilizer dose interactions were found to be significant at the level of 1% on the obtained plant height values and the effects of the interactions of these factors were found to be significant at the level of 5% (Table 4).

The ammonium sulfate and urea fertilizers used were taken quickly by plants with their fast dissolution properties in the soil and had a positive effect on growth. 20-10-10 and 15-5-20 compound fertilizer types which oscillate controlled affected grass performance values more slowly. According to the results of the research, fast-dissolving pure nitrogen fertilizers in the soil had an immediate effect on the height of the lawns and enabled them to grow faster than composite fertilizers. For all fertilizer types, as the dose given increased, the height of the plants gradually increased.

According to Table 5, in terms of grass plant height, 15-5-20 fertilizer was found in the lowest amount with an average of 13.2 cm in the fertilizer type averages, while urea fertilizer was found in the highest amount with 16.5 cm. In terms of fertilizer dose averages, the 8 g m^{-2} fertilizer dose was the highest at 22.3 cm, and the control fertilizer dose remained at the lowest value at 9.9 cm. Regarding the interactions between fertilizer type and fertilizer dose, the control fertilizer dose remained the lowest in the fertilizer types with values of 9.3 cm, 9.3 cm, 10 cm, 11 cm, respectively, while the 8 g m^{-2} fertilizer dose of urea fertilizer was found the highest value with 25.6 cm.

3.4. Color Tone

Nitrogenous fertilizer forms and fertilizer doses were found to be significant at 1% level based on the color tone values obtained (Table 6). The color tone performances obtained with nitrogen fertilizer forms and doses are given in Table 7. According to Table 7, urea and ammonium sulfate fertilizers reached the highest color tone performance with 6.3 points in the fertilizer type average in terms of grass color tone, while 15-5-20 and 20-10-10 fertilizers scoring 5.6 and 5.8 points respectively, and remained at the lowest color performance value. In terms of fertilizer dose averages, while the 8 g m^{-2} fertilizer dose reached the highest value with 6.6 points, the control fertilizer dose remained at 5 points, which is the lowest color tone performance value. In terms of fertilizer type and fertilizer dose interactions, while 8 g m^{-2} fertilizer doses of ammonium sulfate and urea fertilizer reached the highest color tone performance with a value of 7 points, control fertilizer doses of all fertilizer types were found to be the lowest color tone performance with 5 points.

According to the research results, the increase in nitrogen dose increased the leaf color tone performance in grass. The lowest color tone scores were obtained from control plots where nitrogen was not applied. The highest values were taken from 8 g m^{-2} nitrogen fertilizer applications. Accordingly, as nitrogen doses increased, a darkening of leaf color tones was detected.

Table 2. The results of the variance analysis of the amount of fresh grass

Sources of variation	Degree of Freedom	Sum of Squares	Square average	F Value
Block	2	5770.0	2885.0	0.4566
Type of fertilizer	3	626551.7	208850.6	33.05**
Dosage of fertilizer	4	3302826.7	825706.7	130.68**
TF x DF	12	355040.0	29586.6	4.68**
Error	38	240096.7	6318	
General	59	4530285.0		

**F values are important in probability limits of P<0.01.

Table 3. The amounts of fresh grass obtained in nitrogen fertilizer forms and doses (g)

	Dosage of fertilizer					
Type of fertilizer	0	2	4	6	8	The average type of fertilizer
20-10-10	366.6 ^{ij}	550.0 ^{gh}	750.0 ^{e}	900.0 ^{cd}	1000.0 ^{bc}	713.3 ^{b}
15-5-20	300.0 ^{j}	450.0 ^{hı}	500.0 ^{gh}	600.0 ^{fg}	766.7 ^{e}	523.3 ^{c}
Ammonium sulfate	303.3 ^{j}	800.0 ^{de}	900.0 ^{cd}	950.0 ^{bc}	1050.0 ^{b}	800.7 ^{a}
Urea	273.3 ^{j}	550.0 ^{gh}	700.0 ^{ef}	900.0 ^{cd}	1200.0 ^{a}	724.7 ^{b}
Average fertilizer dose	310.8 ^{e}	587.5 ^{d}	712.5 ^{c}	837.5 ^{b}	1004.2 ^{a}	
Fertilizer type LSD Value	58.75					
Fertilizer dose LSD Value	66.69					
int. LSD Value	131.39					

Fertilizer type LCD Value = t value x std err. Dif. = 2.02439 x 29.0249 = 58.75; Fertilizer dose LCD Value = t value x std err. Dif. = 2.02439 x 32.4508 = 66.69; Fertilizer type x Fertilizer dose LSD value = t value x std err. Dif. = 2.02439 x 64.9016 = 131.39.

Table 4. Results of variance analysis for plant height

Sources of variation	Degree of Freedom	Sum of Squares	Square average	F Value
Block	2	5.03	2.5	1.32
Type of fertilizer	3	232.9	77.6	40.81**
Dosage of fertilizer	4	1101.8	275.5	144.77**
TF x DF	12	108.9	9.1	4.77*
Error	38	72.3	1.9026	
General	59	1520.9		

*The F value is important at the probability limits of P<0.05; **The F values are important at the probability limits of P<0.01.

Table 5. Plant height obtained in nitrogen fertilizer forms and doses (cm)

	Dosage of fertilizer					
Type of fertilizer	0	2	4	6	8	The average type of fertilizer
20-10-10	9,3 ^{k}	12,0 ^{ij}	17,0 ^{f}	20,0 ^{cd}	22,0 ^{bc}	16,1 ^{b}
15-5-20	9,3 ^{k}	11,6 ^{ij}	13,0 ^{hi}	14,3 ^{gh}	17,6 ^{ef}	13,2 ^{c}
Ammonium sulfate	11,0 ^{ijk}	17,6 ^{ef}	19,6 ^{de}	21,3 ^{cd}	24,0 ^{ab}	18,7 ^{a}
Urea	10,0 ^{jk}	12,0 ^{ij}	15,6 ^{fg}	19,3 ^{de}	25,6 ^{a}	16,5 ^{b}
Average fertilizer dose	9,9 ^{e}	13,3 ^{d}	16,3 ^{c}	18,8 ^{b}	22,3 ^{a}	
Fertilizer type LSD Value	1,02					
Fertilizer dose LSD Value	1,14					
int. LSD Value	2,28					

Fertilizer type LCD Value = t value x std err. Dif.. = 2,02439 x 0.50367 = 1.02; Fertilizer dose LCD Value = t value x std err. Dif. = 2.02439 x 0.56312 = 1.14; Fertilizer type x Fertilizer dose LSD value = t value x std err. Dif. = 2.02439 x 1.12624 = 2.28.

Table 6. The results of the variance analysis of the color tone

Sources of variation	Degree of Freedom	Sum of Squares	Square average	F Value
Block	2	0.03	0.015	0.14
Type of fertilizer	3	4.98	1.66	13.62**
Dosage of fertilizer	4	23.23	5.81	47.64**
TF x DF	12	2.10	0.18	1.44^{NS}
Error	38	4.633333	0.122	
General	59	34.983333		

**F values are important at P<0.01 probability limits, NS=F value is insignificant.

Table 7. Color shades obtained in nitrogen fertilizer forms and doses (points)

	Dosage of fertilizer					
Type of fertilizer	0	2	4	6	8	The average type of fertilizer
20-10-10	5.0	5.3	6.0	6.3	6.3	5.8 [b]
15-5-20	5.0	5.0	6.0	6.3	6.0	5.6 [b]
Ammonium sulfate	5.0	6.0	7.0	6.6	7.0	6.3 [a]
Urea	5.0	6.0	6.6	6.6	7.0	6.3 [a]
Average fertilizer dose	5.0 [d]	5.6 [b]	6.4 [a]	6.5 [a]	6.6 [a]	
Fertilizer type LSD Value	0.258					
Fertilizer dose LSD Value	0.29					
int. LSD Value	0.58					

Fertilizer type LCD Value = t value x std err. Dif... = 2.02439 x 0.1275 = 0.258; Fertilizer dose LCD Value = t value x std err. Dif. = 2.02439 x 0.14255 = 0.29; Fertilizer type x Fertilizer dose LSD value = t value x std err. Dif = 2.02439 x 0.28511 = 0.58.

3.5. Grass Quality

The effects of nitrogenous fertilizer forms on the obtained grass quality values were not found to be significant, while the effects of fertilizer dose were found to be significant at the 1% level. The interaction between the two was found to be significant at the 5% level (Table 8).

The grass quality performances obtained with nitrogen fertilizer forms and doses are given in Table 9.

According to Table 9, in terms of grass quality, urea and ammonium sulphate fertilizers reached the highest grass quality performance with 7.8 points in the fertilizer type average, as it has been detected that 20-10-10 and 15-5-20 fertilizers had a low grass quality performance value with 7.4 points. In terms of fertilizer dose averages, the 8 g m^{-2} fertilizer dose reached the highest grass quality performance with 9 points, while the lowest grass quality performance was determined with 5.2 points at the control fertilizer dose. In terms of fertilizer type and fertilizer dose interactions, 8 g m^{-2} doses of all fertilizer types reached the highest value with 9 points, and the control dose had the lowest values with 5, 5.3, 5.3 and 5 points, respectively.

3.6. Coverage Rate

The effects of fertilizer dose on the obtained leaf width values were found to be significant at the 1% level, while the effects of the interactions of these factors were found to be insignificant (Table 10).

The coverage rates obtained with nitrogen fertilizer forms and doses are given in Table 11.

According to Table 11, all fertilizer types reached the highest values in terms of coverage rates with averages of 88.7, 88.1, 87.5 and 86.6 (%), respectively. In terms of fertilizer dose averages, 8 g m^{-2} fertilizer dose reached the highest value with 94.6%, and the control fertilizer dose reached the lowest value with 70.4%. In terms of fertilizer dose and fertilizer type interactions, ammonium sulfate and urea fertilizers had the highest value with 95%, while the control doses of all fertilizer types had the lowest values with 70, 70.3, 70.7 and 70.7 (%), respectively.

Table 8. The results of the variance analysis of the grass quality

Sources of variation	Degree of Freedom	Sum of Squares	Square average	F Value
Block	2	0.3	0.15	0.52
Type of fertilizer	3	2.4	0.8	2.76[NS]
Dosage of fertilizer	4	122.9	30.73	105.82**
TF x DF	12	7.77	0.65	2.23*
Error	38	11.0	0.29	
General	59	144.4		

*The value of F is important at the probability limits of P<0.05, **F values are important at P<0.01 probability limits, NS=F value is insignificant.

Table 9. Grass qualities obtained in nitrogen fertilizer forms and doses (points)

	Dosage of fertilizer					
Type of fertilizer	0	2	4	6	8	The average type of fertilizer
20-10-10	5.0 [f]	6.7 [e]	7.7 [cd]	8.7 [ab]	9.0 [a]	7.4
15-5-20	5.3 [f]	5.7 [f]	8.0 [bcd]	9.0 [a]	9.0 [a]	7.4
Ammonium sulfate	5.3 [f]	7.3 [de]	8.3 [abc]	9.0 [a]	9.0 [a]	7.8
Urea	5.0 [f]	8.0 [bcd]	8.0 [bcd]	9.0 [a]	9.0 [a]	7.8
Average fertilizer dose	5.2 [d]	6.9 [c]	8.0 [b]	8.9 [a]	9.0 [a]	
Fertilizer type LSD Value	0.40					
Fertilizer dose LSD Value	0.45					
int. LSD Value	0.89					

Fertilizer type LCD Value = t value x std err. Dif. = 2.02439 x 0.19676 = 0.40; Fertilizer dose LCD Value = t value x std err. Dif. = 2.02439

x 0.21998 = 0.45; Fertilizer type x Fertilizer dose LSD value = t value x std err. Dif.= 2.02439 x 0.43996 = 0.89.

Table 10. The results of the variance analysis of the coverage rate

Sources of variation	Degree of Freedom	Sum of Squares	Square average	F Value
Block	2	42.2	21.1	1.9
Type of fertilizer	3	34.8	11.6	1.04^{NS}
Dosage of fertilizer	4	4923.9	1231	110.6**
TF x DF	12	77.7	6.5	0.6^{NS}
Error	38	423.1	11.1	
General	59	5501.7		

**F values are important at P<0.01 probability limits, NS=F value is insignificant.

Table 11. Coverage rate obtained in nitrogen fertilizer forms and doses (%)

	Dosage of fertilizer					
Type of fertilizer	0	2	4	6	8	The average type of fertilizer
20-10-10	70.0	89.0	92.3	94.3	94.7	88.1
15-5-20	70.3	83.3	92.0	94.0	93.7	86.6
Ammonium sulfate	70.7	87.7	94.7	95.7	95.0	88.7
Urea	70.7	88.7	89.7	93.3	95.0	87.5
Average fertilizer dose	70.4 ^{c}	87.2 ^{b}	92.2 ^{a}	94.3 ^{a}	94.6 ^{a}	
Fertilizer type LSD Value	2.47					
Fertilizer dose LSD Value	2.76					
int. LSD Value	5.52					

Fertilizer type LCD Value = t value x std err. Dif. = 2.02439 x 1.21843 = 2.47; Fertilizer dose LCD Value = t value x std err. Dif. = 2.02439 x 1.36224 = 2.76; Fertilizer type x Fertilizer dose LSD value = t value x std err. Dif.= = 2.02439 x 2.72448 = 5.52.

4. Conclusion

When evaluated as the amount of fresh grass, it was observed that ammonium sulfate fertilizer showed its effect with an increasing trend between the doses of 0-2 g m^{-2}. This dose keeps plant growth at the desired level and is recommended for economic and ecological benefits.

Looking at the results of plant height values, it is seen that ammonium sulfate fertilizer is more effective, but doses of 2 and 6 g m^{-2} are recommended for the desired development in grass areas.

When the fertilizer dose averages were examined in terms of leaf color, 8 g m^{-2} doses of ammonium sulfate and urea fertilizers (with 6.6 points) exhibited the highest performance. The same fertilizer varieties have 4 g m^{-2} doses (6.4 points) of performance and are recommended because they have values close to the highest performance in terms of lawn color.

When examined in terms of grass quality, it was found that the higher the nitrogen dose, the higher the grass quality performance. The grass quality reached the highest values in all parcels where monthly doses of 6 g m^{-2} and 8 g m^{-2} were used. It has been found that the dose of 4 g m^{-2} of urea fertilizer has an above average value, and it is recommended because it exhibits the desired performance for this type of fertilizer.

When evaluated in terms of coverage rate, it has been understood that ammonium sulfate fertilizer is more prominent than other fertilizers. When the values of 4 g m^{-2} of urea and ammonium sulfate fertilizers were examined, it was found that the coverage rates were more than 90%, and ammonium sulfate fertilizer is recommended considering the economic and ecological benefits.

When all the results are evaluated, 6 and 8 g m^{-2} doses of ammonium sulphate fertilizer are recommended for structuring of grass plants in alkaline soils in a short time in Igdir ecological conditions and continuing the development by achieving the desired color, texture, structure, and quality features and also balancing the pH values of the soil by considering both economic and ecological benefits.

Author Contributions

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

	C.M.	İ.H.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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