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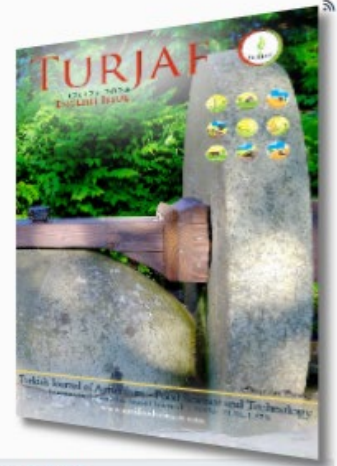
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■ Araştırma Makalesi ■ Deneme ■ Ölgü Sunumu ■ Diğer ■ Düzeltme



## Variation of Secondary Metabolites, Chlorophyll Contents, and Antioxidant Activity in Six Medicinally Important Plants in Bangladesh

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### ABSTRACT

Plant phenolics and flavonoids function as antioxidants that act as scavengers of free radicals in the human body. This study aimed to determine the total phenolics and flavonoids contents, ferric-reducing antioxidant power (FRAP), free radical scavenging ability, chlorophyll contents, and total amounts of carotenoids of six medicinal plants viz. *Anisomeles indica* (L.) Kuntze, *Eclipta prostrata* (L.) L., *Glinus oppositifolius* (L.) Aug.DC., *Litsea glutinosa* (Lour.) C.B.Rob., *Origanum vulgare* L., and *Oxalis debilis* Kunth. The results reveal that *L. glutinosa* has the maximum amount of total phenolic content (TPC) (1.906 mg GAE g<sup>-1</sup> FW) and total flavonoid content (TFC) (13.933 mg QUE 100g<sup>-1</sup> FW), while the lowest TPC (0.2803 mg GAE g<sup>-1</sup> FW) was observed in *O. vulgare* and the least TFC (1.6 mg QUE 100g<sup>-1</sup> FW), was observed in *A. indica*. The leaves of *L. glutinosa* showed excellent antioxidant properties (IC<sub>50</sub> = 6.24 mg mL<sup>-1</sup>), and *G. oppositifolius* showed the least antioxidant potential (IC<sub>50</sub> = 18.423 mg mL<sup>-1</sup>). Pigment content such as chlorophyll-a was highest in *E. prostrata* (1.5963 mg g<sup>-1</sup>), while *L. glutinosa* has the highest chlorophyll-b (2.176 mg g<sup>-1</sup> FW), chlorophyll-(a+b) (3.6157 mg g<sup>-1</sup> FW), and carotenoids (1.61 µg 100g<sup>-1</sup> FW) content. A strong linear correlation (DPPH, R<sup>2</sup> = 0.8688, FRAP, R<sup>2</sup> = 0.8595) was found between TPC and antioxidative capability. *L. glutinosa* contains significant amounts of phenols and flavonoids, which have antioxidant qualities, suggesting the possibility of using this species in phytotherapy and pharmacy.

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## Introduction

People have been utilizing plants to treat a variety of illnesses/ailments from ancient times; because they contain a number of active chemicals with significant pharmacological effects. Natural antioxidants, like flavonoids and polyphenols, are commonly used in medicine to treat a variety of serious diseases due to their well-known ability to reduce oxidative stress and neutralize free radicals (Yen and Chuang, 2000). High concentrations of phenolics and flavonoids with potent antioxidant qualities found in leafy greens have been linked to a lower incidence of diabetes, cancer, heart disease, and neurological illnesses, according to epidemiological research (Adebooye et al., 2008). In addition, phenolic and flavonoid compounds naturally produced in plants have numerous functions including anti-inflammatory, antimicrobial, antiviral, neuroprotective, antitumor, antipyretic, antimalarial, analgesic, antioxidant activity with free-radical scavenging capacity, anti-proliferative

agents, antiangiogenic, etc. (Hakim et al. 2021; Ullah et al., 2020). Consequently, as compared to artificial phenolic antioxidants like butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate, consumers prefer to eat fruits and vegetables because of their high antioxidant content and low toxicity (Jideani et al., 2021).

Bangladesh is home to more than 1,200 medicinal plants (Uddin and Lee, 2020) that have been used to treat human as well as veterinary ailments for a long time (Sarwar, 2020). Among them six important medicinal plants, naturally available and which are traditionally used as therapeutic medicine by the urban and tribal peoples of Bangladesh viz. *Anisomeles indica* (L.) Kuntze, *Eclipta prostrata* (L.) L., *Glinus oppositifolius* (L.) Aug.DC., *Litsea glutinosa* (Lour.) C.B.Rob., *Origanum vulgare* L., and *Oxalis debilis* Kunth, were used for the study. People have been using these medicinal herbs extensively for many years to cure common illnesses like cough, fever,

asthma, diarrhea, allergies, inflammation, skin problems, jaundice, hair loss, abdominal pain, gastrointestinal problems, dyspepsia, healing of cuts and wounds, colic, rheumatic arthritis, etc. throughout the world (Akber et al., 2011; Kadir et al., 2013; Sofowora et al., 2013). Therapeutically and industrially essential chemical compounds like alkaloids, flavonoids, phenolic compounds, and others can be significantly identified as a new source of crude drugs from their phytochemical constituents (Mangoale and Afolayan, 2020; Velu et al., 2018). The experiments were carried out with the aforesaid plant parts to investigate the status of phytochemicals (total phenolics, flavonoids, and carotenoids) along with their overall antioxidant enzyme activity and to quantify the green leaf pigments available in them.

## Materials and Methods

### Plant Samples

Three different plants harbored at the Bangladesh Agricultural University Botanical Garden, each representing a replicate from each species, were marked (Table 1). The study area is located at 90°26'29.6" E and 24°43'26.8" N, 29 meters above sea level (Sarwar, 2020). A humid subtropical monsoon climate (Köppen *Cwa*) prevailed in this area with an average annual rainfall of about 273.5 cm (<https://en.wikipedia.org/wiki/Mymensingh>). The temperature falls below 15°C in winter and reaches as high as 40°C in summer.

Their tender twigs were picked up with a pair of scissors and stored in a Ziploc bag before chemical analysis. Tender leaves from each replicate were cut into small pieces, and the chemical analysis was performed with the freshly harvested samples following the standard protocols.

### Chemical Reagents

Analytical-grade chemical reagents and solvents were used, including HCl, FeCl<sub>3</sub>.6H<sub>2</sub>O, Quercetin, Aluminium Chloride, Sodium Acetate Trihydrate, Gallic Acid (GA), Acetic Acid, TPTZ (2,4,6-tripyridyl-s-triazine), Folin-Ciocalteu Reagent, Potassium Acetate, and DPPH (2,2-diphenyl-1-picrylhydrazyl).

### Determination of Total Phenolic Contents (TPC)

TPC was determined by the Folin-Ciocalteu technique slightly modified from the protocols described by Adebooye et al., (2008). 2.5 g of leaf sample and 50 mL of methanol were combined in a 250 mL beaker. The samples were then homogenized using an OV-5 homogenizer (VELP, Italy) for two to three minutes. The mixture was centrifuged for 5 minutes at 5000 rpm to remove the

supernatant for phenol analysis after being left in the dark for 60 minutes. Gallic acid (GA) solutions were used as the standard. A 50 mL test tube was filled with precisely 0.5 mL of various Gallic acid solutions or plant extract concentrations. Then 2.5 mL of the Na<sub>2</sub>CO<sub>3</sub> (7.5%) solution and 2.5 mL of the Folin-Ciocalteu reagent were added. The mixture was kept at 25°C in dark condition for half an hour. At 760 nm, absorbance was then measured.

### Total Flavonoid Content (TFC) Determination

To determine TFC a modified version of Kumaran and Karunakaran's (2007) protocol was followed. Quercetin was used as standard. One milliliter of Quercetin solutions or plant extracts at different concentrations was added to 50 ml test tubes separately followed by the addition of 5.6 mL of distilled water, 200 mL of 1M Potassium Acetate, 200 µl of 10% AlCl<sub>3</sub>, and 3 mL of Methanol in each tube. After 30 minutes of dark keeping at 25°C, the mixture's absorbance at 420 nm was assessed using a spectrophotometer. By plotting the concentration against the respective absorbance values, a standard curve was established for calculating the total flavonoid content as QUE per 100 g FW (Figure 1b).

### Assessment of Ferric Reducing Antioxidant Power (FRAP Values)

The method outlined by Kumari and Padmaja (2012) was used to compute the FRAP value. The absorbance value was recorded at 593 nm. A standard curve was constructed after the FRAP values were calculated for various standard antioxidants. The outcomes were determined using the following equation and expressed as µg AAE per mg of the fresh plant extract.

$$AAE = [(Absorbance \text{ at } 593 \text{ nm} / 0.002) - 0.004].$$

FRAP value of sample (µM) = (Change in the absorption of sample from 0 to 4 minute/change in absorbance of standard from 0 to 4 minute) × FRAP value of the standard (100 µM) and expressed as µ mol Fe(II)/g (Benzie and Strain, 1999).

### DPPH Radical Scavenging Capacity Assay

A stable free radical DPPH was used to measure the extracts' capacity to scavenge free radicals. 50 mL of methanol and 2.5 g of samples were combined, and the mixture was then exposed to darkness for 30 minutes. The mixture was centrifuged at 5000 rpm for 5 minutes to separate the extract. To make the assay up to 5 mL of volume, 2 ml of plant extracts and 3 ml of 40 g ml<sup>-1</sup> DPPH in methanol were employed. After the mixture was well combined, it was left in the dark at 25°C for 30 minutes.

Table 1. List of the plants used in this study.

Common Name	Scientific Name	Family
Gobura	<i>Anisomeles indica</i> (L.) Kuntze	Lamiaceae
Kalokeshi	<i>Eclipta prostrata</i> (L.) L.	Asteraceae
Gima	<i>Glinus oppositifolius</i> (L.) Aug.DC	Molluginiaceae
Kharajura	<i>Litsea glutinosa</i> (Lour.) C.B.Rob.	Lauraceae
Oregano	<i>Origanum vulgare</i> L.	Lamiaceae
Amrul	<i>Oxalis debilis</i> Kunth	Oxalidaceae

Table 2. Chlorophyll a, chlorophyll b, and total chlorophyll content in leaves.

SL No.	Medicinal plant species	Chl. a	Chl. b	Total Chl.
1	<i>Anisomeles indica</i>	1.5663 <sup>a</sup>	1.9493 <sup>b</sup>	3.5157 <sup>a</sup>
2	<i>Eclipta prostrata</i>	1.5963 <sup>a</sup>	1.7923 <sup>b</sup>	3.3950 <sup>ab</sup>
3	<i>Glinus oppositifolius</i>	1.5153 <sup>a</sup>	1.5047 <sup>c</sup>	3.0200 <sup>b</sup>
4	<i>Litsea glutinosa</i>	1.4957 <sup>a</sup>	2.1760 <sup>a</sup>	3.6517 <sup>a</sup>
5	<i>Oreganum vulgare</i>	0.3290 <sup>b</sup>	0.1897 <sup>c</sup>	0.5187 <sup>d</sup>
6	<i>Oxalis debilis</i>	0.0750 <sup>c</sup>	1.2170 <sup>d</sup>	1.2920 <sup>c</sup>
LSD <sub>0.05</sub>		0.2478	0.2021	0.3905

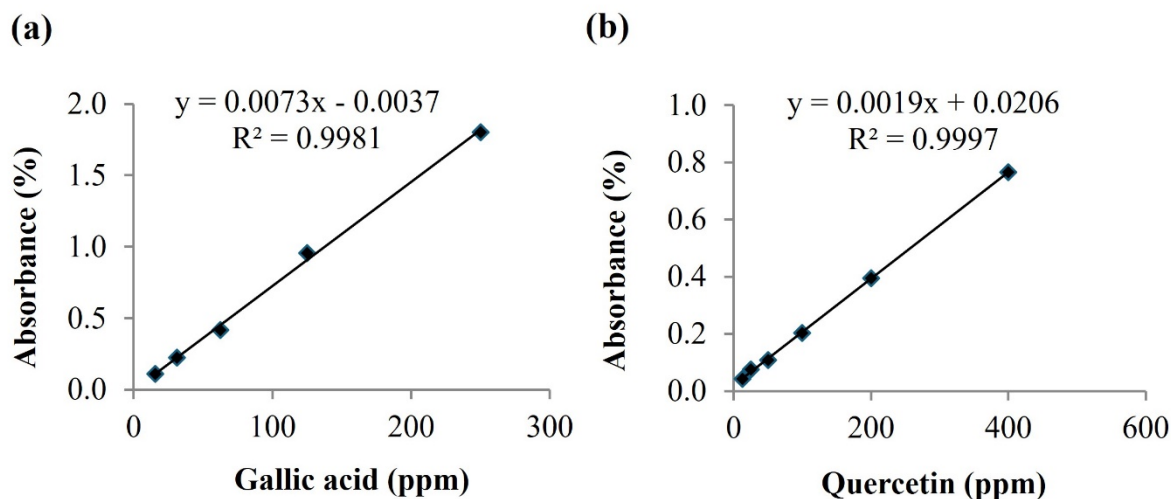


Figure 1. (a) Gallic acid and (b) Quercetin standard curve concentrations using ascorbic acid.

Methanol served as the negative control, ascorbic acid served as the positive control, and an extract devoid of DPPH served as the blank. The absorbance measurement was made at 517 nm, and the % of inhibitory activity was calculated as follows:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Here,

$A_0$  = absorbance of the blank sample,

$A_1$  = absorbance of the plant sample.

By plotting the percentage inhibition against the concentration of the corresponding extract, the  $IC_{50}$  value. The calibration curve and linear regression were used to determine the  $IC_{50}$  value, which is the amount of antioxidants required to reduce DPPH by 50%. The results are expressed as a percentage decrease in DPPH absorption. Antioxidant activity is inversely proportional to the  $IC_{50}$  values.

#### Pigments Determination

The pigments were extracted according to Abugri et al. (2013). 1.0 g grinded samples and 25 mL of methanol (99.9%) were agitated and kept in the dark for seven days. Water was eliminated using Whatman No. 1 filter paper and anhydrous Sodium Sulfate ( $Na_2SO_4$ ). Absorbance was measured with a spectrophotometer (DR-6000, Hach, USA) at 470 nm, 649 nm, 664 nm, and 760 nm. The amount of chlorophyll (a and b), total chlorophylls (a+b), and carotenoids were calculated following the formulas:

$$\text{Chlorophyll a (Chl a)} = (13.36 A_{664} - 5.19 A_{649}) \times 25 / \text{FW}$$

$$\text{Chlorophyll b (Chl b)} = (27.43 A_{649} - 8.12 A_{664}) \times 25 / \text{FW}$$

$$\text{Total Chlorophyll (Chl a+b)} = \text{Chl a} + \text{Chl b}$$

$$\text{Carotenoids (CX+C)} = (4.785 A_{470} + 3.657 A_{664} - 12.76 A_{649}) \times 25 / \text{FW}$$

Where,

$A_{649}$  = absorbance at 649 nm,

$A_{664}$  = absorbance at 664 nm,

$A_{470}$  = absorbance at 470 runs,

FW = Fresh weight of plant leaves (mg)

#### Statistical Analyses

One-way ANOVA analysis was employed to assess whether the difference between the mean values of each parameter studied was statistically significant or not using the free software Statistix 10. Additionally, Microsoft Excel was utilized to prepare graphs and process data.

#### Results

##### Total Phenolic Content

The TPC of the leaves of six different plants varied significantly, ranging from 0.4073 to 1.739 mg GAE 100g<sup>-1</sup> fresh weight (Figure 3a). *Litsea glutinosa* had the highest total soluble phenolic concentration (1.739 mg GAE 100g<sup>-1</sup> FW), followed by *A. indica* (1.222 mg GAE 100g<sup>-1</sup> FW). The lowest phenol was detected in *O. vulgare* (0.4073 mg GAE 100g<sup>-1</sup> FW) leaves.

Assuming the phenolic content of *L. glutinosa* (1.739 mg GAE 100g<sup>-1</sup> FW) to be 100 per cent, the relative phenolic contents in *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* were as 23.421%, 55.664%, 49.896%, 33.105%, and 70.270%, respectively.

### Total Flavonoid Content

The amount of flavonoids varied considerably from 1.6 to 13.933 mg QUE 100g<sup>-1</sup> fresh weight (Figure 3b). The highest flavonoid was found in *L. glutinosa* (13.933 mg QUE 100g<sup>-1</sup> FW), while the least was found in the leaves of *A. indica* (0.2873 mg QUE 100g<sup>-1</sup> FW). Assuming the phenolic content of *L. glutinosa* (13.933 mg QUE 100g<sup>-1</sup>

FW) as 100 per cent, the relative phenolic contents in *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* were as 76.08%, 54.55%, 83.86%, 63.16%, and 11.48%, respectively.

### Leaf pigments

The chlorophyll-a content of leaves varied considerably, ranging from 0.075 to 1.5963 mg g<sup>-1</sup> FW. The leaf of *E. prostrata* contained the highest chlorophyll-a (1.5963 mg g<sup>-1</sup> FW), followed by *A. indica* (1.5663 mg g<sup>-1</sup> FW). The lowest chlorophyll-a concentration was in the leaf of *O. debilis* (0.075 mg g<sup>-1</sup> FW).

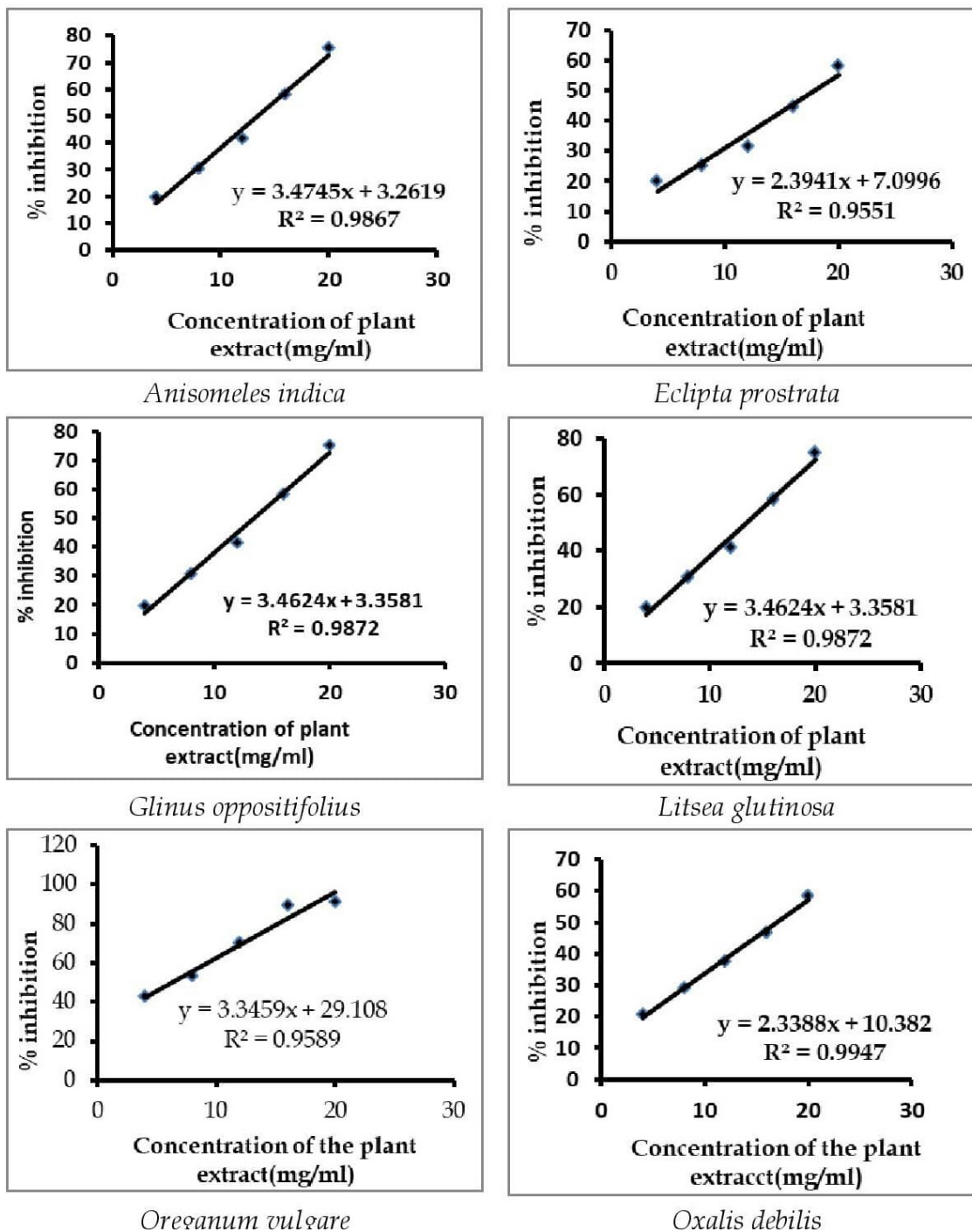


Figure 2. Construction of linear regression equation ( $y=mx+c$ ) by plotting different concentrations of the plant extracts on the X-axis and the % inhibition on the Y-axis

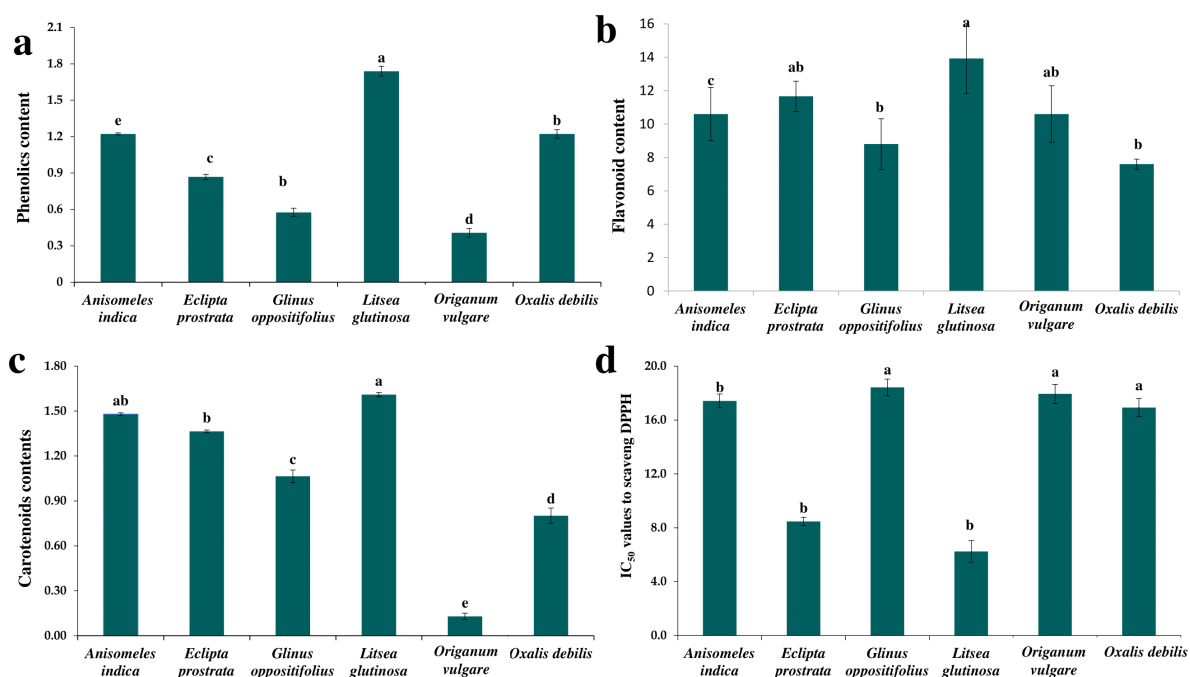


Figure 3. (a) Total soluble phenolic content, (b) Total soluble flavonoid content, (c) Amount of carotenoids, (d) IC<sub>50</sub> values for methanolic extracts. The data points are the mean of 3 replicates ± SEM. Bars with different alphabets are significantly different at P ≤ 0.05.

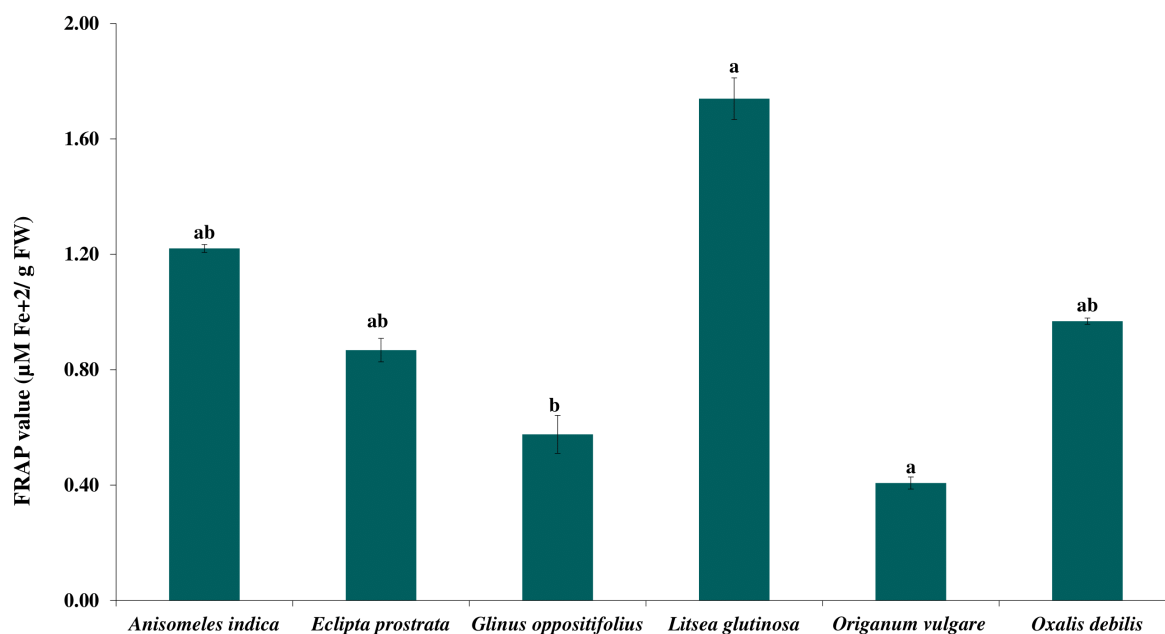


Figure 4. The FRAP values of leaf extracts from six different medicinal plants for DPPH radical scavenging. The data points are the mean of 3 replicates ± SEM. Bars with different alphabets are significantly different at P ≤ 0.05.

Assuming *E. prostrata*'s chlorophyll-a content (1.5963 mg g<sup>-1</sup> FW) as 100 percent, the relative chlorophyll-a contents in *A. indica*, *G. oppositifolius*, *O. debilis*, *L. glutinosa*, and *O. vulgare* were 98.12%, 94.925%, 4.7%, 44.164%, 93.697%, and 20.610%, respectively. The chlorophyll-b content of leaves ranged from 0.1897 to 2.1760 mg g<sup>-1</sup> FW. The leaf of *L. glutinosa* contained the highest chlorophyll b (2.1760 mg g<sup>-1</sup> FW), followed by *A. indica* (1.9493 mg g<sup>-1</sup> FW). The lowest level of chlorophyll-b was encountered in the leaf of *O. vulgare* (0.1897 mg g<sup>-1</sup> FW). Assuming the chlorophyll-b content of *L. glutinosa* leaf as 100 percent, the chlorophyll-b

contents of *O. vulgare*, *O. debilis* var. *corymbosa*, *L. glutinosa*, *G. oppositifolius*, and *A. indica* were 8.718%, 55.93%, 82.37%, 69.14%, and 89.58%, respectively. The chlorophyll (a+b) values ranged from 0.5187 to 3.65 mg g<sup>-1</sup> FW. The leaf of *L. glutinosa* had the highest chlorophyll (a+b) content (3.65 mg g<sup>-1</sup> FW), followed by *A. indica* (3.51 mg g<sup>-1</sup> FW). *Origanum vulgare* leaf chlorophyll (a+b) content is the lowest amount (0.5187 mg g<sup>-1</sup> FW). Others such as *O. vulgare* (0.5187 mg g<sup>-1</sup> FW), *O. debilis* (1.2920 mg g<sup>-1</sup> FW), and *E. prostrata* contained chlorophyll (a+b) (3.395 mg g<sup>-1</sup> FW).

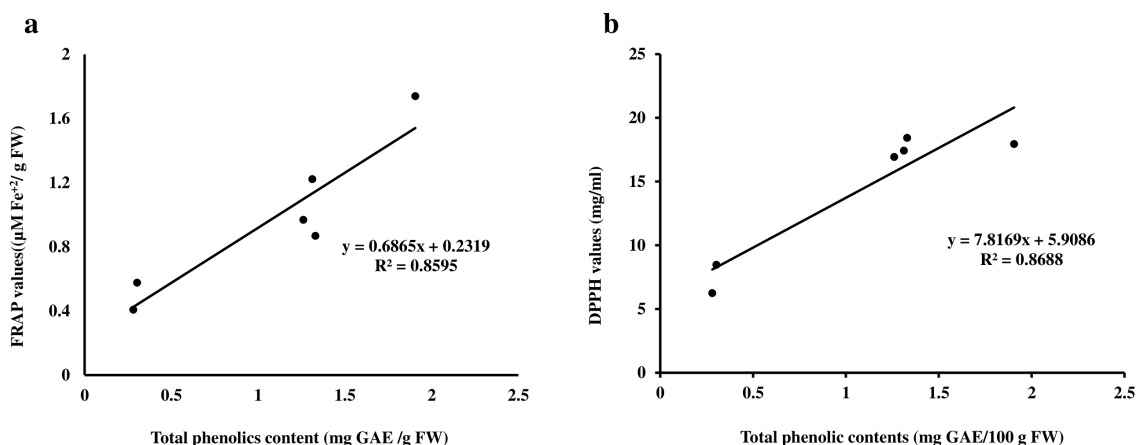


Figure 5. (a) Correlation between the FRAP values and the total phenolics contents. (b) Relationship between DPPH scavenging assay and total phenolic compounds in six medicinal plants.

The chlorophyll (a+b) content of *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* was determined to be 14.204%, 35.380%, 92.970%, 82.701%, and 96.275%, respectively, when compared to the chlorophyll (a+b) content of *L. glutinosa* leaf, assumed at 100%.

#### Carotenoid Content

Total carotenoid content ranged from 0.1290 to 1.610  $\mu\text{g g}^{-1}$  FW (Figure 3c). *Litsea glutinosa* leaf showed the highest carotenoid content (1.610  $\mu\text{g g}^{-1}$  FW), followed by *A. indica* (1.4787  $\mu\text{g g}^{-1}$  FW). The lowest value was detected in *O. debilis*, while *G. oppositifolius* (1.0647  $\mu\text{g g}^{-1}$  FW) and *E. prostrata* (1.3643  $\mu\text{g g}^{-1}$  FW). Assuming the carotenoids content of *L. glutinosa*'s leaf as 100%, and the relative carotenoids in *O. vulgare*, *O. debilis*, *G. oppositifolius*, *E. prostrata*, and *A. indica* were 8.012%, 50.329%, 66.130%, 84.739%, and 91.844%, respectively.

#### DPPH Radical Scavenging Assay

The DPPH scavenging abilities of the chosen medicinal plants were employed to investigate their total antioxidant activities. The linear regression equation ( $y=mx+c$ ) was constructed using % inhibition. Concentrations and the  $\text{IC}_{50}$  values were calculated using the linear regression equation (Figure 2). The  $\text{IC}_{50}$  values of leaf extract varied significantly and ranged from 6.24 to 18.423  $\text{mg mL}^{-1}$  leaf methanolic extract (Figure 3d). *Oxalis debilis*, *E. prostrata*, and *L. glutinosa* had  $\text{IC}_{50}$  values for leaf extracts to scavenge DPPH that are statistically identical (Figure 3d). Assuming  $\text{IC}_{50}$  values of leaf methanolic extract of *L. glutinosa* leaves (6.24  $\text{mg mL}^{-1}$ ) to scavenge DPPH as 100%, the relative  $\text{IC}_{50}$ , *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* were as 287.5%, 271.66%, 135.68%, 295.24%, and 279.17%, respectively.

#### Ferric Reducing Antioxidant Power (FRAP)

The testing method and the chemical makeup of the extract both have a significant impact on the plant extract's antioxidant capability. It cannot be fully assessed by a single approach because it can be influenced by a multitude of things. To assess the diverse antioxidant action pathways, numerous measures of antioxidant characteristics are necessary. So, the antioxidant potential of six medicinal plants was also assessed using the FRAP

test. Ferric (III) ions are changed into ferrous (II) ions in this test to measure antioxidant capacity. A simple technique, the FRAP assay could be used with both alcoholic and aqueous plant extracts. The antioxidant capabilities of these plants ranged from 0.40733 to 1.7392  $\text{M Fe}^{2+}/\text{g}$ , as illustrated in Figure 4. With 1.73930  $\mu\text{M Fe}^{2+}/\text{g}$ , *L. glutinosa* had the maximum antioxidant capacity, whereas *O. vulgare* had the lowest value (0.40733  $\mu\text{M Fe}^{2+}/\text{g}$ ). Other species' leaves displayed FRAP contents of 0.5757  $\mu\text{M Fe}^{2+}/\text{g}$  for *G. oppositifolius* and 0.9680  $\mu\text{M Fe}^{2+}/\text{g}$  for *O. debilis*. The relative FRAP content of *O. vulgare*, *O. debilis*, *E. prostrata*, *G. oppositifolius*, and *A. indica* were 23.421%, 56.354%, 49.896%, 33.105%, and 70.270%, respectively, when the value of the FRAP content in *L. glutinosa* leaf was taken into consideration.

#### Relationship between Antioxidant Capacity and TPC

Despite notable variations in the total antioxidant capacities and phenolic contents of the chosen plants, Figure 5a shows that there are linear-positive relationships ( $R^2 = 0.8595$ ) between the FRAP values and the TPC. The results of the two methods used to measure total phenolic content and antioxidant capacity showed strong correlations, suggesting that phenol molecules play a significant role in these plants' antioxidant activities and may therefore be important to their beneficial properties (Feng et al., 2016). A comparable robust connection ( $R^2 = 0.8688$ , Figure 5b) was seen between the medicinal plants' chosen phenolic content and their capacity to scavenge DPPH. The findings demonstrated that the DPPH scavenging capacity of medicinal plants is positively correlated with their phenolic content, indicating the plants' efficacy in harnessing the phytochemicals they contain.

#### Discussion

Plant extracts contain phenolic compounds, which have been demonstrated to significantly increase a system's antioxidant capacity and to be important in preventing several oxidative stress-related diseases, including cancer (Shahidi and Ambigaipalan, 2015). Recent *in vitro* studies have shown that phenolics are more efficient than carotenoids, vitamins C and E, and other nutrients (Rodríguez-Roque et al., 2013). By scavenging lipid free



radicals or preventing hydrogen peroxides from turning into free radicals, they demonstrate antioxidant function. In this study, the phytochemical content of each plant is screened, and the variations in phenolics, flavonoids, chlorophyll contents, and antioxidant activity are also evaluated. Among six medicinal plants studied, total phenolics content was found in the order of *O. vulgare* < *G. oppositifolius* < *E. prostrata* < *O. debilis* < *A. indica* < *L. glutinosa* (Figure 3a). The plant with the highest phenolic content was *L. glutinosa*, a member of the Lamiaceae family, which is applied to treat diabetes, edema, colds, arthritis, asthma, and traumatic injuries (Wang et al., 2016). Phenolic compounds are characterized by multiple phenol groups; some of them chelate metal ions and donate hydrogen atoms or electrons to neutralize free radicals in aqueous solutions (Petti and Scully, 2009). Furthermore, phenolic compounds have a range of biological properties that may be related to their antioxidant action, including antibacterial, anticancer, and antimutagenic properties (Shui and Leong, 2002).

Flavonoids, which comprise flavones, flavonols, and condensed tannins, are typical secondary plant metabolites. Epidemiological studies suggest that eating foods rich in flavonoids may protect individuals against illnesses linked to oxidative stress (Li et al., 2023). Numerous plant sources of flavonoids have demonstrated their anti-oxidant stress and anti-free radical capabilities *in vitro* (Shen et al., 2022). The amount of flavonoid in the leaf's methanolic extracts was found in the order of *A. indica* < *O. debilis* < *G. oppositifolius* < *O. vulgare* < *E. prostrata* < *L. glutinosa* (Figure 3b). The plant *E. prostrata* had the second-highest amount of phenolics. Studies have shown that *E. prostrata* (syn. *Eclipta alba*) is an excellent cancer fighter, antioxidant, anti-mycotoxin, anti-hyperglycemic, and immune modulator (Ayyakkannu et al., 2020).

According to Yen and Duh (1994), antioxidants are chemicals that, in the presence of ambient oxygen or reactive oxygen species, can either block or slow down the oxidation processes. They serve as a protective barrier against diseases caused by free radicals (Kumari and Padmaja, 2012). Endogenous antioxidants comprise non-enzymatic substances such as uric acid, bilirubin, albumin, and metallothionein as well as enzymes like glutathione peroxidase, catalase, and superoxide dismutase (Mironczuk-Chodakowska et al., 2018). Antioxidants from outside the body, such as dietary supplements or medications containing the active component of an antioxidant chemical, are necessary when the body's defenses against reactive oxygen species (ROS) are insufficient to fully protect it (Pisoschi and Negulescu, 2011). Recent studies reported that synthetic antioxidants harm human health (Lourenço et al., 2019). Thus, scientists have been searching more actively in recent years for safe, effective natural chemicals that combat free radicals. In combination with the body's antioxidant defenses, consuming antioxidant-rich foods and plants seems like a smart idea. Antioxidants come primarily from the food we eat and other parts of plants (Lobo et al., 2010). Natural antioxidants can be found in abundance in traditional medicinal herbs. Many medicinal plants comprise antioxidants that protect cells from the cellular damage induced by ROS, superoxide, and peroxy and hydroxyl ions, among other free radicals (Ramesh and Ilyas, 2017).

The theory underlying oxidative stress holds that imbalances between the generation ROS and antioxidant defenses create oxidative stress which is responsible for a variety of illnesses, including rheumatism, diabetes, carcinogenesis, aging, arthritis, asthma, and various neuroscience disorders (Xu et al., 2017).

In the DPPH test, antioxidants convert the DPPH radical to a yellow compound, diphenyl-picryl hydrazine, with the extent of the reaction depending on the antioxidants' hydrogen-donating capacity (Frezza et al., 2019). According to Chohra et al. (2020), The plant extract's antioxidant activity and its IC<sub>50</sub> values are inversely correlated. A reliable and consistent technique for evaluating antioxidants' capacity to scavenge free radicals is the DPPH assay. (Zaman et al., 2020). A higher IC<sub>50</sub> value implies a poorer scavenging activity of the scavengers, whereas a lower IC<sub>50</sub> value shows that the extract is more successful as a DPPH scavenger since more scavengers were needed to achieve a 50% scavenging reaction (Olugbami et al., 2014). Of the six plants examined, *G. oppositifolius* displayed the lowest level of antioxidative ability due to its greatest IC<sub>50</sub> value, while *L. glutinosa* had the highest level of antioxidative activity and the lowest IC<sub>50</sub> value (6.24 mg mL<sup>-1</sup>). The body's regular biochemical activities produce free radicals that are linked to several conditions, including diabetes, edema, traumatic damage, arthritis, asthma, and gastrointestinal problems such as dyspepsia, diarrhea, and dyspepsia (Wang et al., 2016).

Carotenoids defend against the sun and may prevent sunburns, photosensitivity, and even some types of skin cancer by altering how fibroblasts move through their cell cycle and aiding in the process of epithelization (Adumanya, 2016). According to this investigation, the leaf extract of *L. glutinosa* exhibited a high concentration of carotenoids, 1.61 mg/100 g FW. The findings of the analysis show that *L. glutinosa* leaf is a source of carotenoids and may hold promise for the creation of pharmaceuticals with anti-oxidant qualities. This study implies that *A. indica*, which has the second-highest carotenoid content, has potential applications in the therapy of rheumatism, AIDS, *Helicobacter pylori*, and cancer.

Photosynthetic pigments, such as porphyrin, are made of substances with very different chemical structures. The green pigment known as chlorophyll is made up of a magnesium ion-containing tetrapyrrole ring. The phytol chain, a long hydrophobic chain, is present in it (Kumari and Padmaja, 2018). Carotenoids and chlorophyll-a and -b make up the entirety of a leaf's pigment and are necessary for photosynthesis. Different species have different foliar pigment concentrations. The productivity of green plants is dependent on photosynthesis efficiency (Venkatesh et al., 2022). Chlorophyll and carotenoid variation in leaves, as well as their relationship, can be influenced by both internal and external factors. Ivanov et al. (2020) reported that chlorophyll and carotenoid content varied as per microclimatic conditions. The response to light and shade conditions of terrestrial plants has been measured by the ratio of chlorophyll-a and chlorophyll-b. Pigments can be analyzed both qualitatively and quantitatively by the absorbance of light (Teng et al., 2020). The amount of chlorophyll-a is expected to be higher than chlorophyll-b.

In this study, Chlorophyll-a content is higher in *E. prostrata* and Chlorophyll-b content in *L. glutinosa*. Comparing the total chlorophyll content of six plants, *L. glutinosa* showed the highest total chlorophyll content. Chlorophyll or its derivatives can be used as photodynamic agents to treat tumors or cancer (Brandis et al., 2006).

The FRAP assay indicates the antioxidant capacity of foods, drinks, and supplements that contain polyphenols. It interacts with a potential antioxidant (Spiegel et al., 2020). The study indicated that *L. glutinosa* contains many phytoconstituents and excellent antioxidant activity by effectively scavenging various free radicals. In this study, *L. glutinosa* shows a high quantity of FRAP values was found to be 1.739  $\mu\text{M Fe}^{+2}/\text{g FW}$  in the leaf extract. The second highest FRAP value contains *A. indica*.

The results of the correlation analysis show that there was a significant correlation between these techniques, suggesting that all three assays were appropriate and trustworthy for estimating the total antioxidant capabilities of plant extracts. Other correlations between DPPH and phenolic compounds are also substantially associated because of the strong correlation that exists between the FRAP values and phenolic compounds.

## Conclusions

The study examined the overall antioxidant capacity, phenolic content, and flavonoid content of extracts derived from six therapeutic plants. Science backs the traditional use of *L. glutinosa* as a readily accessible source of naturally occurring antioxidants with major health advantages. This is especially true given the plant's high level of antioxidant activity. This study does not clarify the specific functions of a plant species to a particular disease rather it generalizes the presence of live-saving essential secondary metabolites in those species. A significant amount of research is required to separate and identify the active components and their efficacy. Furthermore, future studies are recommended to explore the *in vivo* antioxidant capabilities of *L. glutinosa* and other medicinal plants under investigation.

## Declarations

### Authors Contributions

MMK, AKMGS, and MA designed the experiment. FF experimented and analyzed the data. All authors checked and validated data and results. FF and MJHJ prepared the draft manuscript. MMK, AKMGS, and MA reviewed and revised the draft. After studying the work, all authors gave their permission for submission.

### Data Availability Statement

The data supporting the findings of this work are publicly accessible at <https://www.doi.org/10.57760/sciencedb.06991> in the Science Data Bank.

### Declaration of Competing Interest

The researchers stated that none of their known conflicts of interest or interpersonal connections could have impacted the published results.

## Ethical Approval

We did not use any human or animal participants in our study. Upheld the highest standards of personal decorum, acting honorably in all of our interactions and undertakings in the workplace. In both our words and deeds, we were being honest. Our actions and decisions were motivated by the University's overall welfare rather than self-interest.

## Acknowledgments

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## Litter Decomposition of Indigenous Agroforestry Tree Species, Jimma Zone, Southwest Ethiopia

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### ABSTRACT

At the Jimma town's Boye nursery site in southwest Ethiopia, three selected indigenous agroforestry tree species were the subject of a study to determine how their foliage litter materials broke down over time. Using Pearson's correlation coefficient, the study used a single exponential model to calculate the rate at which decomposing litter materials decayed and the litter chemical quality indices that were investigated. *C. macrostachus* had a rate constant of 0.0400 day<sup>-1</sup>, which was substantially faster than that of *F. vata* and *E. abyssinica*. It took 17.3 and 29.7 days, respectively, for *F. vata* and *E. abyssinica* to lose 50% of the initial dry matter. While nitrogen and phosphorus were found to be facilitators, the parameters of the chemical quality of the litter were found to be impeding biochemical parameters. The species with the fastest rate of nitrogen return in a short amount of time was found to be *E. abyssinica*, followed by *C. macrostachus* and *F. vasta*. *C. macrostachus* decomposes quickly, which may limit its ability to improve soil fertility over an extended period of time. It is important to take into account *F. vasta* and primarily *E. abyssinica* for the long-term accumulation of soil organic carbon.

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## Introduction

The benefits of agroforestry include preserving soil erosion, enhancing the water-holding capacity of soil, and improving soil fertility through nitrogen fixation (Yadessa et al., 2001). The process of breakdown is crucial for the recirculation of our planet (Shi, 2013). The breakdown of tree species biomass, particularly foliage litter, can enhance soil fertility in tropical agroforestry systems.

Agroforestry land-use systems continue to benefit from selecting and employing desirable species based on their leaf litter decomposition characteristics. Litter materials with rapid decomposition characteristics are associated with slow to low quality (Palm et al., 2001). High-quality materials could be used for short-term soil fertility correction, whereas low-quality materials are used for long-term soil organic matter maintenance.

The most important factors influencing litter decomposition are litter quality and climatic factors like rainfall and temperature patterns (Mubarak et al., 2008). According to Mafongoya et al. (1998), the chemical parameters nitrogen (N), phosphorus (P), lignin, cellulose, hemicellulose, lignocellulosic index (LCI), and C/N are more important than nitrogen. Nevertheless, the

composition of these chemicals can greatly differ depending on the species, growth stage, plant parts, and environmental factors in which the species are cultivated. Nevertheless, the composition of these chemicals can greatly differ depending on the species, growth stage, plant parts, and environmental factors in which the species are grown (Abidemi, 2017). There are both fresh and senescent plant materials in agroforestry, but in natural forests and agricultural systems, the senescent material dominates. Purposeful pruning of foliage material is more common than natural litterfall in agroforestry land use systems (Moumita et al., 2016; Nair et al., 1999).

The multipurpose tree *E. abyssinica* is used in traditional medicine by the native population to treat a wide range of illnesses, including infections, bronchitis, asthma, inflammation, cough, and malaria (Cui et al., 2008). Raising stall-fed sheep and goats to supplement low-quality diets with protein during the dry season and fix nitrogen from the atmosphere through their root nodules was an inexpensive and effective way to resource poor Ethiopian farmers (Aerts et al., 2008).

The tree species *F.vasta* can be used to fuel wood and livestock feed. The present result revealed that *F.vasta* exhibits a favorable effect on soil properties studies, indicating that the tree is crucial for enhancing soil fertility in the Hawassa Zuria district (Zelege et al., 2015).

In order to conserve soil, *C. macrostachus* is used to provide shade and shelter. The useful shade that trees provide is what makes them popular for planting. The study by Manjur et al. (2014) revealed a significant alteration in maize yield under the canopy of *C. macrostachus*. There is a higher concentration of nutrients in foliage materials, which are thought to have superior potential to improve soil fertility. Despite this, there are few attempts to track the decomposition pattern of intentionally harvested agroforestry species' foliage biomasses. The objective of this study was to assess the rate of decay of foliage litter materials from three particular native Agroforestry tree species, namely, *C.macrostachus*, *F.vasta*, and *E.abysinica*, grown at the Jimma Boye nursery site in southwest Ethiopia. The study also sought to ascertain the effect of foliage litter's chemical makeup on decomposition rates.

## Materials and Methods

### Study Site

The experiment took place at the Jimma Town Boye plant nursery. The research site is located about 4.5 km to the west of the administrative town of Jimma, lying between latitudes 7°40' N and 36°50' E.

### Climate Conditions and Soil Types of the Study Area

The study area was located within the climatic zone, locally known as Weynedega, which is very suitable for agroforestry practices as well as human settlement (Abdela et al., 2017). The mean annual temperature ranges from 14 to 29 °C, with the mean annual rainfall ranging from 1200 to 2000 mm (Getachew et al., 2021). There is maximum precipitation during the three months from June to August, with minimum rainfall in December and January (Alemu et al., 2011).

According to Jafer et al. (2018), the study areas' most common soil types are nitosols (35%), vertisols (15%), cambisols, and miscellaneous (50%) with a pH of 4.5. Nitosol dominates the area for experimental investigations.

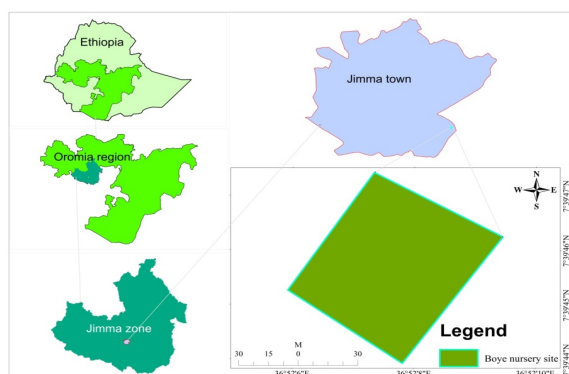


Figure 1. Location of the study area

### Species Selection

Due to their ability to adapt to the conditions of the research area, *Ficus vasta*, *Croton macrostachus*, and *Erythrina abyssinica* were chosen for this study. In addition to its adaptability, *Erythrina abyssinica* was also selected for its capacity to preserve nitrogen.

### Foliage Collection and Sample Preparation

The collection and preparation of foliage biomass was carried out following a routine sample process of wood perennial plant tissue, (Jones Jr., 2019). The species' most recent and completely formed live leaf biomass was collected. This leaf was exposed to direct sunlight before or at the start of the species reproductive stage. Three replicate leaf samples were obtained and allowed to air dry for three further days (Gindaba et al., 2004). The 50 g of dried plant biomass was put in nylon litter bags with a 2mm mesh size.

### Experimental Design and Treatment Arrangement

A factorial RCBD design was used in the installation of the experiment. The main factor in the experiment was the species type (*C. macrostachus*, *F. vasta*, and *E. abyssinica*), while the sub-factors included incubation periods of (15, 30, 45, 60, 75, and 90 days, all with three replications. Each of the 3 blocks was assigned all the treatments, resulting in a combined total of 54 litter bags. As decomposition is faster in sub-soil, litter bags are placed beneath the soil at a depth of 0 - 15cm (Nair et al., 1999).

### Litter Bag Collection

The litter bags were randomly picked up block by block, 15 days apart, for three consecutive months. After being recovered and transported to the laboratory, the bags were placed inside a paper envelope with a label. Samples were air dried and cleaned from attached soil particles carefully by hand and hair brush, then oven dried at 70°C until constant weight (Gindaba et al., 2004). The mass loss pattern of litter residues was analyzed by weighing oven-dried samples individually. At various sampling times, the proportion of ash-free dry matter that persisted was calculated using the following functions (Hossain et al., 2011):

$$\text{Mass remaining (\%)} = \frac{DM_t}{DM_0} \times 100 \quad (1)$$

The dry matter at the time of sampling is referred to as DM<sub>t</sub>, and the initial dry matter of the litter sample kept for breakdown is referred to as DM<sub>0</sub>. The decomposition rate constant (k) of litter residues was determined using a single exponential model (Olson, 1963) as shown below.

$$W_t = W_0 e^{-kt} \quad (2)$$

Where W<sub>t</sub> is weight remaining at time t, and W<sub>0</sub> initial weight, 'e' is the base of natural logarithm, k is constant, and t is time (days).

### Chemical Analyses

Standard analytical procedures were used for the chemical analysis of the samples. Therefore, cellulose content was determined using sequential heating fiber extraction methods (Soest & Wine, 1967). The extraction was done in the order of acid detergent fiber to estimate cellulose.

The plant tissue was analyzed using the dry ashing technique to determine the carbon content. To do so, one gram of milled foliage litter biomass was weighed into a crucible and calcinated at 450°C for 3 hours in Muffle Furnas. In the study, fifty percent of the ash-free dry matter was considered organic carbon (Anderson & Ingram, 1994). Additionally, the Kjeldahl distillation and (Olson, 1963) were used to determine the amount of nitrogen and phosphorus respectively.

**Statistical Analysis**

Statistical analyses were conducted using R. V. 3.6.3 (Wieduwilt et al., 2020) and Microsoft Excel computerized programs. We evaluated model fit using tree species in the sj plot and incubation period, tested for significance using the ANOVA function in the packaging car, and decomposition rate of selected agroforestry tree species using emmeans package (Wieduwilt et al., 2020). Any results declared statistically different were done at a 5% level of error tolerance (95% confidence interval). The correlation between decomposition rate and certain biochemical composition parameters was investigated using Pearson’s correlation coefficient

**Results and Discussions**

**Foliage Decomposition Rate of Croton macrostachus, Ficus vasta, and Erythrina abyssinica**

The two parameters that have been used to describe the results for the decomposition pattern are loss of dry matter (percentage of original mass) and litter decomposition rates. The results of the Analysis of Variance (ANOVA) showed that the species type and incubation period had an influence on both parameters ( $p < 0.001$ ).

**The Effect of Tree Varieties on Mass Loss During a 90-Day Period**

The results showed that during the first fifteen days of the study, *C. macrostachus* litter lost the most dry matter mass, while *Erythrina abyssinica* lost the least (Fig 2). The mass that was still in the litter bag at the time ranged from 41.87% in *C. macrostachus* to 63.81% in *E. abyssinica*. Because of their morphological characteristics, *F. vasta*'s foliage dry matter dropped from 59.13% to 21.53% more quickly during that period than that of *C. macrostachus* and *E. abyssinica* after fifteen days. Other researchers have also stated that the structural differences in the leaves of plants present a possible source of variability in the amount of mass lost to soil (Prescott & Vesterdal, 2021). By the 60th day, the remaining mass of *F. vast* foliage was 5.08g, representing 10.16% and being less than the other two species. Specifically, *E. abyssinica* had approximately

22.07% (11.035 g) of decaying leaf litter left at the conclusion of the experiment.

As a result, during the monitoring periods, the species showed a biphasic weight loss pattern, with a rapid initial phase and a slower subsequent phase. Sarkar et al. (2016), the early stage’s rapid mass loss may be due to leaching and microbial action on water-soluble components, whereas the later stage’s slower mass loss may be caused by an increase in the recalcitrant fraction.

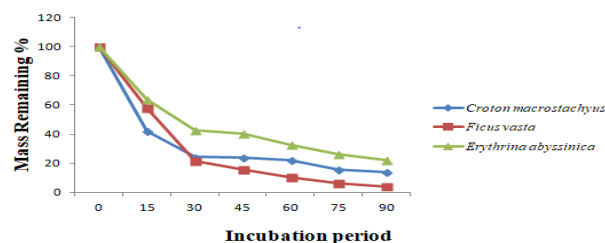


Figure 2. portrays the litter mass that stays during the decomposition of foliage litter materials in a span of 90 days.

**The Effect of Various Tree Species Over A 90-Day Period on the Decomposition Rate Constant (K)**

The decomposition rate ( $\text{day}^{-1}$ ) of the leaf litter materials under examination showed different patterns. The researchers verified that, over each 15-day sampling period, *C. macrostachus* had the fastest decomposition rates, ranging from 0.0357 to 0.0404  $\text{day}^{-1}$ . Additionally, it shows that it takes 17.3 days to lose 50% of its initial mass, which is the maximum net mean daily decomposition rate constant ( $k = 0.0400$ ) (Table 1). Both *F.vasta* and *E.abysinica* have the same decomposition rates of 0.0233  $\text{day}^{-1}$  over each 15-day sampling period and are slower than *C.macrostachus*. Both *F. vasta* and *E. abyssinica* showed varying decomposition rate constants of 0.0266 to 0.0200 and 0.0200 to 0.0100  $\text{day}^{-1}$  at 30 to 45 days. From the 60th day until the end of the incubation period, all three species (*C. macrostachus*, *F. vasta*, and *E. abyssinica*) exhibited a consistent decomposition rate constant of 0.0100 days. The study proved that both the C/N ratio and cellulose content are good quality indicators in decomposition studies. In this respect, *C.macrostachus* contains less cellulose. Liu et al. (2007) also suggested that in the initial stages of litter decomposition, N can be a good predictor of decomposition rate, whereas in later stages, chemical compounds such as lignin can play a more important role. This study also showed that the decomposition rate constants of *C.macrostachus* and *F.vasta* are significantly higher than those of *E.abysinica*. This was indicated by fast weight loss.

Table 1. Impact of different species decomposition rates at various decomposition stages

Species	Incubation period (days)					
	KD ( $\text{day}^{-1}$ )					
	15	30	45	60	75	90
<i>C.macrostachyus</i>	0.0400 <sup>a</sup>	0.0266 <sup>b</sup>	0.0200 <sup>c</sup>	0.0100 <sup>d</sup>	0.0100 <sup>d</sup>	0.0100 <sup>d</sup>
<i>F.vasta</i>	0.0233 <sup>bc</sup>	0.0266 <sup>b</sup>	0.0200 <sup>c</sup>	0.0133 <sup>d</sup>	0.0100 <sup>d</sup>	0.0100 <sup>d</sup>
<i>E.abysinica</i>	0.0233 <sup>bc</sup>	0.0200 <sup>c</sup>	0.0100 <sup>d</sup>	0.0100 <sup>d</sup>	0.0010 <sup>d</sup>	0.0100 <sup>d</sup>
LSD	0.00504277					

Table 2. Relationships between some litter chemistry parameters of the species foliage litter material and the decomposition rate constants (KDC.m, KDF.v, and KDE.a).

Parameter	Correlation Coefficient		
	KDC.m	KDF.v	KDE.a
	r	R	r
Cellulose	0.4ns	-0.53**	-0.79***
Nitrogen	0.78*	0.71*	0.98***
Phosphorus	0.218 <sup>ns</sup>	0.69***	0.425ns
C/N	-0.58**	0.78**	0.95***

\* Significant when  $P < 0.05$ , \*\* significant when  $P < 0.01$ , \*\*\* significant when  $p < 0.001$ , insignificant when NS is included. kDM.c=rate of *C.macrostachyus*, kDF.v=rate of *F.vasta*

### Mean Values in Columns with Matching Letters are Not Significantly Different.

The examined species' overall KD values range from 0.0100 to 0.0400 days<sup>-1</sup>, which is comparatively smaller than the domestic ranges for *S.sesban* and *F.macrophylla* that have been reported (Bekele et al., 2020). Since the experiment was conducted on a single site with consistent climatic conditions, the observed variation in K values may be explained by the differences in litter quality among the species (Table 1). This validates the results of the Mazaka and Shoko (2013) study on leaf litter from related legume tree species in Zambia. "The chemical compositions of *Leucaena leucocephala* and *Senna siamea* leaf litter materials influenced their decomposition rates more than climate factors," they concluded.

### The Influence of Litter's Chemical Composition on Rates of Decomposition

The relationship between the breakdown rate and a few litter chemical parameters of the *C. macrostachyus*, *F. vasta*, and *E. abyssinica* tree species was examined using the Pearson correlation coefficient. The species was found to be influenced by more than one litter chemical parameter (Table 2). As confirmed, *F.vasta* and *E.abysinica* were significantly influenced by cellulose (-ve), C/N ratio, and nitrogen (+ve), whereas *C.macrostachyus* influenced by N (+ve) and C/N (-ve). Accordingly, cellulose and the C/N ratio were reported as more reasonable parameters for retarding the decomposition rates of *F.vasta* and *E.abysinica* than *C.macrostachyus* whereas nitrogen's decomposition facilitation effect was more pronounced in the latter species. The negative effects of cellulose and the C/N ratio were more pronounced in *F.vasta* and *E.abysinica* than *C.macrostachyus* whereas the positive effect of nitrogen was more pronounced in the latter species foliage litter. This is attributable to the level of litter chemical variations observed among the tested species (Table 2).

The observed variation in the initial level of litter chemical quality indices among the tested species may be the reason for this (Bekele & Nigatu, 2019). The higher N content for both *E.byssinica* and *C.macrostachyus*, and the probable higher P content for *F. vasta*, are based on the author's significantly higher cellulose report for *E. abyssinica*. This lends credence to the theory that, under some circumstances, one of the most significant determinants of foliage litter decomposition is its chemical quality, and that the effects may differ depending on the species (Sarkar et al., 2016).

### Summary and Conclusion

Despite a few attempts to use native species, the litter decomposition of agroforestry tree species has not been thoroughly studied. The study examined the breakdown of three agroforestry tree species foliage litter town's at the Jimma town's Boye nursery site in southwest Ethiopia. The three species were *E. abyssinica*, *F. vasta*, and *C. macrostachyus*. To achieve this, full-grown, green leaves with petiole foliage were collected from the entire crown of the mother plant and left to air dry for three days. After that, the litter-bag method was used to conduct the experiment in an open field. The impact of litter chemical quality indices on decomposition rates was also examined in this study.

The findings verified noteworthy ( $p < 0.001$ ) variations in the amount of ash-free dry matter lost and the rates of decomposition among the various species. After the study, the final mass of leaf litter remaining varied from 4 to 22.07%, with an average of 13.05%, and the average daily decomposition rate was 0.025. Our knowledge of how the metabolic makeup of leaf litter material influences its rate of decomposition has also been broadened by the work. In fact, it was found that cellulose and C/N were impeding factors, while N and P were related to facilitation.

According to the study, *C. macrostachyus* foliage litter decomposes the fastest, followed by *F. vasta* and *E. abyssinica*, in that order. The rapid decomposition of *C. macrostachyus* litter may limit its long-term capacity to accumulate organic matter. As an alternative, *F. vasta* and mostly *E. abyssinica* may be taken into consideration for these reasons. Despite the fact that the experiment was only conducted in one place and one season, a more thorough investigation is suggested before making any firm recommendations. Furthermore, the study only looked at the foliage; more investigation is needed into the roots, stems, buds, and flowers.

### Declarations

All information in this paper is true and correct to the best of our knowledge and belief. We hereby declare that all the above information is correct and accurate. I solemnly declare that all the information furnished in this document is free of errors to the best of my knowledge.

### Author Contribution Statements

Kasu and Dargo conceived of the presented idea. Kasu, Dargo and Solomon developed the theory and performed the computations. Kasu and Dargo verified the analytical methods. Kasu, Dargo and Solomon to investigate [a specific aspect] and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Kasu, Dargo and Solomon conceived and planned the experiments. Kasu and Dargo. carried out the experiments. Kasu and Dargo contributed to sample preparation. Kasu contributed to the interpretation of the results. Kasu took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

#### Competing Interest Statement

We declare that there is no conflict of interest

#### Funding Declaration

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## Climate Change Trends and Vulnerabilities in Bangladesh's Crop Sector: A Review of Crop Production Challenges and Resilience Strategies

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### ABSTRACT

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Climate change has become one of the most important threats to worldwide agricultural production systems. This paper evaluates how Bangladesh, a prominent developing country in the low-lying Ganges-Brahmaputra-Meghna delta, is susceptible to climate change and assesses present agricultural practices that target sustaining production under these threats. The study synthesizes the ongoing research findings of climatic change tendency, involving the rising of temperature, alteration of precipitation pattern, along with the onward frequency of extreme weather incidence and their complication to crop production. It discusses the key susceptibilities of Bangladesh's crop sector, such as a shortage of irrigation water, the impact of rising temperatures, increasing sea levels and the loss of biodiversity. Moreover, the study explores the resilience strategies and measures adopted by farmers, policymakers and researchers to alleviate the hostile effect of climate change on crop production. With all these considerations, the paper aimed to analyse the current climate change trend, adverse effects to agricultural sectors and existing resilience practices in Bangladesh as well as future strategies against climate change.

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### Introduction

Bangladesh is known for being extremely prone to climate instability and alterations (Harmeling, 2008; Agrawala et al., 2003). The nation is located on low-lying floodplains at the point of convergence of three significant Asian rivers, the Meghna, the Ganges, and the Brahmaputra, collectively with their additional tributaries. The economic situation of Bangladesh as well as the country's progress closer to sustainable development are under a great deal of strain due to the country's distinctive geographic location with shifting environmental conditions (MoEFCC, 2022). The estimated population is expected to be 9 crores, or almost 56% of the total, living in "high climate exposure areas," while 5.3 crores are expected to dwell in "very high climate change areas" (The Daily Star, 2023). Bangladesh comes up at number seven in worldwide rankings when its vulnerability to disasters is considered (Eckstein et al., 2021). Climate change is rewriting the rules of existing crop production technologies, inducing both biotic and abiotic stresses where traditional farming practices struggle to cope with the increasing pressure of extreme weather events, soil degradation and pest attack. Elevated temperatures cause heat stress in plants, which decreases yields, particularly in

cereal crops such as rice, wheat, maize, and barley during the flowering stage, by disrupting chlorophyll synthesis, enzymatic reactions and protein metabolism (Lobell et al., 2021). When temperatures rise by 4°C, the result of global warming affects the production of wheat by approximately 66%, along with the output of rice by 28% (MoEF, 2009). Unpredictable patterns of precipitation cause extended droughts, which lower soil moisture content, restrict root growth, disrupt the absorption of nutrients, reduce yield and make the plant more vulnerable to insect and disease infestations (Dai, 2013). According to Anjum et al. (2011), drought stress brought on by climate change severely slows down the rate of CO<sub>2</sub> absorption, stomatal conductivity, and plant water relations, all of which lower yield. Moreover, increased frequency and intensity of excessive precipitation raises the frequency of flooding and prolonged waterlogging, which results in abnormal root functioning, lowering soil oxygen levels, promoting root rot, and decreasing crop yield (Furtak & Wolinska, 2023). Sea level rise due to climate change increases saltwater intrusion in fresh land, including rivers and groundwater sources, which reduces crop yield through an increment of salinity levels in the soil (Baten et al., 2015).

Approximately 11.20 % of the nation's GDP in FY 2022-2023 came from agriculture (BER, 2023). By 2050, fluctuations in the climate with severe events like waterways floods in mid-Bangladesh, along with a shortage of water throughout north-western Bangladesh may cause nearly a third of Bangladesh's agricultural GDP to be permanently lost (World Bank, 2022).

Climate change aggravates ecological conditions by changing several environmental factors, which worsen biodiversity loss by increasing insect pests and weed infestations (Skendžić et al., 2021). High temperatures promote the metabolism and proliferation of disease-causing insects, pests and pathogens, leading to a rapid population explosion (Shahzad et al., 2021). It has the possibility to modify plant blossoming times, mismatch in pollinator flying periods, leading to a species endangered that relies on pollinators for their effective reproduction process (Vanbergen & Initiative, 2013). Nitrogen deposition, uplifting temperature, and CO<sub>2</sub> increment could act together to unsettle this critical mutualism by changing plant chemistry in ways that change flower attraction or nutritional composition for pollinators (Huo et al., 2020). It has been concluded that climate change affects plant microorganisms and crop quality through the anthropogenic introduction of xenobiotics (polycyclic aromatic hydrocarbons, pesticides, etc.), presenting dangers to soil well-being by upsetting soil microbial enzyme activities and system structures (Singh et al., 2019). Moreover, weeds are serious destroyers of crop yields, which can be influenced by the changing conditions of the climate. Generally, any effects of higher CO<sub>2</sub> or climate change that alter the growth or competitiveness of weeds and crops will shift their interactions (Singh et al., 2011). Climate change will likely cause shifts in weed community compositions, their population dynamics, life cycle, phenology, and infestation pressure, resulting in some weed species going extinct while others becoming more aggressive invaders (Anwar et al., 2021). It is projected that by 2095, temperatures in Bangladesh will rise by up to 2.2°C. This increase is expected to result in a doubling of leaf folder pests affecting rice. Conversely, the population of brown plant hoppers is predicted to remain unchanged, while the incidence of potato late blight is expected to decrease (Salam et al., 2019).

This review aims to analyse the present patterns of climate change affecting agricultural production. It will also highlight the agricultural sector's estimated vulnerability to climate change, considering the socioeconomic implications. Additionally, the current study will examine existing climate resilience methods in greater detail in order to maintain crop productivity in the face of climate change, along with an overview of the policies that have been implemented to counteract it.

## Materials and Methods

This review studied a plethora of scholarly literature, reviewing over 300 articles concerning the evolving climatic trends and subsequent impacts on Bangladesh. From this extensive pool, a judicious selection process singled out the 50 most seminal works for inclusion. In pursuit of comprehensive insights, an array of data was

meticulously gathered from various governmental authorities and esteemed international organizations, including but not limited to the BBS, World Bank, USAID, IFRC, IPCC, UNDP, and WMO. Moreover, a thorough scrutiny of diverse newspaper sources augmented this data gathering endeavour.

## Result and Discussion

### *Climate Changing Trend in Bangladesh*

Bangladesh, a subtropical nation in south Asia, has unique geographical features (due to its low-lying topography) that make it vulnerable to climate change. The risks, dangers, and future forecasts related to the spotted climate patterns are elaborated on based on numerous indicators that indicate the topographical and hydrological changes.

#### *Temperature*

Bangladesh is located in a subtropical area and highly vulnerable to the adverse effect of climate change, which are mainly expressed as a rise in air temperatures. Bangladesh's historical climate has included yearly mean temperatures of about 26°C, with annual variations ranging from 15°C to 34°C. The wintertime period (December-February) is cooler and less humid than the summer season (April-September), which coincides with the highest temperatures. The mean temperature has risen significantly during the last thirty years by 0.5°C from 1976 to 2019, which is a cause for concern (World Bank, 2021a). When analysing the years from 1980 to 2022, the yearly average temperature of Bangladesh shows a clear upward trend, with values ranging from 25.09°C to 26.6°C, regarding minor deviations. With only a few tiny variations, the average yearly temperature stays at 25.58°C (DoE, 2023). However, Figure 1 shows that some years had greater departures over the average, suggesting significant variations in temperature throughout the period in question. The trend of rising temperatures has picked up dramatically in the last several decades. The average temperature increased by 0.39°C during the years 1991 to 2000. Between 2001 and 2010, the bump in temperature became more significant, rising by 0.53°C. The increase persisted between 2011 and 2019, with a notable peak of 1.06°C (Figure 1). Additionally, there has been a rise in the lowest temperatures during the winter and monsoon seasons, with increases of 0.45°C and 0.52°C, respectively (DoE, 2023). According to Ahmed et al. (1999), Bangladesh's expected temperature rise from the base year of 1990 is predicted to reach 1.3°C by 2030 and 2.6°C by 2075. In a different study, IOC (1993) predicted that by 2030, there will be fluctuations of 0.7°C in the monsoon months and 1.4°C in the winter. According to projections, there would be changes in the winter of 2.1°C and the monsoon of 1.7°C in 2075 (Yu et al., 2010). Making use of baseline data from 1961 to 1990, Karmakar (2000) showed that between 2050 and 2100, yearly average maximum temperatures are predicted to rise by 0.40°C and 0.73°C, respectively. The pattern of rising temperatures emphasizes how urgently comprehensive measures to mitigate the effects of climate change in Bangladesh are needed.

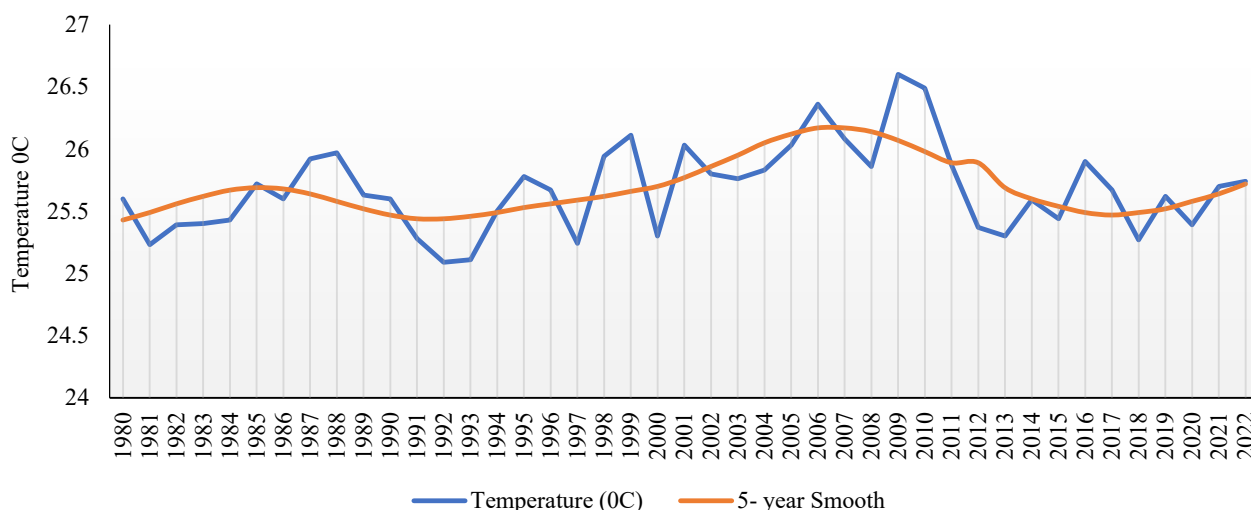


Figure 1. Average temperature in Bangladesh (1980-2022)  
Source: World Bank (2021a)

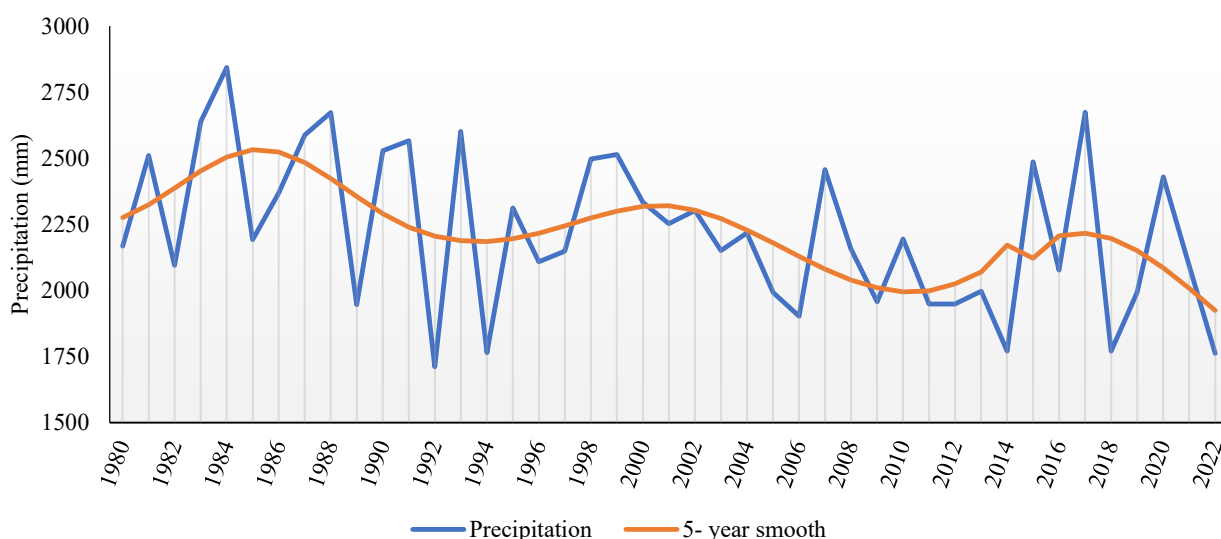


Figure 2. Average annual rainfall in Bangladesh (1980-2022)  
Source: World Bank (2021a)

### Rainfall

Climate change is predicted to alter the kind and quantity of rainfall, while the exact degree of this alteration is yet unknown (Wasimi, 2009). This change is expected to increase the unpredictable nature of extreme weather events, making it more difficult to estimate the frequency of extreme rainfall events because there might not be a consistent set of values for statistical extrapolation (Linacre, 1992). Based on data from 1990, it is projected that the average annual evaporation in Bangladesh will increase slightly by 2075 while remaining relatively stable up to 2030 (Ahmed et al., 1999). Although the country as a whole receives 2320 mm of rain on average each year, some areas receive up to 6000 mm on average (Hossain et al., 1987b). The average amount of precipitation has been steadily dropping during the previous forty years. The average rainfall from 1981 to 1990 was 2413.50 mm. Right after that, there was a decline with an average of 2250.38 mm in the following decade (1991-2000). The mean annual precipitation throughout the years 2001-2010 and also

2011-2020 showed lowering patterns at 2157.83 mm and 2109.11 mm, respectively (Figure 2). This declining tendency continued throughout the next two decades. The ongoing decade, 2021-2030, sees this negative pattern continuing (World Bank, 2021b). Precipitation is expected to increase somewhat in the winter and moderately in the summer of 2030. Nonetheless, a notable increase in winter evaporation is anticipated by 2075. More precipitation is expected during the monsoon season, while less precipitation is expected in the winter. This suggests that more summertime flooding may result from heavier rainfall, whereas winter drought conditions may be exacerbated by less precipitation and warmer weather. Moreover, significant upward trends in precipitation are anticipated for the northeastern and southeastern areas of Bangladesh, with expected increases ranging from 10 to 80 mm during the near-future period (2015-2044), 40 to 200 mm in the mid-century timeframe (2045-2074), and 40 to 260 mm in the far-future era (2075-2100) (Bhattacharjee et al., 2023).

*Sea level rising*

Due to its distinct geographic location and ongoing environmental changes, Bangladesh is among the world's most at-risk nations for the effects of sea level rise. Bangladesh constitutes a very low-lying country, with the majority of its landmass located three meters or less below sea level and a large portion of its inhabitants residing around the coast (Nishat & Mukherjee, 2013). In accordance with the Government of Bangladesh's Coastal Zone Policy (CZPo, 2005), 19 of the 64 districts, or 147 Upazilas, are located in the coastal zone. More than 35 million people live in the Ganges-Brahmaputra-Meghna delta, which makes up the majority of Bangladesh's coastal region. Sea level fluctuations affect about 30% of the nation's arable land because its coastal location (Haque, 2006). According to tidal measurements collected along Bangladesh's coast, sea level rise is occurring far more quickly than the world average (Khan et al., 2000). It has been revealed that, sea level rise rates vary across the Bay of Bengal, with the Ganges coastal floodplains experiencing an increase of 5.3 to 5.8 millimeters per year, the Meghna estuary floodplains rising by 4.2 to 5.3 millimeters annually, and the Chattogram coast showing a slower rise of 3.7 to 4.2 millimeters per year, highlighting the necessity for region-specific adaptation strategies (Partha Shankar Saha, 2024). Moreover, sea levels are expected to rise by 10 cm, 25 cm, and 75 cm by 2020, 2050, and 2100, respectively (Table 1) which will affect the agricultural production system adversely (World Bank, 2000).

*Flood*

Due to its low-lying geography and high population density, Bangladesh faces severe flooding risks

exacerbated by climate change, with annual floods affecting 30% to 70% of the country (Litchfield, 2010). Furthermore, the UNDP has ranked the nation as the world's sixth most flood-prone nation (Rawlani & Sovacool, 2011). Between 1972 and 2022, Bangladesh witnessed 86 instances of catastrophic flooding throughout the nation, covering a geographical region of 747,230 km<sup>2</sup>. The resulting floods claimed 42,279 lives and significantly impacted the lives of over 396 million people (DoE, 2023). The floods that occurred in 1954, 1955, 1974, 1987, 1988, 1998, 2004, 2007, 2017, 2019 and 2020 all resulted in significant destruction of property along with a significant number of fatalities (BWDB, 2021). The majority of those incidents occurred during monsoon floods in riverine areas. Other forms of flooding are additionally rather prevalent, including floods caused by storms or cyclones, urban flooding, riverbank flooding, etc. (Baten et al., 2018). Approximately one-third of the nation experiences extreme flooding each decade, and in extreme cases of flooding, almost 60% of the nation is submerged (Brouwer et al., 2007). According to the investigation, by 2070-2099, compared to 1971-2000, the amplitude of maximum water levels might grow by 16% in a low-emission situation while 36% annually under a high-emission situation (Dhaka Tribune, 2023). Bangladesh's substantial humanitarian and financial consequences from flooding are predicted to rise dramatically as a result of climate change unless more is done to develop endurance and implement mitigation strategies. Figure 3, displays percentages of the entire Bangladeshi territory that has experienced flooding since 1980.

Table 1. Possible impacts of different forms climate change in Bangladesh

Year	2020	2050	2100
Sea level rise	10 cm	25 cm	75 cm
Land below SLR	2% of the total land=2500 km <sup>2</sup> .	4% of land=6300 km <sup>2</sup> .	17.5% of land =25000 km <sup>2</sup> . Patuakhali, Khulna and Barisal regions most affected.
Storm surge	-	A cycle like 1991 happens again with a 10% increase in intensity, storm surge goes from 7.1 to 8.6 m with 0.3 m SLR	Storm surge goes from 7.4 to 9.1 m with 1 m SLR
Salinity	Increase	Increase	Increase
Flooding	20% increase in areas subject to flooding.	Increase flooding in Meghna and Ganges floodplain.	Both inundation area and flood intensity will increase tremendously. Devastating flood may cause crop failure for any year.

Source: World Bank (2000)

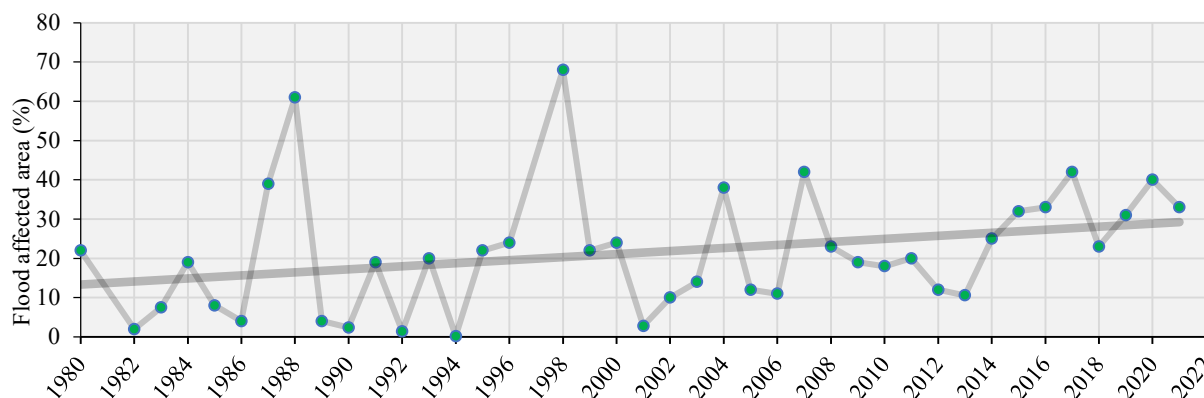


Figure 3. Annual flood affected area (%) in Bangladesh (1980-2021)  
Source: Bangladesh Water Development Board, (Annual Flood Report 2021)

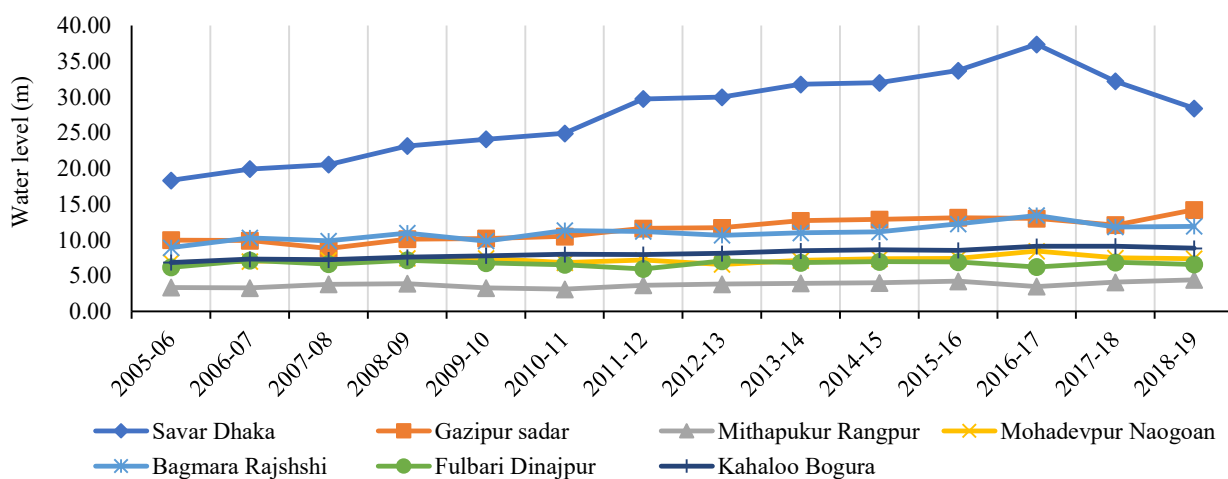


Figure 4. Changes in the depth of groundwater (Jan- Mar) over time

Source: BADC 2020, Minor irrigation survey report 2010-19

### Cyclone

Bangladesh's positioning in the northern half of the Bay of Bengal and the North Indian Ocean (NIO) makes it particularly susceptible to cyclones (Ali, 1996a). The northern portion of the Bay of Bengal's funnel-shaped topology exacerbates the frequency of tropical cyclones, which annually impact thousands of people. In addition to having all four categories of cyclones; cyclonic storm, severe cyclonic storm, very severe cyclonic storm, as well as super cyclonic storm, the nation witnessed roughly 0.93% of all tropical cyclones worldwide (Ali, 1999b). In Bangladesh, the frequency of catastrophic cyclones has risen dramatically in the past few decades. Bangladesh was struck by 36 tropical cyclones (TCs) between 1970 and 2023 (Hossain & Mullick, 2020; World Bank, 2024). Based on available data, Bangladesh experiences on average 1.15 tropical cyclones annually. Since 1970, cyclones have wiped out the lives of over 450,000 individuals living in Bangladesh. Among these most deadly storms are Cyclones Gorky (1991) and Bhola (1970), which claimed the lives of 140,000 individuals as well as 300,000 people, respectively (Saha & James, 2017). Furthermore, the Intergovernmental Panel on Climate Change notes that as upcoming tropical cyclones intensify, Bangladesh's coastal flooding caused by cyclonic storm surges will probably get worse (IPCC, 2007). The present setting entails 14% and 69% additional areas than the present baseline assumption that are susceptible to flooding depths of over a meter and 3 meters, correspondingly. In the severe case, a cyclone with a 10-year return time is expected to become significantly stronger by 2050 and would impact 43% of the entire exposed area, which is 17% greater than it does now (BDP2100, 2018).

### Groundwater Depletion

Groundwater plays a crucial role in the struggle against impoverishment, ensuring the availability of nutritious food and drinking water, the advancement of good jobs, growth in socioeconomic status and the ability of economies and society to withstand the effects of global warming. Bangladesh is primarily dependent on underground water for agriculture; between 71 to 94% of its groundwater is abstracted for this purpose (Margat & Van der Gun, 2013). It is commonly known that the

overuse of groundwater over the past ten years has resulted in a significant depletion of water bodies. According to UN (2022) estimates, Bangladesh is the sixth-most-extracted groundwater-producing nation in the world annually. Water levels reach their lowest extent near the end of the summer season due to groundwater removal along with other diversions (Khaki et al., 2018; Mojid et al., 2019). In addition to these various causes, lowering levels of soil water could additionally be the consequence of reduced precipitation along with the dissipated replenishment it generates, as well as additional variables like altered agricultural practices or reduced river flows. (Pena-Arancibia et al., 2020). The recognized patterns of the depth of groundwater drops in certain parts of northwest Bangladesh have been attributed to widespread irrigation practices using groundwater (Kirby et al., 2015; Mojid et al., 2019; Salem et al., 2017). Bangladesh's agricultural sector relies heavily on the growing of rice. Boro rice, which might require up to 11,500 m<sup>3</sup> of water per hectare for production, accounts for over 70% of the agricultural output during the driest times of the year (Chowdhury, 2010). The hydrograph was created using data collected in AWLRs (Automatic Water Level Recorders) from various Bangladeshi areas between 2006 and 2018, to understand the groundwater depletion trend. Gazipur Sadar in the Gazipur district saw the greatest reduction, as can be apparent in the illustration (Figure 4). The greatest groundwater depth declines during a dry period occurred in March 2017 that increased by 8.96 meters over the preceding two years.

### Impact of Climatic Changes in Agriculture Temperature and Rainfall

Numerous regions of Bangladesh are currently experiencing agricultural disruptions as a direct consequence of temperature and rainfall variations. Climate change would have a significant negative influence on Bangladesh's agricultural output; yields of rice and wheat are predicted to fall by 28% and 68%, respectively (Rezvi, 2018). Crops require precise temperature ranges for optimal growth, and a 10% rise can significantly decrease monsoon rice yields by 2.94, 53.06, and 17.28 tons per hectare during development,

reproduction, and maturation stages, respectively (Islam et al., 2008a). The possibility of cultivating wheat and potatoes could be greatly hampered by changes in temperature (by 2<sup>o</sup> and 4<sup>o</sup> degrees Celsius), and losses from production could be more than 60% of the potential yields (Karim, 1993). The probability of growing wheat and potatoes would be substantially reduced with temperature changes of 2 and 4 degrees Celsius (Hossain et al., 2020a). Furthermore, temperature sensitive crop yield has the potential to be significantly impacted by temperature increases, particularly Bangladesh's rabi crops. The increased respiration efficiency of cultivated crops due to climate change causes them to require more carbon for consumption, accelerate phenological improvement, and degrade tissue more quickly. Seasons are also impacted by temperature variations, which can potentially cause plants to become more susceptible to harmful thresholds along with enhancing the probability of evapotranspiration (Solomon, 2007; Hatfield et al., 2011). Bangladesh produces 0.8 million tons of pulses, 0.9 million tons of oilseeds, and around 9.0 million tons of potatoes annually. The ideal temperature range for all of the above crops is 18-25°C. Such crops are extremely susceptible to changes in moisture, fog and clouds. An increase in temperatures will probably lead such crops to become discontinued (Biswas et al., 2018). Rising temperatures (3<sup>o</sup>C) have reportedly been linked to changes in Bangladesh's GDP, which have been predicted to range around -0.854% in 2027, reaching -2.491% in 2047, followed by -7.591% in the near future (Kompas et al., 2018).

Climate factors, such as variations in the current pattern of precipitation, can lead to a number of challenges in agriculture, such as crop failure in the earliest phases of development, and they can also have an impact on Bangladesh's key crops long-term productivity. Due to the rapidly declining groundwater level, which is now the biggest threat to the production of boro rice, the task of guaranteeing food security is going to be extremely difficult for the nation. Upcoming average monthly precipitation could fluctuate greatly from normal levels, depending on environmental change situation forecasts. It is assumed that monsoon precipitation will increase by 27% in 2070 and by 11% in 2030 (Biswas et al., 2018). A one-millimeter increase in precipitation throughout the development, reproductive, and aging stages reduced the yield of monsoon rice by 0.036, 0.230, and 0.292 tons, respectively (Amin et al., 2014). Rahman and Lateh (2017) carried out a spatial-temporal assessment over a 40-year period (1971-2010) involving 34 meteorological stations in Bangladesh. They concluded that Bangladesh's recent climate change is predicted to raise its average temperature by 0.2°C every ten years. Regarding precipitation, they recorded an increasing yearly tendency of 7.13 mm from

1971 to 2010, but a decreasing premonsoon (-0.75 mm) along with a post-monsoon (-0.55 mm) every year. The findings indicate that there is considerable fluctuation in pre-monsoon and post-monsoon rainfall (44.84-85.25%). This variability is likely to have an adverse effect on the agricultural sector, as pre-monsoon rains are crucial for preparing land for rainfed rice production. Floods and waterlogged conditions brought on by heavy precipitation may additionally result in the destruction of crops. The anticipated deviations in the country's temperature and precipitation are shown in the following Table 2.

#### *Flood and Cyclone*

In Bangladesh, flooding is widely acknowledged as the most frequent and damaging climate related calamity. The most susceptible sector of the economy to flooding is agriculture, resulting in massive crop devastation annually (Yu et al., 2010). The environmental conditions of Bangladesh are responsible for an increase in the incidence and extent of floods, according to Rahman and Salehin (2013). On average, between 30 and 70 percent of the nation floods each year (Ahmed, 2006). There are significant occurrences of flooding, including the 1998 flood that submerged 68% of the country, caused 33 million people to be relocated, and destroyed 100,000 km<sup>2</sup> of agricultural land (Rashid, 2000). Meanwhile, catastrophic flooding incidents such as the 2004 and 2007 monsoonal floods impacted 38% and 42% of the land area, respectively. It was determined that 89% of the Aus rice area, 93% of the total rice-growing area, and 73% of the jute-growing area were severely disrupted because of the 2004 flood. In 2007, a flood harmed 14 million people, inundated about 42% of the nation's terrain, and plunged 2.1 million hectares of fertile land (Huq et al., 2010). The most devastating floods in recent times devastated Bangladesh's northeast in June 2022, mostly affecting the regions of Sunamganj and Sylhet. This catastrophic incident completely destroyed 83,394 hectares of crops; it resulted in financial losses to cattle of 28.1 million US dollars and it additionally destroyed sanitation facilities and waterways (Sheikh, 2022). Approximately 27,000 hectares of agricultural land in the Chattogram area had flooded. According to estimates, Tk135 crore worth of destruction was caused by the flood to Aman crops on 14,000 hectares of cropland, Aus on 4,500 hectares, Aman seedbeds on 3,700 hectares, and vegetables on 5,100 hectares (Azad, 2023). The projected agricultural loss is indicated in the accompanying Table 3 from 2007 to 2022.

Crop productivity is severely harmed by cyclones. Due to its natural surroundings and proximity to a tropical cyclone, Bangladesh's coastal region is especially prone to cyclonic storms, floods, and other natural calamities (Ahmed & Rahman, 1999).

Table 2. Projected temperature & rainfall changing trend in Bangladesh

Year	Temperature Changes (°C) mean			Precipitation Changes (%) mean		
	Annual	DJF	JJA	Annual	DJF	JJA
2030	1.0	1.1	0.8	5	- 2	6
2050	1.4	1.6	1.1	6	- 5	8
2100	2.4	2.7	1.9	10	- 10	12

Note: DJF represents the months of December, January and February, usually the winter months. JJA represents the months of June, July and August, the monsoon months Source: National Adaptation Programme of Action, Bangladesh (2005)

Table 3. Loss in production (M. ton) due to major floods from 2007 to 2022

Crops name	Loss in production (M. ton) due to floods from 2007 to 2022								
	2007-08	2008-09	2009-10	2011-12	2014-15	2015-16	2017-18	2020-21	2021-22
Aus	94164	990	-	7519	7077	17395	64482	1255.26	54569
Aman	230681	113465	-	70014	125250	102254	734905	29364.34	-
Boro	-	-	369591	-	-	97592	-	-	21304
Jute	46145	-	-	-	-	-	33895	4498.01	-
Sugarcane	116387	-	-	-	-	-	-	5166	-
Vegetables	99614	670	-	5242	79	-	41275	3682.31	-
Banana	7014	-	-	-	-	-	352	405.72	-
Pineapple	2815	-	-	-	-	-	-	-	-
Papaya	2386	30034	-	-	-	-	-	-	-
Maize	1743	745	-	-	-	-	-	-	-
Ginger	1084	-	-	-	-	-	-	-	-
Chilies	2204	622	-	2542	-	-	740	378.39	-
Betel leave	230	-	-	-	-	-	4129	692.42	-

Source: BBS 2014, BBS 2023, (\*M. ton= Metric ton)

Table 4: Cropped area damaged by several cyclones in Bangladesh

Name of the cyclone	Year	Damaged agricultural land
Sidr	2007 <sup>a</sup>	Approximately 644 000 hectares
Aila	2009 <sup>b</sup>	More than 123,000 hectares
Mahasen	2013 <sup>c</sup>	Approximately 32,633 hectares
Fani	2019 <sup>d</sup>	Approximately 63,063 hectares
Bulbul	2019 <sup>d</sup>	Approximately 22,836 hectares
Amphan	2020 <sup>d</sup>	Approximately 1,76,007 hectares
Yaas	2021 <sup>e</sup>	Approximately 12,151 hectares
Sitrang	2022 <sup>f</sup>	Approximately 10,200 hectares
Midhili	2023 <sup>g</sup>	Approximately 29,000 hectares

(<sup>a</sup> Relief Web, 2007), (<sup>b</sup>Relief Web, 2009), (<sup>c</sup>Parvez, 2013), (<sup>d</sup>Dhali, 2020), (<sup>e</sup>Islam *et al.*, 2021d) (<sup>f</sup>The Business Standard, 2022), (<sup>g</sup>Ali, 2023c).

Cyclones have the most detrimental effects on human life since they destroy crops, homes, animals, and transportation infrastructure, in addition to driving up the number of accidental human deaths. When it comes to devastating cyclones in Bangladesh, the 1991 storm devastated 280,000 acres of cultivated land, 60% of livestock and 80% of poultry stocks. Having a maximum speed of 225 Km<sup>h</sup><sup>-1</sup>, the 1991 cyclone struck Bangladesh and annihilated roughly 138,000 people, with total losses estimated at US\$ 4 billion (Kausher *et al.*, 1996). According to Ali (1996a), the average wind velocity of the cyclone will fluctuate between 248 Km<sup>h</sup><sup>-1</sup> and 275 Km<sup>h</sup><sup>-1</sup> wherever the temperature rises by 2 °C and 4 °C. Any rise in storm surge water could quickly flood the low-lying, flat areas of the nation. Such unusual incidents will seriously harm the infrastructure, food safety, the agricultural sector, the economy, and eventually the survival of the nation. However, in 2007, cyclone Sidr killed 468,000 cattle and destroyed 16,10,000 hectares of arable land, resulting in a massive economic loss assessed to be worth US\$ 3 billion (Shamsuddoha & Chowdhury, 2007). The destructive hurricane Amphan made landfall in the coastal area in 2020. By May 22, approximately 2,233 km<sup>2</sup> of land had been flooded. In Bangladesh and India, there have been reports of devastation to some 3,200 km of roads and 400 km of riverbanks, which have hampered response operations and exposed additional areas to flooding (IFRC, 2020). The agricultural loss in Bangladesh as a result of many cyclones is displayed in Table 4 due to different cyclones.

#### Scarcity of groundwater

Groundwater level is currently being drastically lowered by climate change, which is now the biggest danger to agriculture productivity. In Bangladesh, the harvesting of groundwater for irrigation during the growing of rice has increased rapidly as a result of the expansion of the cultivable area with irrigation, especially for dry season irrigated rice. Groundwater accessibility in Bangladesh is undoubtedly being impacted by climate change, as evidenced by reports of declining groundwater depth in the country's Northern regions (Mustafa *et al.*, 2019). To ensure the sustainable use and/or effective execution of groundwater, the outflow of water in the northwest regions of Bangladesh must be reduced by 60% of its current level. The groundwater table is expected to drop five to six times more quickly in 2026-2047 compared to the baseline era (1985-2006) if the existing expanding upward trend in the removal of groundwater persists. This is because both climate change and increased groundwater extraction are having an impact on the extent of groundwater (Hossain *et al.*, 2020a). Furthermore, according to Mustafa *et al.* (2019), in order to maintain the environmental sustainability of groundwater in Bangladesh's northwest, withdrawal of groundwater requires being reduced by more than 50% of current uses. In the years 2016-17, 2017-18, 2018-19, 2019-20, 2020-21, 2021-22, and 2022-23, the overall area under irrigated crop was 55.27, 55.57, 55.87, 56.27, 56.54, 56.88, and 57.20 lakh hectares, respectively (BBS, 2023). Because

there was absolutely no replenishment from precipitation or above-ground flows, such massive extraction has reduced subsurface water stores. Many researchers have indicated that the world's greatest groundwater declines are found in northern Bangladesh and northern India, up to Assam (Tiwari et al., 2009; Rodell et al., 2009; Wada et al., 2010). Therefore, the state of groundwater would be far-reaching for additional extraction of groundwater owing to agriculture, considering this unpredictability and massive groundwater extraction for irrigation. Various environmentally friendly farming methods, along with additional water management techniques should be implemented in order to guarantee the appropriate and sustainable use of groundwater.

#### *Drought*

Due to its geographical position, Bangladesh is one of the most vulnerable countries in the world to natural disasters (Afrin, 2016). Every five years, there are significant nationwide droughts in Bangladesh (Selvaraju & Bass, 2007). Up to 47% of the nation, where 53% of people currently reside, is allegedly at risk of drought (Biswas et al., 2018). One common catastrophe throughout the nation is drought. In 1973, 1978, 1979, 1981, 1982, 1992, 1994, 1995, 2000, 2006, and 2009, Bangladesh was hit by severe droughts (Al Mamun et al., 2024). The production rates of a variety of crops are adversely affected by climate change, groundwater scarcity, irregular rainfall, and high temperatures, especially in Bangladesh's northwest (Mishra & Singh, 2011). Drought is a major threat to crops grown in the pre-kharif (mid-March to mid-May) and Rabi (mid-November to mid-March) seasons (Mohsenipour et al., 2018). Approximately 2.32 million hectares of land have been damaged by droughts of various severity per year (Habiba et al., 2011). Drought not only damages agriculture but also has adverse impacts on society and the environment, including loss of livelihoods, migration, increased food prices, decreased biodiversity, disease, destruction of land, and a host of other issues. A common drought occurrence damages 2.32 million hectares (ha) of cropland each year (Kharif season) and 1.2 million hectares (ha) per year (Rabi season) (CEGIS, 2013). According to research by Rahman et al. (2007), the northwest of Bangladesh had a 25-30% decrease in agricultural production due to drought. Each year, drought causes about 17% of the Aman harvests to be lost (Alam, 2015). Drought-related yield decreases range from 45-60% for rice and 50-70% for highland crops in extremely severe cases. In mild droughts, rice yield loss ranges from 10-30%; in severe droughts, it could reach 70-90% (Biswas et al., 2018). An extreme climatic change scenario (60 percent water stress) could result in a 55-62 % reduction in dry season or boro rice production (Selvaraju & Baas, 2007).

#### **Sustainable Agricultural Practices in Bangladesh to Mitigate The Global Warming Effect**

Bangladesh is currently dealing with the effects of global warming, including rising temperatures, unpredictable rainfall and prolonged droughts. These factors are putting forth it difficult for farmers to maintain crop productivity. In response to current challenges, Bangladeshi farmers are implementing climate-resilient

agriculture techniques and devising creative ways to ensure their survival. Climate-smart agriculture (CSA) is an environmentally conscious farming approach designed to promote sustainable agricultural practices and rural development while addressing the challenges posed by environmental deterioration to food production. This approach recognizes that reducing greenhouse gas emissions while simultaneously boosting resistance to the consequences of climate change is necessary to feed a growing population. Currently used sustainable farming methods include the following:

#### ***Coastal Region***

##### *Sack Method*

In coastal areas, government officials and local NGOs implemented the sack method to prevent fruits and vegetables from being carried away by floods. Families found great success with sac farming in assisting them in adjusting to situations of getting waterlogged and saltwater intrusion (Angrish et al., 2006). The process of sac farming involves putting manure in a bag with nutrient-rich soil, adding a few gravels to the bottom for optimum drainage, leaving a few holes in the interior for air circulation, and then placing plant material on top to thrive. Sack farming is used to grow vegetables such as bottle gourd, gourd, brinjal, cucumber, and chili. Sack farming's key advantages are its portability, efficiency, profitability, improvements to nutritional stability and decreased agricultural risk (IWMI, 2015).

##### *Sorjan Method*

This unique approach is intended for regions in which conventional crop cultivars are unable to produce acceptable yields due to significant soil salinity and extended inundation. Raised beds are created by dividing the area into raised subplots and removing dirt from nearby locations. Whereas the submerged sections or ditches are utilized for periodic fish production throughout the monsoon, raised planting beds are employed for the year-round production of vegetables, fast-growing fruits, and other crops. The Sorjan is surrounded by ditches or canals that supply irrigation and water throughout dry periods. The land is split up into multiple subplots in this instance. There's an interminable trench to hold water between two distinct subplots. Each subplot is elevated by removing soil from the side that borders it. The plot's ideal dimensions are 8.0 × 2.5 × 4.5 meters. A number of factors, including the extent of the plot, might alter it. The raised bed is mostly used for growing fruits and vegetables, sugarcane cultivation, and dhaincha. In the channels that separate the two distinct beds, indigenous fish are raised. While more deeper permits rice-fish or rice-duck farming in addition to vegetable and nursery cultivation, shallow Sorjan is best suited for the continuous cultivation of vegetables (Sattar & Abedin, 2012). Sorjan has proven to have major financial advantages. In the first year, for example, it yielded Tk. 121,227 in net earnings from 0.55 ha of land, compared with Tk. 15,570 from a single crop of aman rice, indicating a 678% rise in net earnings (Chaki et al., 2023).

##### *Double Layer Mulching*

Double-layered mulching, implementing straw at the top and bottom of a small hole was demonstrated to lessen soil salinity by approximately 30 percent. In the country's coastal region, sweet gourd is produced using a double-



layer mulch technique, which results in a better yield (18.5 t ha<sup>-1</sup>) effectiveness (Shawkhatuzamman et al., 2023). Under this approach, producers get ready for their field by using a power tiller and tilling it three times before planting. Pits are sufficiently excavated when there is a 2.0-meter gap between each pit and between each line. Throughout the pit setup, compost along with a low amount of fertilizer are administered. Next, sprouted seeds are put down in a pit. Intercultural operations that are required are carried out when they are.

#### *Pitcher (Kolosh) irrigation to grow watermelon*

Salinization rises and groundwater for watering becomes scarce on land throughout the summertime. During the dry period, water is added to crops such as watermelon, sweet gourd, bitter gourd, etc. using pitchers, popularly called as Kolosh. Farmers living in Batiaghata Upazila in Khulna and Fakhirhat Upazila in Bagherhat district are making good use of the pitcher irrigation technology (also known as Kolosh irrigation) that SMRC and SRDI implemented for areas that became available by November. Pitchers are pierced to create a bottom hole that is roughly one inch in diameter. A jute string that is between half and one-meter long is placed into the hole. After that, the pitchers of water are lowered to a depth of 5 to 9 cm into pits (known as mada in this area), and the jute strips are stretched out at an identical level to allow the saturated jute to constantly hydrate the earth and lower the salinity surrounding the root zone. Three to four crop seeds are sown in each of the pits surrounding the pitcher. Producing watermelon, sweet gourd, and cucumber in pits during the dry season is a low-cost approach that involves growing rabi crops in locations with mild salinity (Mada).

#### *Ring Method*

The strategy was largely used in homestead gardening, where farmers would plant vegetables using a reinforced concrete toilet ring as a defense against saltwater and flooding. Local NGOs have just re-established it in Shyamnagar, Satkhira in 2021. It possesses the capacity to boost output, flexibility, and a reduction in greenhouse gas emissions.

#### *Usage of raised shrimp farm bund for year-round cropping*

Using this method, fruits, vegetables, and some types of trees are grown along the outer edge of shrimp farms. Aman rice with shrimp and/or fish has been transferred onto certain areas known locally as "Gher," or shrimp farms. To protect water and fish throughout dry periods, farmers excavated a ditch near the plot's centre, along the field's edge, or at any corner. Farmers may utilize water from shallow tube wells to keep fish alive. Occasionally, non-saline and mildly (S2) to considerably (S3) saline areas are utilized for boro or winter rice. It is referred to as the "Lockpur model" in the Khulna-Bagerhat district. The trenches around the border, corner, or center are 60-90 cm deep, and the boundary is erected around 60-90 cm higher than the level of floodwaters. Producers utilize nylon netting as a trail for creeping vegetation that is held up by strings, bamboo, or dhaincha in order to nurture crops. Precipitation removes the saline from the soil in the bunds, making it easier to grow vegetables. About a decade ago, the SRDI program, through SMRC, created this particular technology.

#### *Farm-Pond (Khamar-Pokor)*

The technique is a recently invented technology or method for preparing a land area for a variety of crops, fruits, and forests by digging up a pond on medium-high (flooded up to 90 cm) to medium-low (flooded 90-180 cm) lands where modern varieties (transplanted Aman rice) are unable to grow because of wetness in the beginning of the dry season or during late drainage. In ponds that have been mined to a depth of 180 to 270 cm, bunds and banks typically measure 90 cm in height and 120-150 cm in breadth. The surface soil remains on top, whereas the mined soil covers the remaining area of the land. SMRC and SRDI advise this kind of land use system, which has already been implemented over the past decade or so in the Khulna district's Batiaghata and Dacope Upazila. It is referred to as "Kuni" technology in Dacope. This kind of innovation aids in removing salt from the soil by washing it away. Aman rice (MV) transplanted, melons, and vegetables cultivated on elevated land; tree (fruit/forestry) crops cultivated on pond bunds or banks; fish cultured in ponds (Talapia: *Oreochromis niloticus*; Silver carp: *Hypophthalmichthys molitrix*).

#### *Dibbling Method*

This kind of technology is typically used on a large scale in Bhola, and on a smaller scale in Noakhali and Chittagong. Soils are generally brittle, either clay loam or loam. Pre-Kharif (Aus) season is when the Shaitta rice variety, a local variation, is grown. In March and April, people do dibble. Salinity in March and April should be avoided, as should germinating issues brought on by surface the formation of crusting. Instead, the in-situ dibbling technique for conserving soil water should be utilized. The soil is left undisturbed and rice seeds are buried at 3-6 cm depth. A cavity in the ground is made using a peg that has a diameter of 2.5 to 5 cm.

#### *Agronomic*

The coastline region is rather level, although there are topographical disparities between its upper and lower regions, which results in stagnant water in the agricultural land. The middle highland has persistent water that is between 15 and 90 centimeters deep. Many salt-tolerant modern cultivars, such as BRR1 dhan47, BRR1 dhan53, BRR1 dhan54, BRR1 dhan55, BRR1 dhan61, BRR1 dhan97, BRR1 dhan99, and BINA dhan-8, BINA dhan-10, are currently offered across the nation by various NARS organizations. It is possible to improve cropping intensity by around 0.602 million hectares in very saline (S1) and slightly saline (S2) locations by using appropriate water and soil conservation techniques and developing salt resistant crop cultivars (Shawkhatuzamman et al., 2023). This technique aids in the leaching of soluble salts, thereby lowering the salinity of the soil because a particular level of irrigation water is maintained in the field for the growth of successful rice harvests. The comparatively lower water level during this time facilitates the dissolution of salts. It is a reality, though, that the region's capacity to cultivate rice harvests throughout the winter is severely limited by the shortage of high-quality water used for irrigation. It is possible to cultivate vegetables and other horticulture crops effectively with raised dyke, ail, or bund.

### **Drought Prone area**

#### *Mini Ponds for rainwater harvesting*

In regions with severe water shortages, ponds have the potential to be excavated again, ideally in High Barind Tract Zones. Such mini-ponds can be used for rainwater gathering to provide additional irrigation on agricultural land lacking a watering base. Small farms should ideally have mini-ponds of 5 m × 5 m × 2 m (length × breadth × depth). In accordance with the specifications, it is further advised to dig bigger ponds (10 m × 10 m × 2 m). However, a few farmers desired the convenience of these small ponds situated in a field's corner. It is necessary to raise enough consciousness among local residents regarding the benefits of ponds. Mini ponds have reportedly been shown to boost profits on the same plot of land by up to 23%. According to BARI research (Sagor, 2017), only a small piece of agricultural land (2.5%) is enough to conserve rainwater to supply irrigation, provided crops are correctly chosen.

#### *Homestead gardening*

It is necessary to support the regional population's traditional wisdom of ecologically appropriate land maintenance. In regions susceptible to drought, home gardening technologies offer a thriving environment that benefits people, animals, birds, cattle, and various other types of biological diversity. Insects are kept from colonizing the land by adding organic material, ash additives, and household waste products. Vegetables from homestead gardening are occasionally grown for commercial use as well as for domestic consumption. Homestead bamboo species can also be planted since they are excellent soil-binding agents and grow quickly. Including homestead gardening throughout the family system helps to guarantee food safety in the family's residence by providing a variety of elements. In regions susceptible to drought, homestead farming is a useful practice for integrating gender issues into the framework for adapting to global warming.

#### *Mango and Jujube cultivation*

A potential replacement crop for managing drought in Barind areas is the mango, often known as Jujube (*Ziziphus jujuba*). Each season, more land is planted with mangoes because the area is renowned for producing higher-quality and more abundant mangoes. The tropical fruit crop known as jujube can tolerate a broad variety of temperatures. The jujube's ability to withstand prolonged drought qualifies as one of its best features. In Barind Tracts, the crop is capable of being grown well with minimal irrigation. Transplanting aman rice has the potential to be interplanted with jujubes across the second kharif season.

#### *Zero tillage wheat and garlic cultivation*

In the northern region of Bangladesh, growing wheat with zero-tillage after rice has proven to be the most effective resource-conservation technique yet. This technique involves planting wheat seeds in the entire field immediately after rice has been harvested, with no tillage. A machine is used to dig the hole, and once the seeds are planted, soil is added by hand. Due to its many benefits, zero-tillage wheat contributes to overcoming some limitations in the rice-wheat system. According to (Erenstein et al., 2008), zero-tillage wheat allows for early planting, assists in preventing unwanted plants, lowers expenses, and conserves moisture. In the low-lying rice growing districts of northern Bangladesh, the practice

of zero-tillage garlic production has become more and more prominent over the past few years. Using this approach, rice straw is utilized as mulch when rice is harvested, and garlic is seeded directly into the bed of soil. For vegetative development, garlic farming requires a cool, humid atmosphere; bulb formation requires a somewhat dry phase. Zero-tillage garlic cultivation under rice straw mulch is regarded as a climate-smart technique that provides a cost-efficient and efficient way to boost garlic yield. The BARI introduced zero tillage, which requires very little disruption of the land, in 2020. It has been demonstrated that this technique efficiently uses the nutrients and remaining water from earlier crops. This method works very effectively for small-scale producers and is a good fit with the Chalanbil regions in the regions of Pabna, Natore, and Sirajganj (Chaki et al., 2023).

#### *Alternative Wetting & Drying (AWD) irrigation*

AWD has been successfully demonstrated to dramatically reduce the consumption of water and arsenic in rice grains, without affecting rice output and releasing less methane throughout the rice-producing process. This technique does not maintain constant flooding of the soil. Instead, following the time the stagnant water has subsided, it is let to run off for a day or longer without getting reflooded (Lampayan et al., 2015). The field water tube used to apply the AWD technique can be formed of bamboo or plastic pipe that is 30 cm long. Its diameter should vary between 10 and 15 cm to ensure the soil is simple to take out using one's hands while the level of the water is apparent. Make multiple openings in the inner tube on every surface to allow water to easily enter and exit within it. So that it sticks out 15 cm over the ground's surface, and drives the hollow tube into the ground. It is important to avoid puncturing the plough pan's bottom. Hand labor is used to remove the dirt within the tube's interior so that the bottom may be seen. The evapotranspiration, water loss, and diffusion that occur in the agricultural field during watering enable the water level to progressively drop. The field's implanted tubes allow for the monitoring of water depths as low as 15 to 20 cm under the surface of the ground. Watering should be used in a crop to restore the groundwater level to an ideal depth of 5 cm after it falls 15 cm under the layer of soil.

### **Floodplain Area**

#### *Agronomic*

Over fifty percent of the country's overall rice acreage—roughly 12.0 million hectares when including the Aus, Aman, and Boro seasons—is dedicated to the cultivation of amaranth rice, the majority of which is produced in rainfed conditions. Aman rice is hence extremely vulnerable to flash floods and the monsoon. Five submergence-tolerant rice varieties, BRRI dhan51, BRRI dhan52, BRRI dhan79, BINA Dhan 11, and BINA Dhan 12, evolved and were encouraged by the Bangladesh Rice Research Institute (BRRI) and the Bangladesh Institute of Nuclear Agriculture (BINA). These rice cultivars are appropriate for cultivation during the Aman season (July–November).

#### *Floating agriculture*

In certain regions of Bangladesh, a distinct and ingenious farming technique called floating agriculture also referred to as hydroponic agriculture is employed to cultivate crops in regions often impacted by inundation or prolonged instances of water stagnation. This is carried out in Bangladesh's southern flooding areas, specifically in the

districts of Barishal, Gopalganj, and Pirojpur (Chowdhury & Moore, 2017; Islam et al., 2019c). Various kinds of crops, including cowpea coriander, ladies' finger, wax gourd, taro, amaranth, pumpkin, spinach, eggplant, hyacinth bean, and tomato, are grown in the floating beds, along with certain spices like ginger, chili, and turmeric (Haq et al., 2004). With only minimal chemical fertilization, output per unit of land could be increased by using this low-tech farming method (Pantanella et al., 2010; Sterrett, 2011). The efficiency of the floating agriculture technique is ten times higher than that of the traditional farming scheme, and it is not dependent on any outside fertilizer to operate (Haq et al., 2004). Because floating gardens facilitate the effective utilization of water hyacinth, which eventually contributes to the maintenance of water quality, reduces mosquito disease outbreaks, and opens up opportunities for fishing, they are ecologically safe (Saha, 2010). Another inherent advantage of floating agriculture includes the utilization of floating platforms as organic substances following crop harvest. Taking into account the average outlay and return, Pavel et al. (2014) calculated the expense and utility of thirty floating gardens. Their research showed that the floating bed sizes had an impact on the benefit-cost ratio (BCR) along with a net return (NR) of 0.26 years. Their analysis also revealed that the projected NR (USD 111.55) and BCR (USD 3.67) for standard (14 × 4 × 3 ft) beds are higher than those for all other dimensions of beds. The results of Abdullah et al. (2014) show that the bed areas of floating gardens and net return have a favourable correlation. Moreover, because this approach is labour-intensive, it also generates employment possibilities in rural regions, which reduces the migration of labour to urban regions. When men and women participate in these activities, gender equity is increased, women are empowered, the development of capabilities occurs, and there are greater social relationships between women (Chowdhury & Moore, 2017; Islam & Atkins, 2007).

## Conclusion

From the review, it is clear that environmental degradation as a result of climate change, is increasing day by day, making agriculture more susceptible. Erratic rainfall, frequent drought, sea level rising, frequency of floods, salt water intrusion, increasing frequency of cyclones, groundwater depletion, etc are the most drastic effects of climatic change in Bangladesh. This changing trend will be more dangerous in the future. It is expected that, within 2100, 17.5% of the area will be drawn under the sea which will make the livelihood more difficult through making the food security more insecure. Effective medium- and long-term adaptation strategies are crucial to reducing negative climate impacts and providing a viable path toward resilience. Useful adaptation technologies include the promotion of rice cultivation systems with better water and nutrient-use efficiencies, rescheduling irrigation and fertilizer application to suit changing conditions, training on interpretation of weather forecasts for effective farm decision-making, promotion of biofertilizers and mineral solubilizers for nutrient supplementation, highlighting the importance of blue-green algae in minimizing methane emission from paddy fields, and use of thermophilic bio-inoculants to sustain nutrient flow dynamics in warmer soils. Altering the time

of sowing or planting can help farmers regulate the length of the growing season to better suit climate change, as well as help the plants to avoid heat stress during critical growth stages. With climate change and variability increasing pressure on available water resources (and especially, net irrigation requirements), improved water management is one of the most important long-term adaptation options that countries must pursue. According to recent estimates, irrigation efficiency in developing countries is extremely low. Maximizing water-use efficiency can be done by retaining crop residue, reducing soil erosion, increasing soil water holding capacity with improved technology, and increasing infiltration with reduced surface runoff. Water-saving rice cultivation methods like aerobic rice and systems of rice intensification can be promoted as effective adaptation strategies in the future warmer climate.

It's a great hope that, the farmers of the country practicing different cropping strategies like sorjan practice, sack method, harvesting techniques of rainwater, floating agriculture, mulching etc to mitigate the effects. Besides these, the government has taken several strategies like, the 8th five-year plan of Bangladesh and climate change, the Climate Change Action Plan, National Environment Policy 2018, Ecologically Critical Areas (ECAs) Management Rules 2016, National Biodiversity Strategy and Action Plan (NBSAP) 2016-2021, Bangladesh Biological Diversity Act 2017, Biosafety Policy of Bangladesh Formulation of National Oil and Chemical Spill Contingency Plan (NOSCOP), Improved Management in Wildlife Conservation and Protection, Adoption of the Bangladesh Delta Plan 2100, etc to ensure food security through the crop protection against climatic change effect. Instead of adopting these strategies, Bangladesh is facing endless difficulties in coping with the adverse effect of global warming. In this situation, government institutions, and national and international NGOs should increase their work efficiently. Besides, these new technologies should be introduced against global warming to ensure the sustainable development of agriculture and the livelihood of farmers.

## Declarations

### Author Contribution

The conceptualization, methodology as well as the project administration together with supervision of the research were handled by Professor Dr. Md. Aminul Hoque. The data collection, investigation, formal analysis and writing of the original draft were undertaken Md. Mejbah Uddin after this. The manuscript was also reviewed and edited by Professor Dr Hoque.

### Conflict of Interest

The authors declare no conflict of interest.

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## Artificial Pollination and Fruit formation in Black Mulberries (*Morus nigra* L.)

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### ABSTRACT

The purpose of this study was to investigate the pollination and fertilization biology of black mulberry (*Morus nigra* L.), with a specific focus on understanding the effects of different pollination treatments on fruit formation and seed formation. Two experiments were designed to evaluate both dioecious and monoecious genotypes. In the first experiment, genotype 25 (dioecious female) was subjected to various artificial pollination treatments using pollen from two male genotypes (genotype 5 and genotype 28), as well as isolation treatments to observe parthenocarpic fruit formation. High fruit formation rates were recorded across all treatments, and no significant differences in fruit size or drupelet number were observed, regardless of the pollen source. The second experiment involved three monoecious genotypes (genotype 1, genotype 30, and genotype 31), where significant variations in fruit formation and size were observed, depending on the pollen source. This study highlights the potential for both fertilized and parthenocarpic fruit formation in black mulberry and underscores the importance of pollen source in determining fruit quality and seed formation.

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## Introduction

Black mulberry (*Morus nigra*) is a species that has spread across temperate and subtropical regions of the northern hemisphere, originating from Asia and the Caucasus, and has adapted well to various ecological conditions. It has long been utilized for its fruit, leaves, roots, and bark, all of which are noted for their medicinal properties (Datta, 2002). In recent years, the economic value of black mulberry fruits has increased due to their use in the food industry (e.g., cakes, confectionery, ice cream) and their rich nutritional content. Despite its increasing commercial importance, research on the pollination and fertilization biology of black mulberry remains scarce.

Research on black mulberry (*Morus nigra*) in Türkiye is not limited to fruit selection studies but spans various disciplines, including the effects of drought stress in vitro (Vijayan et al., 2014), propagation through tissue culture (Švagr et al., 2023), and preservation techniques such as genetic conservation through collection orchards (Uzun & Bayır, 2009). Recent studies have explored the genetic diversity, adaptability to different environmental conditions, and physiological responses of black mulberry under various stress factors, contributing to its agricultural and ecological importance (Gnanesh et al., 2023; Abbas & Rehmat, 2020). These multidisciplinary approaches provide a deeper understanding of *Morus nigra* and its potential for further use in breeding and conservation programs. In addition to the collection orchards of different mulberry species established in various parts of the world,

including India, a mulberry conservation orchard has also been established in Türkiye. Although Tokat hosts a significant population of black mulberry (*Morus nigra*), there are no established black mulberry orchards, and the exact number of trees and genotypes remains unknown. Studies are primarily focused on the preservation and improvement of local mulberry genotypes. Hybridization studies and conservation efforts are being conducted to enhance the genetic diversity, adaptability, and fruit quality of mulberry species (Das & Krishnaswami, 1965; Dwivedi et al., 1989; Tikader & Dandin, 2007).

In order to maximize the benefit from the existing genetic richness in Turkey, it is necessary to initiate large targeted breeding plans in which other breeding methods will be used in addition to selection studies. For this purpose, it is essential to examine the flower structure and fertilization biology of mulberries in detail. Black mulberry flowers are typically unisexual, with male and female flowers found on separate trees (dioecious) or occasionally on the same tree (monoecious) (Gnanesh et al., 2023). The first condition for seed and fruit formation is the formation of healthy male and female flowers and then successful fertilization. For successful fertilization, pollen emitted from the male organ must reach the style of the female flower, where it germinates, develops a pollen tube, and transfers the generative nucleus to the ovary (Janick & Moore, 1996; Thompson, 1996).



In addition to fertilization, black mulberry can also form fruits through parthenocarpy, where fruit develops without fertilization, resulting in seedless fruit. This phenomenon is important for ensuring fruit production under conditions where pollination may be unreliable. Parthenocarpy can be naturally induced or stimulated through specific hormonal changes, such as increased levels of auxin or gibberellin, which promote fruit growth in the absence of seed development (Griggs & Iwakiri, 1973). The occurrence and implications of parthenocarpy in black mulberry were further supported by Gustafson (1942), who extensively discussed natural and artificial parthenocarpy. Parthenocarpy in black mulberry thus provides a potential advantage for stable fruit yields, as it allows fruit to form even when pollinators are scarce.

Understanding parthenocarpy in black mulberry is crucial for improving fruit yield and quality, as well as for developing more efficient breeding programs. Studies in other fruit-bearing species have shown that parthenocarpy can improve fruit set and size, providing a means to mitigate the risks associated with fluctuating pollination conditions (Wilcock & Neiland, 2002; Young & Young, 1992). This study focuses on the reproductive biology of black mulberry, with an emphasis on artificial pollination and fruit formation in monoecious and dioecious genotypes, to enhance agricultural strategies and the breeding potential of black mulberry.

## Materials and Methods

In this study, due to the absence of dedicated black mulberry orchards in Tokat, artificial pollination was conducted using black mulberry trees located in individual orchards across the region. The female genotype (genotype 25) used in the experiment was characterized by a larger fruit size and an early harvest date, typically around. The hybridisation study did not alter the harvest date. Phenological observations, including bud burst, first flowering, and end of flowering, are provided in Table 9. The male genotypes (genotypes 5 and 28) were selected based on their floral characteristics, including the number of male flowers per inflorescence, as reported by Demirel and Yıldız (2021). Two different experiments were established for this purpose to study the effects of these genotypes on fruit formation and seed development.

### Experiment 1

In the first experiment, we selected one dioecious female tree (genotype 25) and used pollen from two dioecious male trees (genotype 5 and genotype 28) for pollination. The flowers on the dioecious female tree (genotype 25) were pollinated with pollen from these male trees to evaluate the effect of different pollen sources.

In this experiment, a single tree of this genotype was used, and 12 branches facing in different directions were selected from this tree. Three of these branches were randomly selected and pollinated with pollen from genotype 28, which produces only male flowers, and isolated so that no other pollen could be taken from outside. Three branches were pollinated with the pollen taken from genotype 5, which forms only male flowers, and isolated so that they do not receive any other foreign pollen from outside. Three randomly selected branches were left open

for open pollination. The remaining three branches were completely covered to observe whether parthenocarpic fruits were formed. The predetermined branches of black mulberries, selected for both parthenocarpy observations and pollination treatments, were isolated with specially made cloth bags according to the branch size, which provide air permeability but do not allow the passage of pollen and other particles

### Experiment 2

Three monoecious genotypes (genotype 1, genotype 30, genotype 31) were used as main plants. On the trees of these monoecious genotypes (genotype 1, genotype 30, genotype 31) and the male genotypes (genotypes 5 and genotypes 28) used for pollination, 12 branches from each genotype, facing different directions, were selected for the experiments. In each of these genotypes (genotype 1, genotype 30, and genotype 31), the flowers on 3 randomly selected branches were artificially pollinated with pollen taken from genotype 28 and were then isolated using specially designed cloth bags. These bags allowed air permeability but prevented any pollen from entering, ensuring no contamination from outside pollen. Three branches were artificially pollinated with pollen from genotype 5 and isolated so that they would not receive any other pollen from outside. Three branches were left open for open pollination. The remaining three branches were emasculated first and then covered to observe whether parthenocarpic fruits were produced or not. Since the identified trees were monoecious, i.e. male and female flowers were in different places on the same tree, special care was taken to ensure that there were no male flowers in the isolated branches.

Pollination was done twice, two days apart, to ensure that pollen reached all flowers in the inflorescence.

During the fruit ripening period, the number of fruits on each branch was calculated as a percentage of the initial number of flowers on that branch. Additionally, the fruit retention rate was expressed as the percentage of flowers that successfully developed into fruits.

Flowers (a total of 712) that were artificially pollinated with genotype 28 and genotype 5, left for open pollination, and isolated to prevent pollen exposure were examined, of which 667 successfully developed into fruits. This larger sample size ensures a robust and reliable basis for statistical analysis. For each treatment, three branches from each genotype were used as replicates. The branches were selected randomly from different directions on the trees to account for variability. Each branch was considered a replication, and the fruits were sampled randomly from these branches.

- The number of seeds was determined by manually extracting the seeds from each drupelet in the inflorescence. The seeds were then counted individually for each drupelet to ensure precise measurement.
- Number of drupelet (number): Total number of drupelet in a cluster
- Fruit dimensions: Including fruit width (mm), fruit length (mm), and fruit weight (g), were measured using a digital caliper for size measurements and a precision electronic balance for weight measurements.

### Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) to determine the significance of differences between treatments. A significance level of  $p < 0.05$  was used for all tests. The statistical analysis was performed using SAS software.

## Results

### Experiment 1

Table 1 shows the fruit formation rates obtained from artificial pollination studies where dioecious genotype 25 (producing only female flowers) was used as the main plant. As seen in the table, almost all pollinated flowers in every treatment developed into fruit, and there was no significant difference in fruit formation rates between the different pollen sources.

There was no significant effect of pollination type or pollen sources on fruit size. The average weight of the fruits taken from the branches pollinated with pollen of genotype 5 was 4.0 g, while the average fruit weight was 2.7 g in the branches that were closed to prevent foreign pollen entry (Table 2). The difference between these two values was not statistically significant. Similarly, it was determined that pollination types (pollination with pollen from genotype 5, genotype 28, free pollination, and isolated branches) did not have a significant effect on fruit size measured as fruit length and fruit width. The fruit width was between 15.2 and 17.0 mm, and the fruit length was between 22.6 and 20.4 mm, depending on the treatments. The number of drupelet (nucs) formed from each flower on an inflorescence (between 18.4 and 18.08) was similar in 4 different pollination treatments.

When the number of seeds formed as a result of fertilization was examined, it was determined that 15.3 and 15.4 seeds were formed in the flowers artificially pollinated with the pollen of genotype 5 and genotype 28, respectively. While this number was 4.3 in the flowers left to open pollination, it was determined that there were no seeds in the flowers that were isolated and not allowed to be pollinated. This result shows that black mulberry can form parthenocarpic fruit without fertilization (Table 2).

### Experiment 2

The results of different pollination treatments applied to the monoecious genotype 1 used as main plant are shown in Table 3. According to this; all of the flowers pollinated with genotype 28 turned into fruits. Out of 43 flowers pollinated with genotype 5, 24 of them turned into fruit and 55.8% fruit formation rate was obtained. Out of 45 flowers left to open pollination, 43 of them turned into fruit. Again, 98.8% of the flowers isolated to prevent flower dusting turned into fruit.

Some characteristics of fruits from monoecious genotype 1, pollinated using different methods (pollination with pollen from genotype 5, genotype 28, free pollination, and isolation to observe parthenocarpy), were showed in Table 4. It was determined that pollination with different genotypes (genotype 5 and genotype 28) caused significant changes in fruit weight. Flowers pollinated with the pollen of genotype 28 formed larger fruits compared to those pollinated with the pollen of genotype 5 and those pollinated with the pollen of genotype 28. The fruit weight in the treatment pollinated with pollen from genotype 5 was 2.33 g, whereas in the treatment pollinated with pollen from genotype 28, the fruit weight increased to 4.35 g. Similar situation was also observed in fruit size. Fruit width and fruit length were higher in fruits formed from flowers pollinated with genotype 28 pollen. The number of drupelets in a fruit varied between 15.4 and 21.3, but the differences were not statistically significant based on an ANOVA test ( $p > 0.05$ ), which was used to compare the effects of different pollination treatments on drupelet number.

The results of different pollination treatments applied to genotype 30 were showed in Table 5. As can be seen from the table, the fruit formation rate of the flowers pollinated with genotype 5, as well as the flowers left for free pollination and completely closed flowers, was over 95%, while the fruit formation rate of the flowers artificially pollinated with the pollen of genotype 28 was 64.3%.

The average fruit weights of the fruits from genotype 30 varied between 3.05 and 3.31 g according to the treatments (Table 6). Fruit width varied between 15.1 mm and 16.7 mm and fruit length between 20.05 and 21.7 mm. There was no statistically significant difference between pollination treatments in terms of both fruit weight and fruit size. When the number of seeds in a fruit was examined, it was determined that no seeds were formed in the covered flowers and the fruits formed were parthenocarp. Fewer seeds (4.3 seeds) were formed in the free pollination treatment compared to those artificially pollinated with the pollen of genotype 5 and genotype 28. In the closed flowers, no seeds were formed, indicating the occurrence of parthenocarpy, where fruit forms without fertilization. The lower seed count in the free pollination treatment may be attributed to lower pollen viability or reduced pollination efficiency. These findings suggest that while black mulberry can form parthenocarpic fruit, successful pollination significantly increases seed production, which in turn positively affects fruit size and quality. Thus, artificial pollination with selected genotypes can be a viable method to enhance fruit characteristics.

Table 1. Fruit formation rates of genotype 25 pollinated in different ways

Pollen Source	Number of Pollinated Flowers	Number of Flowers Turning into Fruit	Fruit formation rate (%)
Genotype 28	58	58	100 a
Genotype 5	51	51	100 a
Open pollination	42	41	97.6 a
Closed	44	43	97.7 a
LSD	ns	ns	ns

The difference between the averages indicated by the same letter is not significant ( $P < 0.05$ ).

Table 2. Effect of pollination method on the fruit characteristics of genotype 25

Pollen Source	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Drupelet Number (per fruit)	Seed Number (per fruit)
Genotype 5	4.0±0.5	17.0±0.7	22.6±0.6	18.4±1.6	15.3±0.5a
Genotype 28	4.0±0.8	16.8±1.2	22.5±1.7	19.6±2.3	15.4±2.8 a
Open pollination	3.0±0.2	15.8±0.6	20.4±1.1	18.3±1.6	4.3± 0.1b
Closed	2.7±0.3	15.2±0.7	20.4±0.8	18.5±0.8	0.0±0.0 c
LSD <sub>0.05</sub>	ns	ns	ns	ns	4.2

The difference between the averages indicated by the same letter is not significant ( $P>0.05$ )

Table 3. Fruit formation rates of genotype 1 pollinated in different ways

Pollen Source	Number of Pollinated Flowers	Number of Flowers Turning into Fruit	Fruit formation rate (%)
Genotype 28	43	43	100 a
Genotype 5	43	24	55.8 b
Open Pollination	45	43	95.5 a
Closed	89	88	98.9 a
LSD	ns	ns	ns

The difference between the averages indicated by the same letter is not significant ( $P>0.05$ ).

Table 4. Effect of pollination method on the fruit characteristics of genotype 1

Pollen Source	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Drupelet Number (per fruit)	Seed Number (per fruit)
Genotype 5	2.33±0.57 b	14.1±1.2 b	18.4±2.2 b	15.4±3.7	10.6±4.5 a
Genotype 28	4.35±0.40 a	17.5±0.2 a	24.1±0.9 a	21.3±1.6	17.7±1.7 a
Open pollination	2.74±0.49 b	15.0±0.5 ab	19.1±1.1 b	17.4±1.4	14.5±2.2 a
Closed	2.84±0.40 ab	15.1±0.8 ab	20.7±1.5 ab	17.6±1.9	0.0±0.0 b
LSD <sub>0.05</sub>	1.53	2.56	4.9	7.6	8.5

The difference between the averages indicated by the same letter is not significant ( $P<0.05$ ).

Table 5. Fruit formation rates of genotype 30 pollinated in different ways

Pollen Source	Number of Pollinated Flowers	Number of Flowers Turning into Fruit	Fruit formation rate (%)
Genotype 28	28	18	64.3 b
Genotype 5	49	47	95.9 a
Open pollination	45	43	95.5 a
Closed	48	46	95.8 a
LSD	ns	ns	ns

The difference between the averages indicated by the same letter is not significant ( $P<0.05$ ).

Table 6. Effect of pollination mode on fruit characteristics of genotype 30.

Pollen Source	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Drupelet Number (per fruit)	Seed Number (per fruit)
Genotype 5	3.13±0.18	16.6±0.3	20.5±0.4	15.9±0.6	12.3± 0.9 a
Genotype 28	3.13±0.42	15.1±2.0	21.0±2.2	18.3±2.2	14.7±2.3 a
Open pollination	3.31±0.24	16.7±0.2	21.7±0.2	16.7±0.5	4.3± 0.4 b
Closed	3.05±0.54	15.9±0.8	21.6±0.7	16.3±0.9	0.0±0.0 c
LSD <sub>0.05</sub>	1.2	2.2	3.8	4.0	4.1

The difference between the averages indicated by the same letter is not significant ( $P<0.05$ ).

Fruit formation rates of genotype 31 pollinated in different ways are given in Table 7. As seen in the table, 78.3% of the flowers pollinated with pollen from genotype 28 resulted in fruit formation, while all the flowers in the other treatments produced fruit.

The weight of the fruits from genotype 31 was between 2.44 g and 2.88 g, fruit length was 18.4 mm and 20.0 mm, and fruit width was between 14.6 mm and 15.8 mm (Table 8). The effect of pollination type on fruit size was found to be insignificant. In this genotype, the number of drupelet

in a fruit varied between 14.2 and 17.5, but the difference was found to be statistically insignificant. When the number of seeds, which is an indicator of successful fertilization, was examined, it was determined that more seeds were formed in artificial pollination treatment with genotype 5 and genotype 28 pollen compared to open pollination. Even though no pollination occurred, fruit still developed in these flowers, but no seeds were formed, indicating the occurrence of parthenocarpy.

Table 7. Fruit formation rates of genotype 31 pollinated in different ways

Pollen Source	Number of Pollinated Flowers	Number of Flowers Turning into Fruit	Fruit formation rate (%)
Genotype 28	23	18	78.32 b
Genotype 5	37	37	100 a
Open pollination	47	47	100 a
Closed	20	20	100 a
LSD	ns	ns	ns

The difference between the averages indicated by the same letter is not significant ( $P < 0.05$ ).

Table 8. Effect of pollination type on fruit characteristics of genotype 31.

Pollen Source	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Drupelet Number (per fruit)	Seed Number (per fruit)
Genotype 5	2.88±0.5	15.8±0.9	19.8±1.9	15.3±1.5	13.7±1.6 a
Genotype 28	2.45±0.3	14.7±1.0	19.7±1.3	17.5±1.1	15.0±1.0 a
Open pollination	2.49±0.3	15.3±0.3	20.0±0.6	16.1±0.9	6.5±1.8 b
Closed	2.44±0.2	14.6±0.3	18.4±1.2	14.2±1.4	0.0±0.0 c
LSD <sub>0.05</sub>	1.0	2.37	4.31	4.01	5.21

The difference between the averages indicated by the same letter is not significant ( $P < 0.05$ ).

Table 9. Some phenological observations of the genotypes

Genotype	Sex Expression	Bud Burst	First Flowering	End of Flowering
Genotype 1	Monoecious	13.04.2019	2.05.2019	30.06.2019
Genotype 5	Dioecious -Male	14.04.2019	5.05.2019	2.07.2019
Genotype 25	Dioecious -Female	13.04.2019	7.05.2019	5.07.2019
Genotype 28	Dioecious -Male	12.04.2019	8.05.2019	9.07.2019
Genotype 30	Monoecious	10.04.2020	5.05.2020	6.07.2020
Genotype 31	Monoecious	09.04.2020	8.05.2020	8.07.2020

## Discussion and Conclusion

In the experiment where dioecious female genotype 25 was used as the main plant, high fruit formation was observed in all pollination treatments. This result reveals that this genotype does not show incompatibility to foreign pollen and can also form parthenocarpic fruits. Cross-pollination has been reported to be common in black mulberry (Abbas & Rehmat, 2020), as well as in other mulberry species (Gnanesh et al., 2023).

When monoecious genotypes were used as main plants, significant differences in fruit formation were observed depending on the pollination method. The flowers of genotype 1 pollinated with pollen from genotype 5 showed lower fruit formation rates compared to the other pollination treatments. While there was a high rate of fruit formation in closed flowers with no pollen (parthenocarpy), the lower fruit formation rate in flowers pollinated with pollen from genotype 5 may be related to excessive pollen load. Although there are no studies on this subject with mulberry species, it has been reported that in some other plant species, excess pollen may prevent fruit formation depending on the incoming pollen source (Wilcock & Neiland, 2002; Young & Young, 1992). In the experiment where genotype 30 and genotype 31 were used as main plants, lower fruit formation was obtained from pollination with genotype 28 pollen. This shows that artificial pollination of mulberries may give different results depending on the pollen source.

The high rate of fruit formation in flowers that were closed to prevent pollen from entering clearly indicates parthenocarpic fruit formation in black mulberry, meaning that these fruits developed without fertilization. The absence of seeds in these fruits indicates the absence of fertilization. Griggs & Iwakiri (1973), in their artificial

pollination studies on *Morus rubra*, found that 89.3% of the fruits obtained from flowers pollinated through controlled pollination—where pollen from a specific source is manually applied to the flowers—contained seeds. In contrast, 87.5% of the flowers subjected to free pollination also produced fruits with seeds, while no seeds were formed in flowers that were isolated to prevent exposure to foreign pollen, indicating parthenocarpic fruit formation.

In addition to the positive effects of the varieties used as pollinators on fruit formation, their contribution to fruit quality parameters is also important. This condition, called metaxenia, is one of the main issues to be considered in the selection of suitable pollinators (Jahed, 2015). Metaxenia refers to the phenomenon where the pollen source directly influences the characteristics of the resulting fruit, such as size, weight, and shape, even though the pollen itself does not contribute genetically to the fruit's formation. In this study, the metaxenia effect was observed only in genotype 1, as the weights and sizes of the fruits varied significantly depending on the pollen source. Specifically, fruits pollinated with pollen from genotype 28 were larger and heavier compared to those pollinated with pollen from genotype 5, indicating a clear influence of the pollen source on fruit development.

There was significant variability in the number of flowers within each cluster that successfully fertilized and formed seeds, both in artificial pollination and open pollination conditions. It was thought that this may be due to the fact that not all of the flowers in an inflorescence are capable of fertilization or that the time of receptivity (able to accept pollen) is different.

It was determined that there was no incompatibility problem in terms of fertilization in the black mulberry trees in the population examined in the study, and that they can form fruit as a result of fertilization, but they can also form parthenocarpic fruit without fertilization. In artificial pollination studies, it was determined that the number of drupelets (individual fruitlets within a single fruit) varied significantly depending on the pollination method used. This indicates that different pollination techniques can influence the development of each flower in an inflorescence into individual drupelets, affecting the overall fruit structure and size. Although it was determined that the cluster can turn into fruit even if no flower is pollinated, it was revealed that the number of seeds increases the fruit size.

The findings of this study contribute to the understanding of reproductive biology in black mulberry, providing valuable information for future breeding and cultivation efforts. The identification of parthenocarpy in black mulberry could help inform breeding programs that aim to improve fruit yield, especially in areas where pollination is unreliable. Additionally, the observed metaxenia effect provides new insights into how pollen source can directly influence fruit characteristics, which could be useful for improving fruit quality in commercial cultivation.

## Declarations

### Author Contribution Statement

**Mehmet Akif DEMIREL:** Conducted the study, data collection, review, formal analysis, and writing the original draft.

**Kenan YILDIZ:** Management, supervision, review, editing, and statistical analysis of the study.

### Conflict of Interest

“We declare that the authors have no conflict of interest.”

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## Vermicompost Leach's Effect on Onion Seed Germination and Seedling Emergence in Response to Drought Stress

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### ABSTRACT

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#### Keywords:

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The Seed Technology Division, BARI, Gazipur, Bangladesh carried out a lab experiment in 2020–2021 to find the best vermicompost treatment for enhancing seed germination and seedling emergence in an environment of drought stress. With 4 replications, the experiment used a 2-factorial completely randomized design. Five different amounts of priming were applied to onion seeds: hydropriming, 5% vermicompost priming (VCP), 10% VCP, 15% VCP, and untreated control. In addition, the onion seedlings were treated with 10% and 15% PEG, two different levels of drought stress. BARI Paj-4 onion was the kind that was used. The findings demonstrated that, under drought-stressed situations, seed priming with vermicompost leach improved seed germination and seedling emergence percent. especially, the vermicompost leach at 10% under 10% PEG drought stress condition showed the best results in terms of promptness index (123), germination stress tolerance index (84.25%), seed germination (90%), seedling emergence percentage (81%), and seedling vigor index (614).

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## Introduction

Crops nowadays face numerous biotic and abiotic challenges, with threats from adverse environmental conditions being the most significant (Jisha et al., 2012; Muhie, 2018). In many countries of the world including Bangladesh, abiotic stresses have a major negative impact on crop production (Joshi and Sawant, 2012). Seed germination and seedling stage are the most critical stages of crop that are influenced by environmental stress. Among the critical environmental factors, drought badly affect seed germination (Lianes et al., 2015, Thirusendura and Saraswathy, 2017). Environmental factors such as drought (Ahmad et al., 2015) and temperature stress have a significant impact on the growth and development of plants, resulting in harmful effects on yield and production (Prasad et al., 2008; Shao et al., 2009), which in turn threatens the survival of plants and ultimately affects yield (Masondo 2017, 2018). These stresses lead to various physiological, biochemical, and molecular changes and reactions that affect different cellular processes (Prasad et al., 2008). Drought affects the rate of transpiration,

stomatal conductance, and relative water status of the leaf (Ullah et al., 2017). This poses a challenge to existing agronomic efforts aimed at addressing hunger, food insecurity, and malnutrition, particularly in countries where subsistence agriculture (Varshney et al., 2014) and commercial agriculture are the main means of livelihood. In agricultural terms, drought is defined as a physiological and edaphic condition that occurs when the available water for crops is insufficient for their transpiration requirements (Tuberosa 2012) and evaporation needs (Ilyas et al., 2021). Additionally, excessive irrigation on the farm is considered uneconomical and unsustainable because it leads to wastage of water, energy, and labor. Risks associated with excessive irrigation include waterlogging and leaching of nutrients, resulting in low crop yield (Ahmad et al., 2015).

Unfavorable soil moisture during sowing often leads to poor seed germination, resulting in inconsistent seedling emergence. This, in turn, impacts stand establishment, leading to reduced yield (Mwale et al., 2003; Okcu et al., 2005). Seed priming is used as a good technique for

improving seed germination and seedling performance under environmental stress condition (Joshi et al., 2012). In different vegetable crops, priming has been used successfully for improving seed quality (Ermis et al., 2016; Saranya et al., 2017). There are many priming agents used in improving seed germination, vermicompost is one of them. Vermicompost priming improved seed germination of *Brassica napus* seeds at salt stress conditions (Benazzouk et al., 2019). Onion (*Allium cepa* L.) is a spice crop and its seed lose viability and vigour at faster rates than seeds of most other crops, even at relatively optimum storage conditions (Ellis et al., 1996; Yapping et al., 2000) and germination of its seeds can be affected by extreme abiotic factors (Thirusendura and Saraswathy, 2017). Poor seed performance is one of the crucial factors that limit onion production and development. For enhancing seed germination and seedling emergence, vermicompost priming showed good result in onion (Muhie et al., 2020), in cucumbers (Edwards et al., 2006), marigolds (Shivsubramanian, 2004), lettuces, tomatoes and cucumbers (Edwards et al., 2006; Arancon et al., 2012), and beans and peas (Ievinsh et al., 2017). In Bangladesh there are 3.50 million hectares under drought condition and onion seed germination under drought stress is badly affected. Vermicompost priming can be a way of solving this problem. But literature about the influence of vermicompost priming enhancing seed germination and seedling emergence of onion under drought in Bangladesh is very sporadic. Therefore, the present study was undertaken to find out a suitable vermicompost leach treatment for better seed germination and seedling emergence under drought stress condition.

## Materials and Methods

This laboratory experiment was conducted during 2020-2021 at Seed Technology Division, BARI, Gazipur, Bangladesh, to investigate the most effective vermicompost treatment on seed germination and seedling emergence under water deficit condition. The experiment was carried out in a 2-factorial completely randomized design. The drought stimulator used was polyethylene glycol with a molecular weight of 6000 (PEG-6000). Drought was applied on onion seeds variety BARI Paj-4 with 10% and 15% PEG-6000 concentrations. A total of five priming levels were applied that included untreated control, 5% vermicompost priming (VCP), 10% VCP, 15% VCP and hydropriming. Onion seeds were subjected to drought stress, using vermicompost starter leach, following the protocols of Muhie et al., (2020).

Vermicompost priming (VCP) was done using a solid matrix priming method. Treatments were conducted by mixing onion seed: vermiculite No. 5: vermicompost leach (w:w:w) at 2:1:3, in plastic boxes. Boxes were kept at 15°C for 2 days in the dark. The same ratio was used with distilled water in hydropriming (HP). Non-primed (NP) dry seeds were used as a control for primed treatments. Seeds were dried near to initial moisture content after VCP and HP treatments. Drought stress was stimulated using polyethylene glycol-6000 (PEG) at different concentrations (W: V) at par treatment. Onion seeds (four replicates of 50 seeds) were placed on filter paper in 90 mm-diameter Petri dishes containing 3 ml of PEG solution.

The dishes were sealed with parafilm to prevent moisture loss. Germination was carried out at 20°C for 12 days according to ISTA (2017). Seeds were considered germinated when the radicle would emerge 2 mm.

The seedling emergence test was carried out in three replicates of 25 seeds each in plastic trays for 21 days. The appearance of the cotyledon above the coco dust was considered as the emergence criterion. Trays were kept at 20±2°C. Trays were watered with the appropriate solutions during the test. The Promptness index (PI) and germination stress tolerance index (GSI) were calculated using the following formulae given by Ashraf et al., (2006).

$$PI = nd_2(1.00) + nd_4(0.75) + nd_6(0.50) + nd_8(0.25),$$

n= is the number of seeds emerged at day d.

$$GSI(\%) = (PI \text{ of stressed seed} / PI \text{ of control seeds}) \times 100.$$

For measuring those indices, a sole non treated control treatment i.e., non-drought and non-primed treatment was conducted excluding 10 priming and drought stress treatment combinations. The seedling vigor index (SVI) was calculated by multiplying the average seedling length (cm) by seedling emergence percent according to Abdul Baki and Anderson (1973). The germination rate index (GRI) was calculated using the formula given by Al-Mudaris (1998).

$$GRI = G_1/1 + G_2/2 + \dots + G_i/I,$$

G1= is the germination percentage at day 1,

G2= is the germination percentage at day 2, and so on.

Recorded data were analyzed statistically with the help of Statistics 10 software. Treatment means were compared following the Least Significant Difference (LSD) Test and t-test.

## Results and Discussion

### Effect of Priming

Priming showed significant differences in seed germination percentage, seedling emergence percentage, average seedling length, seedling vigor index, and germination rate index of onion seed irrespective of drought stress (Table 1). Vermicompost priming had a positive impact on seed germination and increased the germination percentage of onion seed over non-primed and hydroprimed seed. Maximum seed germination (78%) was observed in 10% vermicompost priming and it was statistically similar with 5% vermicompost priming (74% seed germination). Non-primed seed showed minimum germination percentage (63%) which was 19.23% less seed germination compared to Vermicompost priming at 10% whereas hydropriming noted 12.82% less seed germination from that treatment. There was an increment of seed germination of onion at 5% VCP and 10% VCP, after that seed germination was decreased. The researcher said that at lower concentrations of VCP, seed germination increased due to increasing enzyme activities, but later on decreased due to the adverse effect of higher salt accumulation. A similar trend was observed in the case of

seedling emergence percentage, seedling length, seedling vigor index, and germination rate index. The highest seedling emergence percentage (71%) was noted in 10% VCP, and lowest (55%) in non-primed seed. Vermicompost priming at 10% improved by 29.09% seedling emergence over non primed seed, whereas hydropriming increased by 9.09% seedling emergence. Stimulatory effects of enhancing seed germination and seedling emergence have been mentioned in different crops such as cucumbers (Edwards et al., 2006), lettuces, tomatoes, and cucumbers (Edwards et al., 2006; Arancon et al., 2012, and beans and peas (Ievinsh et al., 2017). The positive effect of VCP enhancing germination and seedling emergence of onion seeds might be due to the high activity of enzymes (Arancon et al., 2012) in addition to the presence of an adequate concentration of macro-and micro-nutrients, growth-promoting hormones like auxin and gibberellins (Pant et al., 2009). The highest average seedling length (6.96 cm) was observed in 10% VCP treatment which was statistically at par with 15% VCP treatment (6.90 cm), but the lowest average seedling length (4.96 cm) was noted in nonprimed control treatment. Maximum seedling vigor index (497) was found in 10% VCP treatment and the minimum was in nonprimed control treatment (278). The highest germination rate index (19.28) was observed in 10% VCP treatment which was statistically at par with 5% VCP treatment (18.09), but the lowest GRI (14.96) was noted in nonprimed control treatment.

#### Effect of Drought Stress

Even with priming, all elements of onion seed germination and seedling growth were significantly impacted by dry stress in the experiment. The results indicated that at 10 PEG, drought stress increased seed germination (82%), seedling emergence (72%), average seedling length (6.80 cm), seedling vigor index (496), and germination rate index (20.07). On the other hand, when compared to 10% PEG, the usage of 15% PEG dramatically decreased the proportion of seeds that

germinated, the emergence of seedlings, the average length of seedlings, the seedling vigor index, and others. Muscolo et al. (2014) also observed that decreased root length, seedling tissue water content, and seed germination % were caused by water stress. The investigation also revealed that the germination process's enzyme activity declined, especially that of  $\alpha$ -amylase and  $\alpha$ -glucosidase, which were most adversely impacted by osmotic stress.

#### Interaction Effect

The Interaction effect of priming and drought stress was found nonsignificant on seed germination percentage, seedling emergence percentage, and germination rate index of onion, but found significant on average seedling length and seedling vigor index (Table 3). Though insignificant, seeds of onion treated with 10% vermicompost priming along with 10% polyethylene glycol gave the highest seed germination percentage (90%) and it was statistically similar with 5% vermicompost priming with 10% polyethylene glycol (86% seed germination). So, there was a great scope for increasing seed germination percentage by using vermicompost leach under drought-stress conditions. Non-primed seeds of onion treated with 15% PEG showed the lowest seed germination percentage (52%) and it was 42.22% lower compared to 10% vermicompost priming along with 10% polyethylene glycol. The highest seedling emergence percentage (81%) was noted in 10% VCP treatment under 10% PEG condition, and non-primed seeds of onion treated with 15% PEG showed the lowest seedling emergence percentage (46%) which was 43.21% lower compared to 10% vermicompost priming along with 10% polyethylene glycol. The longest average seedling length (7.58 cm) was observed in 10% VCP treatment under 10% PEG condition, and it was statistically similar (7.48 cm) with 15% VCP treatment under 10% PEG condition, and non-primed seeds of onion treated with 15% PEG showed the shortest average seedling length (4.15 cm). Similarly, the highest seedling vigor index (614) was noted in 10% VCP treatment under 10% PEG condition.

Table 1. Effect of priming on seed germination, seedling emergence, seedling length, seedling vigor index and germination rate index of onion

Priming	Seed germination (%)	Seedling emergence (%)	Seedling length (cm)	SVI	GRI
Non Priming	63	55	4.96	278	14.96
5% VCP	74	65	6.38	417	18.09
10% VCP	78	71	6.96	497	19.28
15% VCP	70	64	6.90	445	17.16
Hydropriming	68	60	5.75	349	16.64
LSD <sub>0.05</sub>	3.8	3.95	0.27	26.0	1.32
CV (%)	5.3	6.15	4.23	6.38	7.48

Where, VCP means vermicompost priming, SVI means seedling vigor index, GRI means germination rate index

Table 2. Effect of drought stress on seed germination, seedling emergence, seedling length, seedling vigor index and germination rate index of onion

Drought stress	Seed germination (%)	Seedling emergence (%)	Seedling length (cm)	SVI	GRI
10% PEG	82	72	6.80	496	20.07
15% PEG	59	53	5.59	298	14.38
t value	2.42	2.50	0.17	16.45	0.84
CV (%)	5.3	6.15	4.23	6.38	7.48

Where, PEG means polyethylene glycol 6000



Table 3. Interaction effect of priming and drought stress on seed germination, seedling emergence, seedling length, seedling vigor index and germination rate index of onion

Drought stress × Priming		Seed germination (%)	Seedling emergence (%)	Seedling length (cm)	SVI	GRI
10% PEG	Non Priming	74	63	5.58	366	18.05
	5% VCP	86	74	6.65	496	20.82
	10% VCP	90	81	7.58	614	22.84
	15% VCP	80	75	7.48	562	19.56
	Hydropriming	78	69	6.50	448	19.08
15% PEG	Non Priming	52	46	4.15	191	11.86
	5% VCP	62	56	6.10	342	15.36
	10% VCP	65	60	6.35	381	15.72
	15% VCP	60	52	6.33	329	14.76
	Hydropriming	58	50	5.00	250	14.21
LSD <sub>0.05</sub>		NS	5.59	0.38	36.78	1.87
CV (%)		5.3	6.15	4.23	6.38	7.48

Where, VCP means vermicompost priming, and PEG means polyethylene glycol 6000

Table 4. Effect of priming and drought stress treatments on promptness index and germination stress tolerance index of onion seed

Drought stress × Priming		Promptness Index	Germination Stress Tolerance Index (%)	Changes in GSI over control (%)
10% PEG	Non Priming	106	72.60	-27.40
	5% VCP	121	82.88	-17.12
	10% VCP	123	84.25	-15.75
	15% VCP	118	80.82	-19.18
	Hydropriming	106	72.60	-27.40
15% PEG	Non Priming	69	47.26	-52.74
	5% VCP	91	62.33	-37.67
	10% VCP	93	63.70	-36.30
	15% VCP	97	66.44	-33.56
	Hydropriming	85	58.22	-41.78
Control		146	100.00	0.00
LSD <sub>0.05</sub>		14.65		
CV (%)		9.70		

Non-primed seeds of onion treated with 15% PEG showed the lowest seedling vigor index (191) and it was 68.89% lower compared to 10% vermicompost priming along with 10% polyethylene glycol. The highest germination rate index (22.84) was noted in 10% VCP treatment under 10% PEG condition. Non-primed seeds of onion treated with 15% PEG showed the lowest germination rate index (11.86) which was 48.07% lower compared to 10% vermicompost priming along with 10% polyethylene glycol.

#### **Effect of Stress Treatments on Germination Stress Tolerance Index**

The promptness index of onion seeds was significantly influenced by priming and drought stress treatments, as shown in Table 4. The highest promptness index (146) was observed in the sole control treatment, which means there was no drought stress and no priming treatment. The promptness index decreased to 21.37% and 40.41% under 10% PEG and 15% PEG, respectively, compared to the sole control treatment. The lowest reduction of the promptness index (15.76%) was noted in the 10% VCP treatment under 10% PEG drought stress conditions. Similarly, the lowest reduction in the germination stress tolerance index (15.75%) was found in the 10% VCP

treatment under 10% PEG drought stress conditions. The reduction in the germination stress tolerance index was lower in 10% PEG compared to 15% PEG, in primed seeds compared to non-primed seeds, and within the priming treatments, 10% VCP was lower compared to hydropriming.

The greater amount of nutrients in vermicompost (VC) may improve germination compared to seeds that are only hydroprimed. This was particularly observed during the seedling emergence test. According to Ievinsh et al., (2017), the beneficial properties of vermicompost are due to the mineral nutrient forms available to plants. Vermicompost contains organic acids such as humic and fluvic acids (Shivsubramanian, 2004). Humic substances from vermicompost have hormone effects on plants (Arancon et al., 2012; Rajashekhar et al., 2017). The improvement obtained from the vermicompost-based primer (VCP) was greater for seed germination (%), seedling emergence (%), seedling length (cm), SVI and GRI. This was observed during both germination and emergence under stress conditions. This suggests that the VCP primer may help restore seed senescence (Burgass and Powell, 1984). In conclusion, vermicompost priming has the potential to improve germination and emergence of onion seed seedlings under abiotic stresses.

## Conclusion

In drought-stressed conditions, seed priming with vermicompost leach has a positive effect on increasing seed germination, seedling emergence percentage, length, seedling vigor index, and germination rate index while lowering less promptness index and germination stress tolerance index. When it came to seed germination (90), seedling emergence (81%), seedling vigor index (614), germination rate index (22.84), promptness index (123), and germination stress tolerance index (84.25%), seed priming with 10% vermicompost combined with 10% PEG performed better than any other treatment combinations. In terms of seed germination (52%), seedling emergence (46%), seedling vigor index (191), germination rate index (11.86), promptness index (69), and germination stress tolerance index (47.26%), non-priming seeds containing 15% PEG performed the worst.

## Declarations

### Competing Interest

Authors have declared that there is no conflicts of interest exist.

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## Boosting the Productivity of Bread Wheat (*Triticum aestivum* L.) Varieties through Optimal Seed Rates and Appropriate Systems for Irrigation Production System of Northwestern Ethiopia

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### ABSTRACT

The availability of high-yielding varieties adapted to diverse agro-ecologies and production systems, preferred by farmers and consumers, is the key factor limiting productivity. Farmers access seeds of different quality levels from various seed systems. Studies on seed systems and rates in relation to yield and yield traits of bread wheat varieties under irrigation are limited. Therefore, an experiment was conducted in 2021 in Northwestern Ethiopia to improve the productivity of bread wheat (*Triticum aestivum* L.) varieties through suitable seed rates and systems under irrigation. The experiment was conducted using a randomized complete block design in a factorial arrangement of two varieties (Kakaba and Ogolcho), three seed systems (formal, intermediate and informal), and three seed rates (125, 150, and 175 kg ha<sup>-1</sup>) in three replications. SAS software was used for analysis. The results showed that the seed system and variety interaction had a significant effect ( $P < 0.05$ ) on productive tiller numbers, days to 50% heading, kernel numbers per spike, and plant height. Additionally, the productive tiller numbers and days to 50% heading were significantly ( $P < 0.05$ ) influenced by the interaction of variety with seed rate. However, the main effects of seed system, seed rate, and variety alone had a significant ( $P < 0.05$ ) impact on physiological maturity, 1000-seed weight, kernel length, grain yield, biomass yield, and harvest index. The highest grain yields were obtained from the following main factors: seed system (intermediate (4.52 t ha<sup>-1</sup>); seed rate (150 kg ha<sup>-1</sup> (4.71 t ha<sup>-1</sup>)); and variety (Kakaba (4.47 t ha<sup>-1</sup>)), which exceeded the average yield in irrigation (4.4 t ha<sup>-1</sup>). It is necessary to conduct experiments involving a greater number of seed rates and varieties over multiple cropping seasons and locations and sowing dates to strengthen the results.

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## Introduction

In Ethiopia, wheat is one of the most vital cereal crops in terms of production and consumption. It significantly contributes to food security, poverty reduction, the supply of raw materials for industries, and the creation of employment opportunities. The national demand for wheat and wheat products is growing at an average rate of 9% per year (Khan et al., 2020), which is higher than the growth rate of local production (7.8%). This is partly due to high population growth, increased urbanization, and changing trends in food consumption patterns and preferences (Tolessa et al., 2019). To bridge the production gaps and meet these demands, Ethiopia has been importing an average of 1.5 million tons of wheat annually at a cost of 700 million dollars in the last five years (Wuletaw et al., 2022). Therefore, achieving self-sufficiency in wheat production is crucial for Ethiopia by enhancing its production and productivity.

Ethiopia is the second largest wheat producer in Africa after Egypt (USDA, 2024). The total production in Ethiopia is 5.2 million metric tons on 1.86 million hectares with a yield of 2.80 metric tons per hectare in the 2023/2024 production season, which is lower than other wheat-producing countries with average yields of 3.54 metric tons per hectare. World wheat production area and total production in the 2023/2024 season were 222.72 million hectares and 787.36 million metric tons, respectively (USDA, 2024). Low yields per hectare in Ethiopia are influenced by environmental factors affecting crop production like temperature, rainfall, wind, and light, which influence flowering, pollination, seed setting, ripening, and disease infection. Inadequate crop management practices such as planting, fertilization, irrigation, and weed control also contribute to low yields. Factors like inappropriate fertilizer and seed rates, lack of

quality seeds for high-yielding varieties adapted to different agro-ecologies, and the use of poor-quality farm-saved seeds are key reasons for low productivity (Yohannes & Mulugeta, 2022). However, using quality seeds from known sources, adopting irrigation and mechanization (Mihratu et al., 2018), and expanding land (EIAR, 2020) are factors that can enhance wheat production and productivity.

Bread wheat (*Triticum aestivum L.*) is a staple crop in Ethiopia, and enhancing its yield and quality is crucial to meet the food requirements of the growing population. The seeding rate is a critical agronomic management practice that significantly affects the growth, yield, and yield components of bread wheat, ultimately influencing crop productivity (Iqbal et al., 2020). High seeding rates can lead to low yields and poor-quality crops due to increased competition for limited resources such as water, nutrients, and sunlight (Jemal et al., 2015). Optimizing the seed rate can help increase production potential, improve crop establishment, and counteract soil fertility decline. This optimization is crucial for ensuring food security, sustaining agricultural productivity, and addressing soil degradation challenges in Ethiopia (Kelemu et al., 2024). It also promotes nutrient availability, sunlight penetration for photosynthesis, appropriate soil conditions for nutrient uptake, and water use efficiency, all crucial for crop vigor, increased yield, and productivity (Amare & Mulatu, 2017).

In Ethiopia, several bread wheat varieties with varying seed size, height, tillering capacity, and maturity have been developed, along with recommended seed rates. However, farmers in the region often use inappropriate seed rates, ranging from 100 kg ha<sup>-1</sup> to 288 kg ha<sup>-1</sup>, for both small and large-sized bread wheat varieties (Dawit, 2012). Identifying the best-performing and well-adapted bread wheat varieties, along with implementing proper agronomic management practices such as seed rate, is essential for boosting productivity. Varieties and seed rates significantly affect the phenology, growth, yield, and yield-related traits of bread wheat (Shigedib & Bitew, 2023).

Farmers access seeds through different seed systems, including formal, informal, and intermediate systems (Dawit et al., 2017). The formal seed system in Ethiopia is mainly government-supported, involving public and private sector institutions (ATA, 2015; Djen, 2021). The informal system enables farmers to select, reproduce, store, utilize, and distribute seeds through social networks and local marketplaces. The intermediate system comprises seed-producing cooperatives (SPCs) and local seed businesses (ATA, 2015). Government offices, development projects, universities, research institutes, ATA, and NGOs are partners that support these systems. The choice of bread wheat varieties, seed sources, and seed rates significantly affects phenology, growth, yield, and yield components (Alemayehu & Mohammed, 2019). Seed systems, both intermediate and informal, have a significant influence on the phenology of barley (Abraha et al., 2020).

Ethiopia has significant potential for irrigated water availability, but only 1.1% of cultivated land is under irrigation (Girmay, 2018). Wheat yield under irrigation in Ethiopia averages 4.4 tons per ha, lower than the attainable yield of 6.5 tons per ha (EIAR, 2020). This is mainly due to crop management practices for irrigation systems being

based on recommendations for rain-fed systems. The scarcity of high-quality certified seeds from reliable sources also affects bread wheat yield. To enhance wheat yield in irrigated systems, it is essential to optimize agronomic practices such as seeding rate and utilize improved varieties from reliable sources. The Ethiopian government is prioritizing the expansion of wheat production in lowland irrigated areas to attain self-sufficiency and enhance productivity in rain-fed agro-ecologies (Tolessa et al., 2019). Understanding the impact of different seeding rates and seed systems on bread wheat growth and yield can provide valuable insights for farmers, researchers, and policymakers. However, many farmers lack information on how inappropriate seed rates and unreliable seed systems affect the productivity of bread wheat varieties in irrigation systems. We hypothesize that high-quality certified seeds and optimal seed rates can significantly influence the quality and productivity of bread wheat varieties. This research aims to investigate the effect of seed systems and seed rates on the phenology, growth, yield, and yield traits of bread wheat varieties. The study also aims to determine the optimum seed rates for optimizing productivity in irrigation production systems in North Western Ethiopia.

## Materials and Methods

### *Description of the Study Area*

The research was conducted at Koga Irrigation Schemes, Ambomesk Kebele, in North Western Ethiopia during the 2020/2021 irrigation production season. It is located between 12° 20' and 12° 31' N latitude and 37° 02' and 37° 08' E longitude, with an elevation ranging from 1807 to 2300 meters above sea level (masl). It has a unimodal rainfall distribution pattern, and the total annual rainfall is 3043.9 mm. The rain usually starts in May and gradually increases in frequency, reaching its peak values in July or August (Desale et al., 2021). The highest and lowest recorded temperatures were 28.01°C and 10.57°C, respectively. The predominant soil type is red nitosol (Likawunt et al., 2012), and the primary production system is crop-livestock mixed farming, where livestock provides the draught power needed for the farming operation. The Koga irrigation dam has a capacity to retain 83 million cubic meters of water on 1,800 ha of land and is designed to irrigate 7,200 ha of land. Wheat, maize, and vegetables are grown under irrigated conditions (Desale et al., 2021).

### *Experimental Materials*

Two bread wheat varieties, Kakaba and Ogolcho, were used for the experiment. Kakaba was released in 2012, and Ogolcho was released in 2010 by the Kulumsa Agricultural Research Center, Ethiopian Institute of Agricultural Research. Kakaba is adapted to lowland to highland areas, while Ogolcho is adapted to lowland to midland areas. The seeds for all varieties were collected from the 2019/2020 harvest.

### *Experimental Treatment and Design*

The experiment consisted of three seed systems (formal, intermediate, and informal), three seed rates (125, 150, and 175 kg ha<sup>-1</sup>), and two bread wheat varieties. It was conducted in a Randomized Complete Block Design (RCBD) in a 3×3×2 factorial arrangement with three

replications, resulting in eighteen treatment combinations to demonstrate the main and interaction effects of seed systems, rates, and bread wheat varieties. Each treatment combination was randomly applied to fixed experimental units. The gross and net harvestable plot areas were 6 m<sup>2</sup> (3 m x 2 m) and 4.16 m<sup>2</sup> (2.6 m x 1.6 m), respectively. The experiment was planted with 10 rows in 0.2 m row spacing. Data collection was done from the middle rows, leaving the outermost two rows on both sides uncollected.

#### Experiment Management and Procedures

The experimental field was plowed and prepared using a tractor, oxen, and hand tools. It was then manually subdivided into blocks and plots based on the design and treatments. Sowing took place on January 8, 2020, by hand drilling in rows and lightly covering the seeds at a depth of 3-5 cm in the soil. All plots were uniformly fertilized with 100 kg ha<sup>-1</sup> NPS (19% N, 38%P, and 7% S) at sowing and 75 kg urea (46% N) at sowing and 75 kg urea at full tillering. Weeding was done manually as needed, and irrigation water was applied at eight-day intervals using the furrow irrigation method, a common practice in Koga irrigation schemes (Schmitter et al., 2017).

#### Data Analysis Methods

Analyzing data was used by SAS software version 9.0. Data normality was tested using Levene's test before subjecting it to ANOVA. Three way ANOVA was used to determine the interaction and main effect of seed system, seed rate and variety. Mean comparisons between treatments were done using Duncan's Multiple Range Test at a 5% level of significance.

## Results and Discussion

#### Days to 50% Heading

The main effects of seed rate ( $P < 0.0001$ ), seed system ( $P < 0.0001$ ), variety ( $P < 0.0001$ ), the seed system with variety interaction ( $P = 0.0087$ ), and variety with seed rate interaction ( $P = 0.0139$ ) had significant ( $P < 0.05$ ) effects on days to 50% heading. Nevertheless, other potential interactions were not statistically significant ( $P > 0.05$ ). The Ogolcho variety with a seed rate of 125 kg ha<sup>-1</sup> showed delayed days to 50% heading (71.44 days), whereas the Kakaba variety with a seed rate of 175 kg ha<sup>-1</sup> exhibited earliness (58.11 days) (Table 1). The Ogolcho variety with the informal system interaction also showed delayed days to 50% heading (71.44 days), while the Kakaba variety

with the formal system interaction exhibited earliness (58.78 days) (Table 3). These early days to 50% heading may be attributed to variations in genetic makeup, seed size and weight, and increased competition for resources at higher seed rates (175 kg ha<sup>-1</sup>), prompting a shift in phenology from the vegetative to the reproductive stage. This has the advantage of escaping terminal moisture stress and adapting well to moisture variability in the growing area. Therefore, varieties with different seed systems and rates that show early heading days may have an advantage in regions where terminal moisture stress is prevalent. These delays in reaching 50% heading may be attributed to reduced plant competition resulting from lower seed rates. In agreement with this, (Abraha et al., 2020) revealed that seeds obtained from the intermediate system (seed-producing cooperatives) and the informal system (farmer-saved seeds) had a significant effect on the days to heading of food barley. This finding is consistent with (Amare & Mulatu, 2017) reported that the Tay bread wheat variety showed a delay in days to 50% heading (73.67 days) when interacted with a seed rate of 100 kg ha<sup>-1</sup>, while the Dinkinesh variety at a seed rate of 150 kg ha<sup>-1</sup> exhibited early heading (50 days). The findings presented in this paper demonstrate that as the seed rate increased from 125 to 175 kg ha<sup>-1</sup>, the days to 50% heading decreased, and vice versa. Similarly, (Kelemu et al., 2024) stated that in bread wheat, as the seed rate increased from 100 to 200 kg ha<sup>-1</sup>, the days to 50% heading decreased from 63.08 to 60.68. This study is also supported by (Wolde et al., 2024), who revealed that as the tef seeding rate increased from 2.5 to 12.5 kg ha<sup>-1</sup>, the days to 50% heading decreased from 56.1 to 51.41, and vice versa.

#### Days to Reach 90% Physiological Maturity

The main effects of seed system ( $P = 0.0005$ ), seed rate ( $P < 0.0001$ ), and variety ( $P < 0.0001$ ) had a highly significant impact on reaching 90% physiological maturity ( $P < 0.05$ ). However, all possible interactions had a non-significant effect on it ( $P > 0.05$ ). The informal system delayed days to 90% physiological maturity (106.89 days), followed by the intermediate system (105.11 days), and earliness was observed in the formal system (104.67 days). The seed rate of 125 kg ha<sup>-1</sup> resulted in delayed days to reach 90% physiological maturity (107.78 days), while the seed rate of 175 kg ha<sup>-1</sup> led to the earliest maturity (103.50 days). Additionally, the Ogolcho variety took longer to reach 90% physiological maturity (109.48 days), while Kakaba had a shorter maturity period (101.63 days) (Table 2).

Table 1. Interaction of variety with seed rate on productive tiller numbers and days to 50% heading

Variety	Seed rate (kg ha <sup>-1</sup> )	Parameters	
		Productive tiller numbers	50% heading days
Kakaba	125	109.67 <sup>a</sup>	63.11 <sup>c</sup>
	150	95.33 <sup>b</sup>	60.78 <sup>d</sup>
	175	84.11 <sup>c</sup>	58.11 <sup>e</sup>
Ogolcho	125	82.78 <sup>c</sup>	71.44 <sup>a</sup>
	150	74.44 <sup>d</sup>	70.11 <sup>a</sup>
	175	65.44 <sup>e</sup>	68.22 <sup>b</sup>
P value (0.05)		0.0347	0.0139
LSD (0.05)		6.5423	1.4607
CV (%)		8.09	2.36

Means with the same letter in the columns were not significantly different at 5%. SL- significance level, CV- coefficient of variation

Table 2. The effect of seed system, variety and rate on physiological maturity (PM), spike length (SL) and thousand kernels weight (TKW) of bread wheat

<i>Factors</i>	<i>Parameters</i>		
Seed system	PM	SL(cm)	TSW (gm)
Formal	104.67 <sup>b</sup>	8.16 <sup>b</sup>	34.94 <sup>b</sup>
Intermediate	105.11 <sup>b</sup>	9.08 <sup>a</sup>	36.18 <sup>a</sup>
Informal	106.89 <sup>a</sup>	7.89 <sup>b</sup>	34.12 <sup>b</sup>
P value (0.05)	< 0.0001	0.0181	0.0006
Seed rate kg ha <sup>-1</sup>			
125	107.78 <sup>a</sup>	9.59 <sup>a</sup>	37.49 <sup>a</sup>
150	105.39 <sup>b</sup>	8.13 <sup>b</sup>	34.96 <sup>b</sup>
175	103.50 <sup>c</sup>	7.40 <sup>b</sup>	32.79 <sup>c</sup>
P value (0.05)	0.0005	<0.0001	<0.0001
Variety			
Kakaba	101.63 <sup>b</sup>	8.84 <sup>a</sup>	36.63 <sup>a</sup>
Ogolcho	109.48 <sup>a</sup>	7.91 <sup>b</sup>	33.53 <sup>b</sup>
P value (0.05)	< 0.0001	0.0099	< 0.0001
LSD (0.05)	1.0885	0.844	0.9853
CV (%)	1.53	14.91	4.20

Means with the same letters in the columns are not significantly different, CV- coefficient of variation

Table 3. Interaction of seed system and variety on productive tiller numbers (PTN), days to 50% heading (HD), plant height (PH) and number of seeds per spike (NSPS)

<i>Interaction</i>		<i>Parameters</i>			
Variety × seed system		PTN	HD	PH	NSPS
Kakaba	Formal	95.56 <sup>ab</sup>	58.78 <sup>d</sup>	82.70 <sup>c</sup>	50.33 <sup>b</sup>
	Intermediate	104.89 <sup>a</sup>	61.00 <sup>c</sup>	83.72 <sup>c</sup>	57.89 <sup>a</sup>
	Informal	88.67 <sup>b</sup>	62.22 <sup>c</sup>	84.13 <sup>c</sup>	44.56 <sup>c</sup>
Ogolcho	Formal	74.89 <sup>c</sup>	69.00 <sup>b</sup>	92.61 <sup>b</sup>	42.44 <sup>c</sup>
	Intermediate	77.89 <sup>c</sup>	69.33 <sup>b</sup>	93.91 <sup>ab</sup>	45.56 <sup>c</sup>
	Informal	69.89 <sup>c</sup>	71.44 <sup>a</sup>	96.20 <sup>a</sup>	40.56 <sup>c</sup>
P value (0.05)		3.79	5.42	4.11	3.35
LSD (0.05)		9.7755	1.87	3.05	4.67
CV (%)		12.09	3.03	3.62	10.55

Means with the same letter in the columns are not significantly different. SL- significance level, CV- coefficient of variation

The early days leading to maturity may be attributed to physiological maturity, which happens when the kernel has accumulated its highest dry matter content, lost its water content, changed color from green to yellowish, and exhibited genetic makeup variability. This early emergence could also be attributed to an increased plant population, leading to plants utilizing growth resources more rapidly through intra-specific competition, which results in earlier maturation. Bread wheat sourced from the informal system (farmer-saved) matured later, while wheat grown from seeds sourced from the formal system (Haramaya University Wheat Research Program) matured earlier (Alemayehu & Mohammed, 2019). This is in agreement with (Bereket, 2022), who revealed that different food barley varieties significantly influenced the days to physiological maturity. Shedho (3381-01) variety took the longest to reach maturity (115.67 days), while the HB 1307 variety matured faster (99.44 days). This study also aligns with (Anbessie et al., 2020 & Birhanu et al., 2020), who reported that the days to reach 90% physiological maturity of bread wheat were significantly influenced by varieties. Increasing the seeding rate from 125-175 kg ha<sup>-1</sup> decreased the number of days to reach 90% physiological maturity of bread wheat, and vice versa (Table 2). This may be due to a higher seed rate contributing the grain-filling period reduction because at a higher seed rate heading and

maturity were hastened compared to a lower seed rate (Alemayehu & Mohammed, 2019). Furthermore, (Kelemu et al., 2024) reported that the number of days to reach physiological maturity was significantly influenced by the seed rate. They noted that as the seed rate increased from 100 to 200 kg ha<sup>-1</sup>, the days to physiological maturity decreased from 110.75 to 107.33, and vice versa. The studies are also supported by (Wolde et al., 2024), who revealed that the seed rate had a significant effect on tef physiological maturity. They reported that as the seed rate increased from 2.5 to 12.5 kg ha<sup>-1</sup>, the days to physiological maturity decreased from 86.75 to 82.92, and vice versa.

#### **Plant Height**

The main effects of seed system ( $P < 0.0001$ ), seed rate ( $P < 0.0001$ ), variety ( $P < 0.0001$ ), and the interaction between variety and seed system ( $P = 0.0248$ ) had a significant impact on plant height. However, other potential interactions had a non-significant effect ( $P > 0.05$ ). The informal system with the Ogolcho variety interaction had the highest plant height (96.20 cm), while the formal system with the Kakaba variety interaction had the shortest (82.7 cm) (Table 3). This might be associated with varietal genetic makeup and species differences. This is in accordance with the findings of (Alemayehu & Mohammed, 2019), who revealed that the tallest plants

were observed in the intermediate seed system (Kersa Local Seed Business Program) of the Digalu bread wheat variety. The shortest plant height was obtained from the formal seed system (Haramaya University Wheat Research Program) of the Qulqull variety. Correspondingly, (Chaluma, 2023) reported that soybean height was significantly influenced by varieties, with the highest height obtained from AFGAT (76.22 cm) and the lowest from Davis (38.32 cm).

### **Spike Length**

The main effects of seed rate ( $P < 0.0001$ ), variety ( $P = 0.0099$ ), and seed system ( $P = 0.0181$ ) significantly influenced ( $P < 0.05$ ) spike length. However, not all possible interaction effects significantly affected spike length. The intermediate system (9.08 cm) had the longest spike length, followed by the formal system (8.16 cm), while the informal system had the shortest (7.89 cm). A seed rate of 125 kg ha<sup>-1</sup> produced the longest spike length (9.59 cm), followed by 150 kg ha<sup>-1</sup> seed rate (8.13 cm), and the shortest from a 175 kg ha<sup>-1</sup> seed rate (7.4 cm). Moreover, the Kakaba variety yielded the highest spike length (8.84 cm), while the Ogolcho variety had the lowest spike length (7.91 cm) (Table 2). The shorter spike length could be attributed to variations in seed quality used for sowing, differences in plant height, genetic makeup variations among varieties, and reduced spacing between plants at higher seed rates (175 kg ha<sup>-1</sup>), leading to shorter spike length and taller plants. Plant height and spike length have an inverse relationship as reported by (Zewdie et al. 2014), shorter plants produce longer spike lengths, while longer plants produce shorter spikes and higher biomass production. This is in agreement with (Khan et al., 2015), who reported that the formal system (Bangladesh Agricultural Research Institute) had the longest spike length (17.5 cm), while the informal system (farmer-saved seed) had the shortest (15.6 cm). Increasing the seed rate of bread wheat from 125 to 175 kg ha<sup>-1</sup> resulted in a decrease in spike length, and vice versa (Table 2). The spike length of bread wheat decreased as the seed rate increased from 100 to 150 kg ha<sup>-1</sup>, and vice versa (Abiot, 2017; Amare & Mulatu, 2017; Bereket, 2022). Furthermore, (Kelemu et al., 2014) revealed that the seed rate had a significant effect on bread wheat spike length. They reported that as the seed rate increased from 100 to 200 kg ha<sup>-1</sup>, spike length decreased from 7.87 to 7.45 cm, and vice versa. In this study, different bread wheat varieties exhibited varying responses to spike length. This is also in agreement with (Bereket, 2022), who reported that the HB 1307 food barley variety produced the longest spike length (8.933 cm), while the Shagee variety produced the shortest spike length (7.289 cm). Similarly, (Abraha et al., 2020) revealed that the variety of food barley significantly influenced spike length. They found that the highest spike length was obtained from Fetina (7.33 cm), while the lowest was from Felamit (6.38 cm).

### **Productive Tiller Numbers**

The main effects of seed system ( $P < 0.0001$ ), seed rate ( $P < 0.0001$ ), and variety ( $P < 0.0001$ ), as well as the interaction between seed system and variety ( $P = 0.0322$ ) and variety and seed rate ( $P = 0.0347$ ), significantly influenced ( $P < 0.05$ ) the number of productive tillers.

However, other potential interactions were not statistically significant ( $P > 0.05$ ). The Kakaba variety with a seed rate of 125 kg ha<sup>-1</sup> had the highest number of productive tillers (109.66), while the lowest number was observed for Ogolcho with a seed rate of 175 kg ha<sup>-1</sup> (65.44) (Table 1). In addition, the intermediate system with the Kakaba variety interaction had the highest productive tiller number (104.89), whereas the lowest was obtained from the informal system with the Ogolcho variety (69.89) (Table 3). The variability in productive tiller production per unit area may be attributed to genetic makeup variation, population density, and spacing. These factors allow plants to utilize more water, light, air, and nutrients, leading to increased photosynthetic activity and ultimately influencing the number of effective tillers. As the seed rate decreased from 175 to 125 kg ha<sup>-1</sup>, the number of productive tillers increased, and vice versa. This might be because a higher seed rate produces many tillers, but it may not result in a high number of productive tillers per unit area. This could be due to competition among tillers for growth factors, leading to the production of fewer productive tillers per unit area. Similarly, (Wolde et al., 2024) revealed that as the tef seeding rate increased from 2.5 to 12.5 kg ha<sup>-1</sup>, effective tillers per plant decreased from 7.16 to 4.00, and vice versa. Similar to the current study, bread wheat varieties sown at lower seed rates (Wondimu et al., 2022) and food barley varieties planted at lower seed rates (Bereket, 2022) exhibited a higher number of productive tillers, and vice versa. Similarly, Alemayehu & Mohammed (2019) reported that the highest productive tiller number of bread wheat was recorded in the intermediate system (Kersa Local Seed Business Program) for the Quluquluu variety (54.56), while the lowest was recorded in the informal system (farmer-saved seed) for the Digalu variety (45.56).

### **Number of Kernels per Spike**

The main effects of seed system ( $P < 0.0001$ ), variety ( $P < 0.0001$ ), seed rate ( $P = 0.0028$ ), and the interaction between seed system and variety ( $P = 0.0463$ ) had a significant impact ( $P < 0.05$ ) on the number of kernels per spike. Other potential interactions did not have a significant effect ( $P > 0.05$ ) on the number of kernels per spike. The intermediate system of the Kakaba variety produced the most kernels per spike (57.89), whereas the informal system of the Ogolcho variety produced the fewest kernels per spike (40.56) (Table 3). The low number of kernels per spike in farmer seeds of the Ogolcho variety may be attributed to its poor quality, limited adaptability, and inadequate management practices during its growth and development. The high number of kernels per spike in the intermediate system of Kakaba may be attributed to the adaptability of the variety to the study area, genetic composition of the variety, high-quality seeds such as thousand seed weight, and other physiological quality parameters including spike length. Similarly, (Alemayehu & Mohammed, 2019) reported that the interaction between seed system and variety had a significant effect on the number of kernels per spike. The Qulqulluu variety seed from the intermediate system (Kersa Local Seed Business Project) yielded the maximum number of kernels per spike at 68.24. Seeds of the Digalu variety from the informal system (farmers saved) yielded the minimum number of kernels per spike (53.31).



Table 4. The effect of seed system, seed rate and varieties on grain yield, biomass yield and harvest index of bread wheat

<i>Factors</i>	<i>Parameters</i>		
Seed system	Grain yield (t ha <sup>-1</sup> )	Biomass yield (t ha <sup>-1</sup> )	HI (%)
P value (0.05)	0.0493	0.0461	<0.0001
Formal	4.31 <sup>ab</sup>	11.87 <sup>b</sup>	36.35 <sup>a</sup>
Intermediate	4.52 <sup>a</sup>	12.32 <sup>ab</sup>	36.74 <sup>a</sup>
Informal	4.21 <sup>b</sup>	12.59 <sup>a</sup>	33.42 <sup>b</sup>
Seed rate kg ha <sup>-1</sup>	P < 0.0001	P < 0.0001	P < 0.0001
125	3.90 <sup>c</sup>	11.15 <sup>c</sup>	35.01 <sup>b</sup>
150	4.71 <sup>a</sup>	12.46 <sup>b</sup>	37.89 <sup>a</sup>
175	4.43 <sup>b</sup>	13.17 <sup>a</sup>	33.62 <sup>c</sup>
Variety			
Kakaba	4.47 <sup>a</sup>	12.021.70 <sup>b</sup>	37.20 <sup>a</sup>
Ogolcho	4.22 <sup>b</sup>	12.491.60 <sup>a</sup>	33.81 <sup>b</sup>
P value (0.05)	0.0194	0.0489	< 0.0001
LSD (0.05)	251.41	572.72	0.8517
CV (%)	8.56	6.91	3.55

Means with the same letters in the column are not significant. HI (%) - harvest index (%), CV- coefficient of variation.

### Thousand Kernel Weight (TKW)

The main effects of seed rate ( $P < 0.0001$ ), seed system ( $P = 0.0006$ ), and variety ( $P < 0.0001$ ) had a highly significant impact ( $P < 0.05$ ) on TKW. However, the remaining potential interactions did not significantly affect TKW ( $P > 0.05$ ). The highest thousand kernel weight (TKW) mean was obtained from the intermediate system (36.18 g), followed by the formal system (34.94 g), and the lowest from the informal system (34.12 g). The highest thousand kernel weight (TKW) was also obtained from the lowest seed rate of 125 kg ha<sup>-1</sup> (37.49 g), followed by 150 kg ha<sup>-1</sup> a seed rate (34.96 g), and the lowest from 175 kg ha<sup>-1</sup> seed rate (32.79 g). Additionally, Kakaba (36.63 g) had the greatest thousand kernel weight (TKW) compared to Ogolcho (33.53 g) (Table 2). The higher thousand kernel weight (TKW) record might be due to the high adaptation to agro-ecology, high-quality seeds, genetic variability of the variety, and longer spike length. Moreover, at a low seed rate, there were fewer plants per unit area. This allowed plants to utilize more light and nutrients from the available space, resulting in the highest thousand kernel weight (TKW) at a reduced seed rate. This might lead to adequate grain filling. Lower seed rates (125 kg ha<sup>-1</sup>) increase the availability of photosynthetic matter and facilitate the transformation of photosynthetic matter into grains, resulting in an increase in thousand kernel weight (TKW). The lowest thousand kernel weight (TKW) produced from the highest seed rate (175 kg ha<sup>-1</sup>) might be due to the higher seed rate resulting in a greater number of spikes per row, which in turn leads to fewer kernels per spike and smaller-sized kernels. This is likely caused by inter-plant competition for limited soil resources. Insufficient photosynthetic matter during the grain filling stage at the higher seed rate (175 kg ha<sup>-1</sup>) could be a contributing factor to the decrease in thousand kernel weight (TKW) due to intense competition. Increasing the seeding rate of bread wheat significantly decreased thousand kernels weight (TKW), and vice versa (Amare & Mulatu, 2017; Eldey et al., 2019; Kelemu et al., 2024). Similarly, (Wolde et al., 2024) reported that the tef seed rate significantly affected the thousand kernel weight (TKW). They observed that as the seed rate increased from 2.5 to 12.5 kg ha<sup>-1</sup>, TKW decreased from 0.37 to 0.32 grams, and vice versa. Similar to the current findings,

(Amare & Mulatu, 2017) and (Anbessie et al. (2020) affirmed that different varieties of bread wheat significantly influenced the thousand seed weight. Food barley varieties had a significant effect on TKW (Bereket, 2022). Similar to these findings, (Khan et al., 2015) reported that the formal system (Bangladesh Agricultural Research Institute) had the highest thousand-grain weight (42.3 g), while the informal system (farmer seed) had the lowest (37.9 g).

### Grain Yield

The main effects of seed rate ( $P < 0.0001$ ), seed system ( $P = 0.0493$ ), and variety ( $P = 0.0194$ ) significantly influenced grain yield ( $P < 0.05$ ). However, other possible interaction effects did not significantly affect it ( $P > 0.05$ ). The intermediate system had the highest grain yield (4.52 t ha<sup>-1</sup>), followed by the formal system (4.31 t ha<sup>-1</sup>), and the informal system had the lowest yield (4.21 t ha<sup>-1</sup>). A seed rate of 150 kg ha<sup>-1</sup> resulted in the highest grain yield (4.71 t ha<sup>-1</sup>), followed by 175 kg ha<sup>-1</sup> (4.43 t ha<sup>-1</sup>), while 125 kg ha<sup>-1</sup> had the lowest yield (3.89 t ha<sup>-1</sup>). Additionally, the Kakaba variety had a higher grain yield (4.47 t ha<sup>-1</sup>) compared to Ogolcho, which yielded 4.22 t ha<sup>-1</sup>) (Table 4). This result indicated a higher grain yield than the average yield (4.4 t ha<sup>-1</sup>) under irrigation in Ethiopia. The highest grain yield might be due to good adaptation to agro-ecology, high seed quality, genetic variability, longer spike length, increased productive tiller number, thousand kernel weight (TKW), and number of kernels per spike. Furthermore, the higher number of primary spikes per unit area reversed the effect of an increase in grain yield per spike obtained at decreased sowing density. This finding is consistent with (Khan et al., 2015), who reported that the highest grain yield was obtained from the formal system (Bangladesh Agricultural Research Institute) (3.5 t ha<sup>-1</sup>), whereas the minimum was from informal system (farmer seed) (2.4 t ha<sup>-1</sup>). Moreover, the highest grain yield was obtained from the intermediate system (Kersa Local Seed Business Project) (5.65 t ha<sup>-1</sup>), while the lowest grain yield was from informal farmers (4.59 t ha<sup>-1</sup>) (Alemayehu & Mohammed, 2019). The current finding is in agreement with (Bereket, 2022) revealing that the highest grain yield was obtained from the HB 1307 food barley variety (4.42 t ha<sup>-1</sup>) while the lowest yield was from Shagee (2.8 t ha<sup>-1</sup>).

This study describes that as the seed rate increased from 125 to 150 kg ha<sup>-1</sup>, grain yield also increased and then decreased (Table 4). This finding is consistent with (Kelemu et al. (2024), who reported that a seed rate of 150 kg ha<sup>-1</sup> for bread wheat resulted in the highest grain yield (3125 kg ha<sup>-1</sup>), whereas 100 kg ha<sup>-1</sup> led to the lowest yield (2674.3 kg ha<sup>-1</sup>). This finding is consistent with the study by Betwoded et al. (2022), which also reported that the seed rate of bread wheat significantly influenced its yield. The study recorded the highest yield of 4.4 t ha<sup>-1</sup> from a seed rate of 150 kg ha<sup>-1</sup>. As the seed rate increased beyond 150 kg ha<sup>-1</sup>, bread wheat grain yield decreased.

#### **Biomass Yield**

The main effects of seed rate ( $P < 0.0001$ ), seed system ( $P = 0.0461$ ), and variety ( $P = 0.0489$ ) had a significant impact on biomass yield ( $P < 0.05$ ). However, other possible interaction effects did not significantly affect it ( $P > 0.05$ ). The informal system had the highest biomass yield (12.59 t ha<sup>-1</sup>), followed by the intermediate system (12.32 t ha<sup>-1</sup>), and the formal system had the lowest yield (11.86 t ha<sup>-1</sup>). A seed rate of 175 kg ha<sup>-1</sup> resulted in the highest biomass yield (13.17 t ha<sup>-1</sup>), whereas 125 kg ha<sup>-1</sup> led to the lowest yield (11.15 t ha<sup>-1</sup>). Additionally, the Kakaba variety had the lowest biomass yield (12.02 t ha<sup>-1</sup>), while Ogolcho had the highest (12.49 t ha<sup>-1</sup>) (Table 4). The highest biomass yield recorded might be due to the plant's height, genetic makeup variability, a greater number of primary stems, and an increase in straw yield per hectare as the seed rate increased. Biomass yield and plant height had a direct association; thus, taller plants resulted in higher biomass yield (Zewdie et al., 2014). Wheat seed sources had a significant effect on aboveground dry biomass yield (Alemayehu & Mohammed, 2019, and Khan et al., 2015). Similarly, (Alemayehu & Mohammed, 2019, Birhanu, 2021), and Nwry et al., 2021) reported that as the seed rate increased, bread wheat biomass yield also increased, and vice versa. Food barley varieties (Bereket, 2022) and malt barley varieties (Senait et al., 2020) had a significant effect on biomass yield.

#### **Harvest Index Percentage**

The main effects of seed rate ( $P < 0.0001$ ), variety ( $P < 0.0001$ ), and seed system ( $P < 0.0001$ ) had a very highly significant effect on the harvest index. However, other possible interaction effects had a non-significant effect on it ( $P > 0.05$ ). The intermediate system (36.74%) and formal system (36.35%) had the highest harvest index, while the informal system had the lowest (33.42%). A seed rate of 150 kg ha<sup>-1</sup> had the highest harvest index (37.89%), followed by 125 kg ha<sup>-1</sup> (35.01%), and 175 kg ha<sup>-1</sup> had the lowest (33.62%). Additionally, the Kakaba variety had the highest harvest index (37.20%), followed by Ogolcho (33.81%) (Table 4). The highest harvest index percentage might be due to higher grain yield, lower biomass yield and plant height, and population density, and an increase in biological yield accompanied by an increase in grain yield at a higher seed rate. This indicates that the harvest index had an interrelationship with grain yield and biomass yield. The harvest index was significantly affected by the seed source of bread wheat (Alemayehu & Mohammed, 2019; Khan et al., 2015). Seed rate had a significant effect on the harvest index by recording the highest yield from 150 kg ha<sup>-1</sup>. Different authors reported the highest harvest index obtained at high

plant population densities. Bread wheat seed rate (Abiot, 2017; Anbessie et al., 2020), and maize plant density (Ion et al., 2015) had a significant effect on the harvest index. Similarly, several researchers stated that the harvest index significantly influenced by bread wheat varieties. Bread wheat varieties had a significant effect on the harvest index (Jemal et al., 2015; Nwry et al., 2021).

#### **Conclusions and Recommendations**

The field experiment showed that seed systems, varieties, and seed rates of bread wheat significantly influenced grain yield and yield traits. The informal seed system had lower grain yield compared to formal and intermediate seed systems. Similarly, Ogolcho bread wheat varieties also had lower grain yield than Kakaba. As the seed rate increased from 125-175 kg ha<sup>-1</sup>, thousand kernel weights, spike length, and the number of kernels per spike significantly decreased. Meanwhile, plant height and biomass yield significantly increased as the seed rate increased from 125-175 kg ha<sup>-1</sup>. Even though grain yield was significantly affected by the main effects of seed rate, seed system, and variety, the highest grain yield was recorded from the intermediate system (4.52 t ha<sup>-1</sup>), Kakaba variety (4.47 t ha<sup>-1</sup>), and 150 kg ha (4.71 t ha<sup>-1</sup>), which are higher than the average bread wheat grain yield in irrigation production systems (4.4 t ha<sup>-1</sup>). Therefore, bread wheat producers and farmers benefit from using seeds from reliable sources, well-adapted varieties, and proper agronomic practices. These experiments were conducted based on some varieties and seed rates at one location and growing season. However, further studies are necessary to conduct experiments considering the number of seed rates and varieties at multiple locations of major bread wheat growing areas for multiple cropping season and year to make a conclusive recommendation for bread wheat producers and farmers. Studies on other bread wheat agronomic management practices such as sowing date and depth, row spacing, nutrient requirements, irrigation water requirement, irrigation scheduling, wheat irrigation critical stages, and partial budget analysis under irrigation are also recommended.

#### **Declarations**

##### **Competing Interest**

The author declares that they have no competing interest.

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## Effects of Dietary Fermented Mealworm Larvae and Stocking Density on the Morphometric Characteristics and Mineral Contents of Tibia Bone of Broilers

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### ABSTRACT

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This study aimed to investigate the effects of the supplementation of defatted mealworm larvae meal fermented with probiotics to the diet of broilers reared under normal stocking density (NSD) and high stocking density (HSD) on the morphometric characteristics and mineral contents of tibia bone of broilers. A total of 450 one-d-old Ross 308 male broiler chicks were randomly distributed into six groups of similar mean weight, each containing five replicates. The experimental treatments were arranged as a 2 × 3 factorial design, incorporating two levels of stocking density (12 birds/m<sup>2</sup>, designated as NSD, and 18 birds/m<sup>2</sup>, designated as HSD) and three different diets in mash form: CONT- a corn-soybean meal-based diet containing no fermented defatted mealworm larvae meal (FDM) (0%); FDMLP- the diet obtained by supplementing defatted mealworm larvae meal (DM) fermented with *Lactobacillus plantarum* to the CONT diet (0.4%); FDMLB- the diet obtained by supplementing DM fermented with *Lactobacillus brevis* to the CONT diet (0.4%). HSD significantly aggravated the morphometric parameters (weight, length, weight/length index, diameter of diaphysis and medullary canal, tibiotarsal index and breaking strength) and decreased mineralization (ash, Ca and P contents) of the tibia of broilers, whereas the FDMLP and FDMLB diets improved tibia mineralization and morphology except its medullary canal diameter and ribusticity index of broilers due to the results of enhanced mineral absorption. In conclusion, the use of FDMLP and FDMLB as new antibacterial feed additives in broiler diets regardless of stocking density was able to improve tibia mineralization and morphology except its medullary canal diameter and ribusticity index of broilers due to the results of enhanced mineral absorption.

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## Introduction

The demand for animal protein for the adequate and balanced nutrition of the rapidly increasing World and Turkey population is constantly increasing. In this respect, the broiler meat production is one of the major agricultural industries that considered as the main source of animal protein all over the World (Altaf et al., 2019). In recent decades, broiler producers are obliged to rear broilers under high stocking density (HSD) to decrease the production costs, produce more kilograms of broilers per unit area for a cheap, safe supply of meat and increase profitability (Khalil et al., 2021). However, HSD impairs broiler welfare especially during the later growing period when the body weight of broilers per m<sup>2</sup> is increased (Zhang et al., 2018). One of the main welfare concerns of

HSD is the restriction in their movements and activities since broilers are reared only in a limited space (Buijs et al., 2012). This lack of exercise leads to development of bone weakness and leg deformation in broilers, as shown by the increased tibial dysplasia (Li et al., 2019) and decreased tibia breaking strength (Sun et al., 2018). HSD also negatively influences microflora and histomorphological structure of the small intestine of broilers (Kridtayopas et al., 2019; Sugiharto, 2022). As it is known, Ca and P are particularly significant for the development and maintenance of skeletal structure, as they are the essential macrominerals of bone tissue of avian organisms (Jabbar et al., 2024). In addition, vitamin D exerts beneficial effects on bone metabolism primarily by

stimulating the absorption of Ca and P absorption in the intestine and promoting their reabsorption in the kidneys, and regulating the bone formation (Zhang et al., 2021). However, HSD reduces the intestinal absorption and bioavailability of minerals such as Ca and P and vitamin D<sub>3</sub> that are necessary for bone especially tibia mineralization (Liu et al., 2020), and induces leg disorders in broilers (Li et al., 2019; Sun et al., 2018; Yan, 2016). Consequently, the increasing leg deformation of broilers reared under HSD increases the susceptibility to fracture during catching and transportation, which causes problems during processing and serious economic losses in the current broiler meat industry (Liu et al., 2020). It is estimated that the poultry industry incurs an annual economic loss of 0.016\$ per broiler in US due to leg disorders (Cook, 2000).

Therefore, maximization of absorption and bioavailability of minerals such as Ca and P from the small intestine by the dietary supplementation of antimicrobial feed additives can be practical for preventing the leg disorders of broilers under HSD (Mohammed et al., 2021; Steczny & Kokoszyński, 2020). In this context, the dietary supplementation of antibiotic growth promoters (AGPs) as antibacterial feed additive to enhance their performance by improving the microbiota and histomorphological structure of the small intestine of broilers under HSD has been investigated in previous times (Hooge et al., 2003). Unfortunately, the use of AGPs for long periods in broiler diets has caused to the appearance of AGPs resistance and residual AGPs in broiler meat, which are harmful to human health and cause increasing public concern (Aslam et al., 2021). The use of AGPs in broiler diets was banned by the European Union in 2006 (Sugiharto, 2022). Likewise, the use of certain substances in animal diets has been prohibited following various amendments, culminating in the enactment of Law No. 25847 on June 16, 2005, Law No. 26056 on January 21, 2006, and Law No. 26511 on May 3, 2007 in Türkiye. These laws establish a complete ban on the use of growth-promoting antibiotics and hormones in all animal diets within Türkiye (Tuncer, 2007). The above-mentioned reasons has increased interest in the use of natural antibacterial feed additives such as probiotics, prebiotics and synbiotics etc. in diets of broilers reared under HSD (Sugiharto, 2022).

Although there are few studies that dietary probiotic or prebiotic supplementation did not influence histomorphological characteristics (Gutierrez-Fuentes et al., 2013; Javid et al., 2022; Mohammed et al., 2021; Steczny & Kokoszyński, 2020) of tibia in broilers, many studies have shown that the dietary supplementation of the natural antibacterial feed additives such as probiotics, prebiotics or synbiotics increased ash, calcium (Ca) and phosphorus (P) contents (Gutierrez-Fuentes et al., 2013; Ortiz et al., 2009; Ziaie et al., 2011) of tibia of broilers reared under normal stocking density.

In this context, dried mealworm larvae meal solid-state fermented with probiotics has been evaluated as a new antibacterial feed additive in broiler diet in a previous study (Islam & Yang, 2017). Compared with animal-derived feed ingredients, insects as a novel feed ingredient have advantages with their ability to convert organic residues into protein more efficiently, need less space and water, the lower environmental impact and high nutritional values

(Lee et al., 2022; Sedgh-Gooya et al., 2022). Presently, insects such as mealworm larvae (M) are not only considered as a nutrient-rich feedstuff (Kwon et al., 2020) but also as an antibacterial feed additive due to their presence of antimicrobial peptides (AMPs) (Benzertiha et al., 2020; Elahi et al., 2022) and chitin (Islam & Yang, 2017; Gasco et al., 2018) for poultry nutrition. It is reported that the chitin amount of M is 4.30-8.91% (Hong et al., 2020). Consequently, chitin in M is partially degraded by the acidic chitinase in the proventriculus and gizzard of chicken to produce chitooligosaccharides, a prebiotic (Sedgh-Gooya et al., 2022) and thereby as a potential antibacterial feed additive for broilers or laying hens (Borrelli et al., 2017). However, the high chitin levels (>2.42 %) in M may impose negative effects on feed intake and protein availability and thereby worsen growth performance in broilers (Mulyono et al., 2019). As a result, both reducing the high chitin content and revealing the antimicrobial components of insects such M and black soldier fly larvae can be performed by solid-state fermentation (SSF) using specific microorganisms that are able to degrade chitin (Mulyono et al., 2019; Hadj Saadoun et al., 2020; Luparelli et al., 2022). Solid-state fermentation (SSF) is a form of microbial fermentation that takes place in conditions with minimal to no free water, since it simulates the natural environment of the selected microorganisms naturally adapted (Peng et al., 2022). Among probiotic bacteria species, Lactic acid bacteria (LAB) is the most used species for M solid-state fermentation (Islam & Yang, 2017). The SSF highlights the using possibility of the solid-state fermented M as an antibacterial feed additive for broilers (Islam & Yang, 2017; Hadj Saadoun et al., 2020; Luparelli et al., 2022).

To our knowledge, there is only a one-week study that was conducted to investigate whether M and super M meal solid-state fermented with only *Lactobacillus plantarum* could be used as antibacterial feed additives in broilers challenged with pathogen bacteria such as *Salmonella* and *Escherichia coli* (Islam & Yang, 2017). Unlike the above study, in the present research, we hypothesized that dietary supplementation of defatted M (DM) subjected to SSF with two different probiotics (*Lactobacillus plantarum* and *Lactobacillus brevis*) with chitinase activity as a new antibacterial feed additive could alleviate the detrimental effects of HSDs on tibia morphometric parameters in broilers by improving the tibia bone mineralization due to their positive effects on microflora and histomorphological structure of the small intestine.

Therefore, the current experiment aimed to compare the effects of the addition of DM solid-state fermented with two different probiotics with chitinase activity as a new antibacterial feed additive to diet of broilers reared under normal- and high-stocking density on the morphometric characteristics and the ash, Ca and P contents of the tibia bone.

## Materials and Methods

### The Location of The Experiment

The experiment was conducted at the broiler chicken research unit within the Tokat Gaziosmanpaşa University Agricultural Application and Research Center Directorate in Tokat, Türkiye.

**Animal Care, Experimental Design and Diets**

On the day of hatching, a total of 450 Ross 308 healthy male broiler chicks, aged one-d, were acquired from a commercial hatchery in Türkiye. During the 6 wk experiment, the broiler chicks were kept on floor pens. Each floor pen was with a 10 cm-thick layer of fresh wood shavings. The temperature of the experimental room was maintained at 32°C for the first wk and gradually reduced and remained at 21°C. A lighting schedule of 23L:1D was employed during overall experimental period. From hatching to 42 days of age, the experimental diets and drinking water were offered *ad libitum*. The present experiment was approved from the Animal Care and Use Committee of Tokat Gaziosmanpasa University, under process number 2019-HADYEK-47.

Experimental treatments consisted of a 2 x 3 factorial arrangement with two levels of stocking density (12 birds/m<sup>2</sup> as normal stocking density (NSD) and 18 birds/m<sup>2</sup> as high stocking density (HSD) (Kridtayopas et al., 2019) and three different mash diets: CONT- a corn-soybean meal-based diet containing no fermented defatted mealworm larvae meal (FDM) (0%); FDMLP- the diet obtained by supplementing DM fermented with *Lactobacillus plantarum* to the CONT diet (0.4%); FDMLB- the diet obtained by supplementing defatted mealworm larvae meal (DM) fermented with *Lactobacillus brevis* to the CONT diet (0.4%). Each treatment comprised 5 replicates. Prior to experimental diet formulation, feed ingredients and DM, FDMLP and FDMLB were analyzed

for their dry matter, crude protein (CP), ether extract, crude ash, starch, total sugar, calcium (Ca) and phosphorus (P) contents according to the methods of the AOAC (2007) at Ankara Food Control Laboratory (Ankara, Türkiye). All diets were formulated by taking into account the analyzed contents of the feed ingredients in accordance with phase feeding practices as the broiler chickens advanced in age and weight, aligned with the guidelines provided by the breeder (Ross 308, 2007). The feeding regimen was structured into three distinct phases: the starter phase, which extended from day 0 to day 10; the grower phase, which occurred from day 11 to day 28; and the finisher phase was from day 29 to day 42. Ingredient composition and nutrition content of the control diet are showed in Table 1.

90-d-old mealworm larvae (M) (*Tenebrio molitor* L.) purchased from a commercial supplier and were freeze-dried. And then the freeze-dried M was ground into the meal using a miller. The M meal obtained was full-fat and produced from the larval stage of yellow meal worms. The crude protein content of M meal in the present study was increased by the chemical defatting process since protein may be utilized as substrates by microorganisms for SSF (Son et al., 2021). The freeze-dried M meal was defatted with petroleum ether using a soxhlet extractor under optimized conditions and then dried. Consequently, the fat content of DM was reduced from 23% to 6.6%, while its crude protein content increased from 44% to 76.2%.

Table 1. Ingredient composition and nutrient content of the control diet (g/100 g, as-fed basis)

Item	Days		
	0-10	11-28	29-42
<b>Ingredients</b>			
Corn	57.30	58.99	64.00
Soybean Meal (44.8 % CP)	34.86	31.49	28.39
Fish Meal (65 % CP)	1.51	2.65	-
Vegetable Oil	1.92	3.35	3.82
Dicalcium Phosphate	2.20	1.85	2.10
Limestone	0.87	0.78	0.80
Salt	0.34	0.32	0.36
Vitamin Premix <sup>1</sup>	0.25	0.25	0.25
Trace Mineral Premix <sup>2</sup>	0.10	0.10	0.10
DL-Methionine	0.36	0.22	0.18
L-Lysine	0.22	-	-
L-Threonine	0.07	-	-
<b>Calculated nutrient content</b>			
Dry Matter	90.10	90.10	90.10
Crude Protein	23.00	22.00	19.00
ME (MJ/kg)	12.66	13.19	13.40
Ca	1.00	0.90	0.90
P available	0.50	0.45	0.45
Methionine+Cystine	1.09	0.94	0.80
Lysine	1.44	1.23	1.01
Na	0.16	0.16	0.16
Tryptophan	0.30	0.29	0.25
Threonine	0.93	0.84	0.72

<sup>1</sup>Vitamin premix/kg diet: 12 000 IU vitamin A; 1 500 IU vitamin D<sub>3</sub>; 50 mg vitamin E; 5 mg vitamin K<sub>3</sub>; 3 mg vitamin B<sub>1</sub>; 6 mg vitamin B<sub>2</sub>; 5 mg vitamin B<sub>6</sub>; 0.03 mg vitamin B<sub>12</sub>; 25 mg niacin; 12 mg Ca-D-pantothenate; 1 mg folic acid; 0.05 mg D-biotin; 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride; <sup>2</sup>Trace Mineral Premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se

Table 2. Nutrient composition and concentrations of microorganisms in DM, FDMLP and FDMLB

Item	DM	FDMLP	FDMLB
Microorganisms' concentrations (log <sub>10</sub> cfu/g)			
Total Mesophilic Aerobic Bacteria	Not detected	3.92	4.29
<i>Lactobacillus</i>	Not detected	2.99	2.45
Yeast-Mold	Not detected	Not detected	Not detected
Nutrient Composition			
Dry Matter, %	95.70	92.00	89.81
Crude Protein, %	76.20	49.28	49.06
Crude Fat, %	6.60	8.14	9.40
Crude Ash, %	7.30	7.81	7.83
Starch, %	3.30	3.73	1.44
Total Sugar, %	0.50	0.36	0.36
Metabolisable Energy, Kcal/kg (for poultry)	3515	2650	2660

Two probiotic bacteria (*Lactobacillus plantarum* and *Lactobacillus brevis*) (Leisner et al. 2008; Islam & Yang, 2017) and *Saccharomyces cerevisiae* (baker's yeast) were used in the SSF of DM with probiotics. *Lactobacillus plantarum* strain and *Lactobacillus brevis* strain were isolated from Çeçil cheese and cheddar cheese, respectively. *Saccharomyces cerevisiae* (baker's yeast) was produced from sugar beet molasses. The probiotics with chitinase activity used in the fermentation were purchased from Neslihan Dikbaş Microorganism Culture Collection at the Agricultural Biotechnology Laboratories of Ataturk University. Chitinase enzyme activity of the purchased probiotics was analyzed in the Agricultural Biotechnology Laboratory of Ataturk University according to the method of Senol et al. (2014). According to the results of the analysis, the chitinase enzyme activities in terms of ammonium sulfate precipitation level were 15.00 U/L and 11.36 U/L for *Lactobacillus plantarum* and *Lactobacillus brevis*, respectively. *Saccharomyces cerevisiae* (baker's yeast) was commercially supplied.

The fermentation of DM with two different probiotic bacteria was carried out by modifying the method of Islam & Yang (2017) in Semi-Solid Phase Fermenter (Infors-HT, Labfors AG, Bottmingen, Switzerland) in the laboratory of Isparta University of Applied Sciences, Agricultural Faculty, Department of Animal Science. Prior to fermentation, DM, distiller's dried grains with solubles (DDGS), defatted rice bran, and water were sterilized by autoclaving at 121°C for 15 min. In the first stage of the fermentation, DDGS (35%), defatted rice bran (35%), DM (30%) and distilled water (80%) were put into the fermenter and then carbon dioxide was added to create an anaerobic environment inside the fermenter. First, 100 ml of incubated *Lactobacillus plantarum* was supplemented to the solid substrate medium in the fermenter and fermented at 38°C for 48 h under anaerobic conditions. After an initial 48-hour fermentation period, a secondary fermentation was conducted using 1.0% *Saccharomyces cerevisiae* (baker's yeast), which had been activated for 1 hour at 37°C in 250 ml of 0.1% peptone water (consisting of 10 g yeast and 90 ml peptone water) at 38°C for an additional 48 hours under anaerobic conditions.

*Saccharomyces cerevisiae* during fermentation enhances the viability and growth of lactic acid bacteria (LAB), since it provides some nutrients, such as amino acids and vitamins to LAB (Menezes et al., 2018; Shi et al.,

2020). Upon completion of the total 96-h fermentation process, the fermented product was dried to a moisture content of less than 15% at 32°C for 24 h using a drying oven. The same fermentation procedure was performed with *Lactobacillus brevis*. To determine microbial concentration, 1 gram of FDMLP or FDMLB was serially diluted in 9 ml of 0.85% sterile saline and thoroughly mixed. Total mesophilic aerobic bacteria counts were determined by plating serial 10-fold dilutions in triplicate onto Plate Count Agar (PCA) (Merck, Darmstadt, Germany), followed by incubation at 30°C for 48 h under aerobic conditions. Lactic acid bacteria (LAB) were enumerated by plating serial 10-fold dilutions in triplicate onto De Man, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany) and incubating at 39-40°C for 5 d under anaerobic conditions. Yeast and mold counts were assessed by plating serial 10-fold dilutions in triplicate onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Merck, Darmstadt, Germany) and incubating at 25°C for 5 d under anaerobic conditions. After incubation, microbial colonies were immediately counted and expressed as log<sub>10</sub> CFU/g. Nutrient composition and concentrations of microorganisms in DM, FDMLP and FDMLB are shown in Table 2. The amount of protein linked to acid detergent fiber (ADF) was determined (AOAC, 2007) and used to estimate the chitin contents of DM, FDMLP and FDMLB (Finke, 2007). The chitin contents of DM, FDMLP and FDMLB were found as 4.20%, 2.74% and 2.81%, respectively.

#### Measurements of The Tibia Morphometric Parameters

At the end of the experiment, the diets were withdrawn 6 h ago prior to slaughter (Xue et al., 2021). After 6 h feed withdrawal, two male broilers, representing the average body weights of each group, from each replicate, were selected (10 male broilers per treatment group) and slaughtered to measure of the tibia morphometric parameters on day 42. The individual left tibias were removed from a total of 60 broilers, labelled and frozen (-20°C) until analysis. The labelled tibias were thawed and immersed in boiling deionized water for 10 min. The left tibias were de-fleshed by hand, defatted for 48 h in ethyl alcohol followed by a 48 h extraction in ethyl ether (Imari et al., 2020). The tibias were subsequently dried to a constant weight in a drying oven at 110°C for 12 h.



Table 3. The effects of the experimental treatments on the morphometric characteristics of the left tibia in 42-d-old broilers<sup>1</sup>

SDs, birds/m <sup>2</sup>	DTs	Weight mg	Length mm	Weight/length index mg/mm	Diaphysis diameter mm	Medullary canal diameter mm	Tibiotarsal index	Ribusticity index	Breaking strength N
12	CONT	7940	97.538	81.413	7.977	4.373	46.864	4.896	305.00
12	FDMLP	8520	99.748	85.498	8.664	3.968	53.887	4.886	335.67
12	FDMLB	8200	99.560	82.434	8.242	4.432	45.815	4.869	316.60
18	CONT	7700	94.585	81.355	7.642	5.062	33.462	4.871	255.40
18	FDMLP	8340	98.574	84.581	8.646	4.745	43.029	4.863	295.60
18	FDMLB	8020	98.338	81.694	7.914	4.952	37.163	4.893	280.50
SEM		120.990	0.509	1.226	0.153	0.106	1.888	0.025	11.473
SDs									
12		8220 <sup>a</sup>	98.949 <sup>a</sup>	83.115 <sup>a</sup>	8.294 <sup>a</sup>	4.258 <sup>b</sup>	48.855 <sup>a</sup>	4.884	319.09 <sup>a</sup>
18		8020 <sup>b</sup>	97.166 <sup>b</sup>	82.543 <sup>b</sup>	8.007 <sup>b</sup>	4.920 <sup>a</sup>	37.885 <sup>b</sup>	4.876	277.17 <sup>b</sup>
SEM		169.398	0.623	1.821	0.207	0.121	2.031	0.038	16.444
DTs									
CONT		7820 <sup>b</sup>	96.061 <sup>b</sup>	81.384 <sup>c</sup>	7.809 <sup>c</sup>	4.717	40.163 <sup>c</sup>	4.884	280.20 <sup>b</sup>
FDMLP		8430 <sup>a</sup>	99.161 <sup>a</sup>	85.040 <sup>a</sup>	8.564 <sup>a</sup>	4.357	48.458 <sup>a</sup>	4.875	315.64 <sup>a</sup>
FDMLB		8110 <sup>a</sup>	98.949 <sup>a</sup>	82.064 <sup>b</sup>	8.078 <sup>b</sup>	4.692	41.489 <sup>b</sup>	4.881	298.55 <sup>a</sup>
SEM		207.469	0.763	2.230	0.254	0.142	2.390	0.047	18.217
P Value									
SDs		0.042	0.049	0.038	0.036	0.001	0.001	0.883	0.010
DTs		0.015	0.019	0.049	0.016	0.186	0.012	0.991	0.045
SDs × DTs		0.994	0.670	0.990	0.979	0.819	0.811	0.918	0.811

<sup>a-c</sup> Values in the same column not sharing a common superscript differ significantly; <sup>1</sup> Data are mean of 10 broilers from each treatment; SEM: Standard Error of Mean; SDs: Stocking Densities; DTs: Dietary Treatments; CONT- a corn-soybean meal-based diet containing no fermented defatted mealworm larvae meal (FDM) (0%); FDMLP- the diet obtained by supplementing DM fermented with *Lactobacillus plantarum* to the CONT diet (0.4%); FDMLB- the diet obtained by supplementing DM fermented with *Lactobacillus brevis* to the CONT diet (0.4%).

Thereafter, tibias were individually weighed using an electronic balance with a precision of 0.001 g (Kern Germany, ABJ 220-4 M model) and their lengths were determined using a digital caliper with an accuracy of 0.001 cm (Mitutoyo, Absolute Digimatic, Mitutoyo, Kruike, Belgium). The bones were scanned at a resolution of 600 dpi and images were then converted to digital media.

To calculate the cortical index (tibiotarsal index), diaphysis diameter of the tibiotarsus was measured with the ImageJ program (Doube et al., 2010). The diameter of the medullary canal was computed from the difference between internal and external diameter of the diaphysis (Midilli et al., 2015). The tibia weight/length index, cortical index and robusticity index were calculated according to the following formulas (Karaarslan & Nazligul, 2018):

$$TW/LI = TW/TL \quad (1)$$

TW/LI : The tibia weight/length index (mg/mm)

TW : The tibia weight

TL : The tibia length

$$CI = [(DD - MCD)/DD] \times 100 \quad (2)$$

(Vahdatpour et al., 2014).

CI : Cortical index (Tibiotarsal index)

DD : The diaphysis diameter

MCD : The medullary canal diameter

$$RI = TL/CRTW \quad (3)$$

(Karaarslan & Nazligul, 2018)

RI : Robusticity index

TL : The tibia length

CRTW : Cube root of the tibia weight

After the above-mentioned measurements, the tibia breaking strength was measured by Warner-Bratzler method using ZWICK/ROELL Z 50 test equipment (Text Xpert Version 3.4) with a speed of 5 mm/min and recorded as Newton (N), the strength value that the bone can withstand at the moment of fracture (Aksit et al., 2017). Afterwards, the tibias were ashed in a muffle furnace at 600°C overnight. Tibia ash was determined as percentage of tibia dry matter weight (Ceylan et al., 2020). The ash of each tibia was analyzed to determine its Ca and P content by using inductively coupled plasma optical emission spectroscopy (ICP-OES; PerkinElmer, Optima 2100 DV), following the procedure outlined by Leske & Coon (2002).

### Statistical Analysis

In the analysis, the univariate general linear model was employed using SPSS version 17.0 (SPSSWIN, 2007) to evaluate the collected data. The model incorporated factors of stocking densities (SDs) and dietary treatments (DTs), along with their interaction effects. Significant differences among treatment means were assessed using Duncan's multiple range test (Duncan, 1955). Statistical significance was determined based on a threshold of  $P < 0.05$ .

## Results and discussion

### The Morphometric Characteristics of Tibia Bone

The effects of the experimental treatments on the morphometric characteristics of the left tibia in 42-d-old broilers were given in Table 3.

HSD significantly ( $P < 0.05$ ) decreased the weight, length and weight/length index of the tibia of broilers when compared with NSD (Table 3). These findings are consistent with those reported by Li et al. (2019), who observed that HSD significantly decreased both the weight and length of the tibia in broilers when compared to broilers raised under NSD. Contrary to these findings,

Karaarslan & Nazligul (2018) who stated that there are no significant differences between the length and the weight/length index of the tibia of broilers under reared NSD and HSD. The observed reduction in the weight and length of the tibia in broilers reared under HSD in the present study may be attributed to the restricted movement in a limited space, which likely resulted in reduced physical exercise, and thereby the increased tibia curvature by adversely affecting tibia proliferation, differentiation and formation compared with those of broilers under NSD (Li et al., 2019).

As shown in Table 3, HSD significantly ( $P < 0.05$ ) decreased diaphysis diameter, increased ( $P < 0.01$ ) medullary canal diameter and decreased ( $P < 0.01$ ) the tibiotarsal index when compared to NSD. These results may be derived from insufficient mobilization in a limited space of broilers (Li et al., 2019). Similarly, Aksit et al. (2017) observed that HSD significantly reduced the diaphysis diameter of the tibia in broilers compared to NSD.

However, the result related to tibiotarsal index in the present study is not consistent with the finding reported by Karaarslan & Nazligul (2018) who stated that there are no significant differences between the tibio-tarsal index of broilers under reared NSD and HSD.

As indicated in Table 3, HSD significantly ( $P < 0.05$ ) decreased breaking strength of the tibia of broilers compared to NSD. This finding is in agreement with the results reported by Sun et al. (2018) and Liu et al. (2020), who observed that HSD significantly reduces the breaking strength of the tibia in broilers. This unfavorable effect of HSD on breaking strength of the tibia of broilers may be the result of a lack of physical activity due to reduced space and unsynchronized development of muscular mass and skeletal system as SD increases, which leads to development of bone weakness, and thereby, the susceptibility to fracture of the tibia is higher (Vargas-Galicia et al., 2017). Contrary to our result, Aksit et al. (2017) and Sun et al. (2018) reported that breaking strength of the tibia of broilers was not significantly affected by SD.

These discrepancies between the results concerning the effect of SD on the morphometric characteristics of the tibia of broilers may be explained by the different experimental conditions such as bird strain, age, rearing systems (in cage or on litter), different numbers of birds per  $m^2$  in pens and cages, environmental conditions, experimental period and type of feed additives (Li et al., 2019).

Moreover, the FDMLP and FDMLB diets exhibited a significant ( $P < 0.05$ ) increase in the weight, length, and weight/length index of the tibia of broilers compared to those fed the CONT diet (see Table 3). The large tibia weight/length index of broilers in the present study showed that feeding with the FDMLP and FDMLB diets of broilers may have caused the denser tibia and thereby increase in tibia weight (Libouban et al., 2001).

The FDMLP and FDMLB diets significantly ( $P < 0.05$ ) increased the diaphysis diameter of the tibia and the tibiotarsal index of broilers compared to the CONT diet, although they did not affect the medullary canal diameter of the tibia of broilers (see Table 3). These results suggest that the supplementation of FDMLP and FDMLB to the diet enhanced the degree of mineralization and

development of the bone. The FDMLP and FDMLB diets significantly ( $P < 0.05$ ) enhanced the breaking strength of the tibia in broilers compared to that of broilers fed the CONT diet.

Since there are no studies on the effects of dietary supplementation of FDMLP and FDMLB on the morphometric characteristics of the tibia of broilers, our results could not be compared with previous studies. However, the improvement in measured tibia parameters of broilers observed in the present study could be attributed to the increased bioavailability and absorption of minerals such as Ca and P for tibia mineralization by the increasing beneficial bacteria in the small intestine due to the combined positive effects of probiotics (Cengiz et al., 2015), chitooligosaccharides as prebiotic and short chain fatty acids (Borrelli et al., 2017) as degradation products of chitin and AMPs (Benzertiha et al., 2020; Mohammed et al., 2021) of FDMLP and FDMLB in the diet.

SDs, DTs and interaction between SDs and DTs did not significantly affect ribusticity index of the tibia of broilers (Table 3).

#### ***The Crude Ash, Ca and P Contents of Tibia Bone of Broilers***

The effects of the experimental treatments on the ash, Ca and P contents of the left tibia in 42-d-old broilers were summarized in Table 4.

HSD significantly decreased the ash ( $P < 0.05$ ) and Ca contents ( $P < 0.05$ ) and P content ( $P < 0.001$ ) of defatted tibia bone in 42-d-old broilers compared to those of broiler reared under NSD (Table 4). This might be explained by decreasing duodenal Ca (calcium-binding protein D28k transcription) and P (type IIb sodium-phosphate co-transporter mRNA) absorption in broilers reared under HSD (Sun et al., 2018). The low Ca content of the tibia also may arise due to the lower serum parathyroid hormone (PTH) concentration in broilers reared under HSD, because PTH is able to absorb bone and increase osteoclasts activity, allowing the broilers to complete the process of bone Ca activation (Wang et al., 2020). The decreasing tibia ash, Ca and P contents of broilers might be attributed to the increase in the synthesis of corticosterone stress hormone in the adrenal glands due to HSD. Excess corticosterone stress hormone level inhibits the osteoblast osteoblastogenesis (Henneicke et al., 2014) and reduced bone mineral density such as Ca and P contents (Kang et al., 2016). Moreover, HSD caused intestinal injury such as reduced villus height and villus surface area in broilers by increasing heat stress, by which heat stress hampers intestinal absorption of Ca and P (Yan et al., 2019). These findings in the present study are partially in line with the previous findings in the litter-floor rearing system, which reported that tibia ash and phosphorus contents of broilers on day 42 were decreased, but, their tibia Ca content was not affected by HSD (Liu et al., 2020).

These results in the present study differed from the previous study that pointed out no effect of SD on tibia ash, Ca and P contents of broilers reared in the litter-floor system (Vargas-Galicia et al., 2017; Sun et al., 2018). Differences in feeding environments (including temperature, relative humidity, etc.), age, broiler strain and feedstuffs in diets might be the cause for this discrepancy among studies.

Table 4. The effects of the experimental treatments on the ash, Ca and P contents of the left tibia in 42-d-old broilers<sup>1</sup>

SDs, birds/m <sup>2</sup>	DTs	Ash, %	Ca, %	P, %
12	CONT	38.95	30.02	8.65
12	FDMLP	40.47	31.10	8.95
12	FDMLB	39.28	30.45	8.91
18	CONT	37.80	29.68	6.53
18	FDMLP	38.87	29.79	7.09
18	FDMLB	38.81	29.70	6.64
SEM		0.332	0.237	0.215
SDs				
12		39.57 <sup>a</sup>	30.53 <sup>a</sup>	8.84 <sup>a</sup>
18		38.49 <sup>b</sup>	29.72 <sup>b</sup>	6.75 <sup>b</sup>
SEM		0.462	0.337	0.132
DTs				
CONT		38.38 <sup>b</sup>	29.85 <sup>b</sup>	7.59 <sup>b</sup>
FDMLP		39.67 <sup>a</sup>	30.45 <sup>a</sup>	8.02 <sup>a</sup>
FDMLB		39.05 <sup>ab</sup>	30.08 <sup>ab</sup>	7.78 <sup>ab</sup>
SEM		0.566	0.413	0.162
P Value				
SDs		0.011	0.011	0.000
DTs		0.029	0.006	0.018
SDs x DTs		0.781	0.715	0.667

<sup>a,b</sup> Values in the same column not sharing a common superscript differ significantly; <sup>1</sup> Data are mean of 10 broilers from each treatment; SEM: Standard Error of Mean; Ash percent (as a percent of dry bone weight); Ca and P percent (as a percent of dry ash weight) of defatted tibia bone on d 42.; SDs: Stocking Densities; DTs: Dietary Treatments; CONT- a corn-soybean meal-based diet containing no fermented defatted mealworm larvae meal (FDM) (0%); FDMLP- the diet obtained by supplementing DM fermented with *Lactobacillus plantarum* to the CONT diet (0.4%); FDMLB- the diet obtained by supplementing DM fermented with *Lactobacillus brevis* to the CONT diet (0.4%).

As shown in Table 4, only the FDMLP diet significantly increased the ash ( $P<0.05$ ), Ca ( $P<0.01$ ) and P ( $P<0.05$ ) contents of the tibia in 42-d-old broilers compared to those of broilers fed the CONT and FDMLB diets. The improvement in the tibia ash, Ca and P contents of broilers in the present study could be related to the increased resorption of Ca and P by the beneficial bacteria in the small intestine due to the combined positive effects of probiotics (Cengiz et al., 2015), chitooligosaccharides as prebiotic and short chain fatty acids (Borrelli et al., 2017) as degradation products of chitin and AMPs (Benzertiha et al., 2020; Mohammed et al., 2021) of FDMLP and FDMLB in the diet, which, in turn, increases availability of serum Ca and P for bone formatting and/or remodeling (Mohammed et al., 2021). Previous studies have evidenced that synbiotics improve intestinal integrity and thereby increased absorption and bioavailability of Ca and P for bone mineralization (Yan et al., 2019).

## Conclusions

Overall, the results gathered in this study indicate that HSD results in reducing tibia morphology and mineralization, whereas the use of FDMLP and FDMLB as new antibacterial feed additives in broiler diets regardless of stocking density was able to improve tibia mineralization and morphology except its medullary canal diameter and ribusticity index of broilers due to the results of enhanced mineral absorption. Nevertheless, further research is needed to investigate the effects of dietary supplementation of FDMLP and FDMLB on the expression of parathyroid hormone-related protein in the tibial growth plate and the gene expressions of calcium-binding protein-D28k (CaBP-D28k) and sodium-

dependent phosphate transporter IIb (NaPi-IIb) in the small intestine of broilers. Further studies should also focus on elucidating the molecular components of FDMLP and FDMLB responsible for their antibacterial activity and clarifying their mechanisms of action to alleviate the adverse effects of HSD on tibial health in broilers.

## Declarations

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### Declaration of Competing Interest

The authors declare no conflict of interest.

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## Evaluating Large Cardamom Agriculture Practices: A Case Study from Panchthar, Nepal

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### ABSTRACT

A study conducted in Phalelung rural municipality, Panchthar, Nepal, in 2023 examined agricultural practices and the economic analysis of large cardamom. A total of 60 households were randomly selected, and data were collected through primary and secondary sources using surveys, focus group discussions, key informant interviews and field observations to assess the large cardamom economy, package of practices and various challenges faced by farmers. The study revealed that 28% of farmers grew the Ramsai cultivar, with over 82% having access to irrigation. Most farmers (77%) managed shade well and maintained their orchards effectively. However, only 50% used specialized harvesting tools, and 63% employed improved kiln (*bhatti*), though more education is needed. 68% of farmers received subsidies for constructing improved kiln (*bhatti*). Notably, 70% of farmers lacked knowledge about cardamom grading, and 90% were unaware of value-added practices. Farmers predominantly used jute bags for packaging. Major issues identified included plant wilt, *furkey*, rhizome rot, and stem borer infestations. The total production cost was NPR. 114,460.5 per hectare, with a BC ratio of 1.54. Having high market value, large cardamom provides promising opportunity to uplift the economic and social condition of farmers and stakeholders. This study provides an overview of the status of large cardamom production, processing practices, best cultivation practices to be adopted, disease pest incidence on large cardamom, storage, grading with various cultural practices to be adopted and feasibility of large cardamom cultivation area. This study would help both governmental and non- governmental agencies to make effective plans for large cardamom cultivation to meet Phyto-Sanitary status and minimum requirements to enter export and import system of cardamom in global market.

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## Introduction

Agricultural activities have stood out as a means of livelihood for most of the population, the main GDP source and an employment opportunity for many. Agriculture employs about 66% of the total population and supports 24.12% of the GDP of Nepal (MoALD, 2022/023). Large cardamom (*Amomum subulatum* Roxb.) is a valuable species of the family Zingiberaceae widely known as “Black Gold” or “Queen of Spices” and more popularly known as “*Kholsa ko sun*” in Nepal (Acharya, 2019).

Large cardamom was first introduced in the Ilam district in 1865, but commercial cultivation began in the late 1950s (Shrestha et al., 2018). Currently, it is grown in 51 districts, mainly in the eastern hill and mountain areas

and gradually expanding to the western districts of Nepal. It is one of the highest commercial products among all Nepal’s exportable products. This plant is a tall, perennial, evergreen herbaceous monocot with a height ranging from 1.5 to 3.0 meters. (Gopal et al., 2012; Bisht et al., 2011). It is a climate-dependent crop; the best production is between the temperature of 4-20°C, annual rainfall of 2000-2500 mm and more than 90% humidity (Chapagain, 2011). Large cardamom is cultivated in shady areas from 600 to 2000 masl (Uma et al., 2014). The plantation of large cardamom begins in June with a planting distance of 1.5 m, and it requires 3-4 years to reach maturity and produce cardamom.

Table 1. Trend analysis of large cardamom from the last 5 years

Year	Nepal			Panchthar District		
	Area (Ha)	Production (Mt)	Yield (Mt/ha)	Area (Ha)	Production (Mt)	Yield (Mt/ha)
2017/18	17004	6849	0.4	1961	829	0.47
2018/19	18273	7954	0.53	2920	1037	0.36
2019/20	18748	9545	0.58	3425	1178	0.37
2020/21	18791	8289	0.53	3432	1196	0.37
2021/22	19144	8714	0.55	3425	1,476	0.47

\*Source: MoALD, (2018); MoALD (2019); MoALD (2020); MoALD (2021); MoALD (2022)

Table 1. represents the trend of large cardamom from the last five years. In Panchthar, the total area dedicated to large cardamom production is 3,425 hectares. Of this, 3,164 hectares were productive, resulting in a total production of 1,476 Mt and a yield of 0.47 Mt/ hectare (MoALD, 2021/22). In the fiscal year 2022/23, Nepal exported 9,991.15 metric tons of cardamom, valued at 8.28 billion Rupees (MOF, 2022/23). Most of the large cardamom produced in Nepal, i.e., more than 95% is marketed to India (Paudel & Malla, 2020). The government of Nepal has initiated the Prime Minister Agriculture Modernization Project (PMAMP) to overcome the problem of low productivity, and higher import rate of Agri-products under ADS (2015-2035) from the Fiscal Year 2015/16. PMAMP has selected the 500 ha of land of Falelung Rural Municipality (Ekteen & Sidin VDC) as the site of the zone area of the project in Panchthar district (AKC, 2022).

In Panchthar, the total area dedicated to large cardamom production is 3,425 hectares. Of this, 3,164 hectares were productive, resulting in a total production of 1,476 Mt and a yield of 0.47 Mt/ hectare (MoALD, 2021/22). In the fiscal year 2022/23, Nepal exported 9,991.15 metric tons of cardamom, valued at 8.28 billion Rupees (MOF, 2022/23). Most of the large cardamom produced in Nepal, i.e., more than 95% is marketed to India (Paudel & Malla, 2020). The government of Nepal has initiated the Prime Minister Agriculture Modernization Project (PMAMP) to overcome the problem of low productivity, and higher import rate of Agri-products under ADS (2015-2035) from the Fiscal Year 2015/16. PMAMP has selected the 500 ha of land of Falelung Rural Municipality (Ekteen & Sidin VDC) as the site of the zone area of the project in Panchthar district (AKC, 2022).

Despite being an important cash crop for Nepalese farmers, the production trend of large cardamom has been stagnant and low because of various challenging factors such as lack of market, lack of improved varieties, disease infestations like *Chirkey* and *Furkey*, lack of suitable processing procedures, and lack of knowledge (Bhandari & Bhandari, 2018).

Thus, this study was conducted to assess the status of large cardamom production practices, major constraints of large cardamom production including disease and pest incidence, suitable storage conditions, along with grading techniques adopted and feasibility of large cardamom cultivation area. This study also indicates the government's role and responsibility in quality control to meet sanitary and Phyto-sanitary standards (SPS) and minimum requirements to enter in export and import system of cardamom in global markets. Thus, this article identifies existing cultivation practices and major production constraints, estimates cost of production and Benefit Cost ratio, discovers existing marketing channel and the post-harvest processes of the large cardamom in the Panchthar

district .Thus, this article identifies existing cultivation practices and major production constraints, estimates cost of production and Benefit Cost ratio, discovers existing marketing channels and the post-harvest processes of the large cardamom in the Panchthar district.

## Materials and Methods

### About the Study Area

Panchthar district is one of the 14 districts of Koshi Province of eastern hilly region of Nepal. It is a hilly district of far east Nepal covering 1,241km<sup>2</sup> (479 sq. meters). According to the census of 2021, 172,400 total populations and Phidim is the district headquarter. The geographical coordinate of this district is 27°06'49.68" North, 87°48'56.88" East.

There are a total of 8 municipalities in Panchthar in which 1 is urban and 7 are rural. The survey research was conducted in Phalelung rural municipality was selected as a study area as being cardamom zone under. Most of the people of this area are involved in cardamom cultivation. The study site selected are Phalelung rural municipality ward 1, 2,4,7,8.

### Preliminary Study

A preliminary study was carried out to collect different information regarding the feasibility of the research. Assessment of the features of the study site was done by direct observation and informal conversations with farmers.



Figure 1. Map of the Panchthar district of Nepal showing the study site

**Sample and Sampling Technique**

A list of farmers growing large cardamom under the command area of large cardamom zone was obtained from the office of PMAMP large cardamom zone and the sampling frame was made of 1000 farmers. A total of 60 households were selected based on simple random sampling. For this study, 60 respondents were selected from Phalelung rural municipality. Simple random sampling was adopted to avoid biases.

**Research Methods and Techniques**

Research instruments like household survey, focus group discussion, key informant interview, field observation, secondary information collection from various sources, etc. were used to collect and triangulate reliable data.

- A) *Household survey*: Household survey was conducted with the help of pre-tested, structured interview schedule as well as via use of telephone. Pretesting of the questionnaire was done among 10 respondents from the area near the study site to check the validity and effectiveness of the interview schedule. Total of 60 samples was taken by using personal interview schedule (PIS). The information on prevailing cultivation practices, production, and productivity, processing practices, problems/constraints faced by farmers on the large cardamom production, processing and marketing in the study area were collected from the farmers by interview.
- B) *Focus Group Discussion*: One Focus Group Discussions was conducted using a pre-determined semi-structured checklist on Chature, Phalelung-08, considering the cultural, gender, and ethnic backgrounds of respondents.
- C) *Key Informant Interview (KII)*: One KII was conducted with progressive farmers, farmer leaders, managers of private farms, and local extension workers to seek some key information about the overall trend of agriculture practices of large cardamom in the study area. about the ongoing situation in agriculture.
- D) *Field Observation*: Frequent visits to farms with keen observations were conducted to assess a brief overview of field conditions throughout the research duration.

**Source of Data**

- A) *Primary Data*: Primary data was collected by direct interviews with farmers through questionnaires and Telephone survey
- B) *Secondary Data*: Secondary data was collected by reviewing relevant journal articles, relevant articles, annual publication reports of governmental authorities, proceedings of I/NGOs and browsing the websites of relevant institutions.

**Data Analysis**

The data was tabulated and analyzed using SPSS, and MS Excel. The total cost of production was calculated as:

Total cost of production = Total fixed cost + Total variable cost

$$\text{Total fixed cost} = \sum C_L + C_E$$

$$\text{Total variable cost} = \sum C_{LP} + C_S + C_F + C_{IO} + C_P + C_H$$

Where,

$C_L$  = Cost of land rent

$C_E$  = Cost of equipment

$C_{LP}$  = Cost of land preparation

$C_S$  = Cost of seedling

$C_F$  = Cost of FYM

$C_{IO}$  = Cost of intercropping operation

$C_P$  = Cost of pesticide

$C_H$  = Cost of harvesting

The BC ratio was also calculated to determine profitability from large cardamom production in the study area:

$$BC = \frac{\text{Total cost (Annual income - Production cost)}}{\text{Total production cost}}$$

$$= \frac{\text{Total benefit}}{\text{Total Cost}}$$

**Indexing**

The Likert scale is used to place different options in ascending or descending order of severity.

The mathematical approach for this index use is

$$I = \frac{\sum S_i f_i}{N}$$

Where,

I= Priority index such that  $0 \leq I \leq 1$

$S_i$ = scale value of  $i^{th}$  priority

$F_i$ = frequency of  $i^{th}$  priority

N= Total number of observations

**Results and Discussion**

**Socio-demographical Results**

The data in Table. 2 presents the gender of the respondent who participated in the survey. Among the respondents, 87% were male while 13% were female.

Figure 3 presents the data regarding the Ethnicity of Respondents. Among the respondents, it was found that 60% respondents were Janajati, 32% were Brahmin and 18% were Chhetri.

Figure 2 presents data regarding the education status of the respondents in the study area. The level of education determines the well-being of the household. Education Status is classified into 4 categories: Illiterate, Primary, Secondary, and Bachelor or Bachelor above. Among the respondents it was found the majority (47%) had completed their primary level education while 37% were illiterate, 15% had completed secondary level education and only 1% had completed bachelor level education.

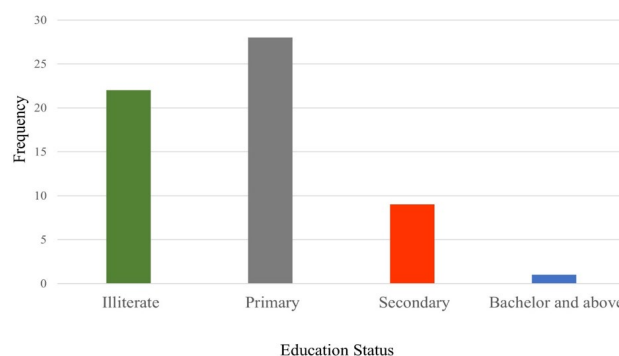


Figure 2. Education status of Respondents



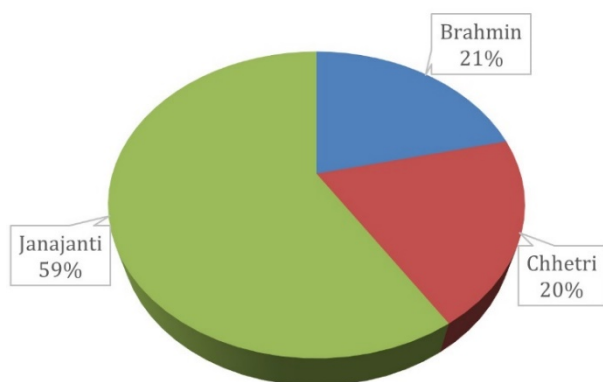


Figure 3. Ethnicity distribution in the study area

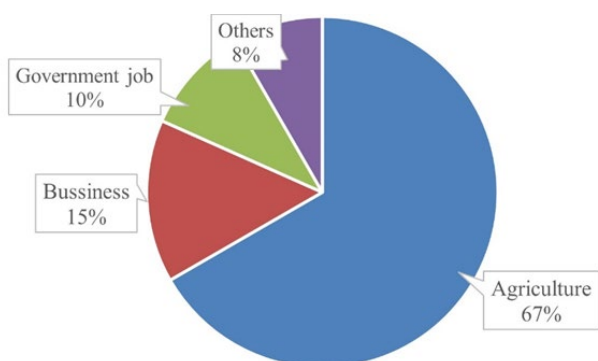


Figure 4. Sources of the household income

Table 2. The gender of respondents in the study area

Variables	Frequency	Percentage
Male	52	87
Female	8	13

Table 3. Distribution of respondents based on land holding size

Land Under Cardamom Cultivation	Frequency	Percentage of total land
(<0.17 hectare)	9	15
(0.17-0.34 hectare)	13	22
(>0.34 hectare)	38	63

Table 4. Land tenancy of the household in the study area.

Category	Frequency	Percentage
Rented	46	77
Owned	14	23

Table 5. Experience of large cardamom-producing farmers

Experience Category	Frequency	Percentage
0-5 years	10	17
5-10 years	31	52
More than 10 years	19	31

**Distribution of Sources of Household Income of Respondents**

The locals perform multiple activities to earn their living. Various activities may include agriculture, business, government jobs, etc. Figure 4 shows that 67% of the respondents had adopted agriculture as their major occupation, followed by 15% who had adopted business as

a major occupation, 10% who adopted a government job, and 8% who adopted other occupations, respectively. It reveals that more than 50% of the households in the study area have adopted farming as their major source of income, and large cardamom farming seems to be the major farming practice supporting them.

The data in Table 3 represents the distribution of respondents based on land under large cardamom cultivation. Among the respondents, it was also found that 63% of farmers cultivate Cardamom in more than 0.34 hectare while 22% of farmers cultivate in 0.17-0.34 kathas while only 15% of farmers cultivate in less than 0.34 hectare.

The data in Table 4. represent the land tenancy of households. Among the respondents, 77% rent up the land while 23% of farmers cultivate up cardamom on their land. The study reveals most farmers usually rent the land to perform cardamom cultivation.

The data in Table 5. represent the experience of large cardamom-producing farmers. Among the respondents, 52% of farmers have 5-10 years' experience, 31% of farmers have more than 10 years' experience, and 17% of farmers have 0-5 years' experience in large cardamom cultivation. The farmers were found to be quite experienced in the study area as the majority of them have been cultivating for more than 10 years.

**Large Cardamom Production Cultural Practices  
Frequency of cultivars in the Study Area**

Figure 5 shows that most farmers use *Ramsai* (28.3%), *Golsai* (25%), and *Chibesai* (20%), but among all other varieties, *Ramsai* is found to be most commonly cultivated due to its suitability in the agro-ecological climatic conditions and easy availability around the Panchthar region. Out of sixteen varieties of large cardamom in the world, six types of large cardamom are cultivated in Nepal, namely *Ramsai*, *Golsai*, *Dambersai*, *Bharlange*, and *Jirmale*. Shrestha et al. (2018) found that in Panchthar, *Ramsai* had higher productivity than *Golsai* and *Chibesai*.

The data in Table 6 represent the planting distance of large cardamom. Most of the farmers in Panchthar prefer the planting distance of 0.92 to 1.22 m; 67% of the farmers are found to follow that spacing, while 28% follow spacing above 1.22 m and 5% follow spacing less than 0.92 m.

Table 7 shows that 82% of farms are provided with irrigation facilities, and the remaining farms were found to be in rainfed condition. 77% of the farmers were found to have good shade management, and 87% of farmers had good orchard management. These data are indicators of good agronomic practices. In the case of large cardamom, shade management is very important. Exposure of large cardamom directly to the sun causes sunburn along with a reduction in the soil's moisture content, so it's important to manage the shade to get optimum productivity. Plants like *Titepati* (*Artemisia vulgaris*), *Bilaune* (*Maesa indica*), *Masysmdal* (*Vigna umbellata*), *Alder* (*Alnus nepalensis*), etc. are used as shading plants for large cardamoms (Shrestha et al., 2018).

Table 8 represents data regarding methods of irrigation. The farmers in the study area mostly use sprinkler irrigation and the Kulo system. It was found that 92% of respondents use a sprinkler system, while only 8% of respondents use *Kulo*.

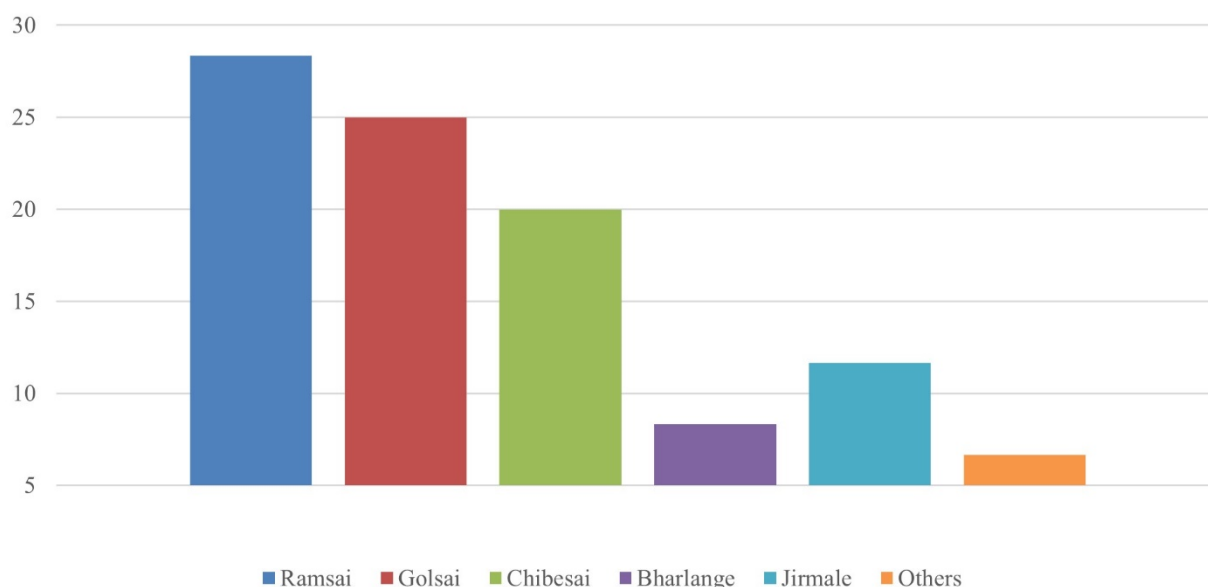


Figure 5. Cultivars in the study area

Table 6. Planting Distance of Large Cardamom

Planting Distance	Frequency	Percentage
Less than 0.92 m	3	5
0.92 to 1.22 m	40	67
Above 1.22 m	17	28

Table 7. Intercultural Practices adopted by the farmers

Intercultural Operation	Yes	No	Total
Irrigation	49 (82%)	11 (18%)	60 (100%)
Shade management	46 (77%)	14 (23%)	60 (100%)
Orchard Management	52 (87%)	8 (13%)	60 (100%)

Table 8. Method of irrigation

Method of Irrigation	Frequency	Percentage
Sprinkler irrigation	55	92
Kulo	5	8

Table 9. Production cost of large cardamom

Activity	Cost (NPR/Ha)	Contribution to Average Cost
I) Fixed Cost		
Land Rent	13835.13	12.08%
Equipment Cost	3387.68	2.95%
Total Fixed Cost	17222.79	15.03%
II) Variable Cost		
Land preparation	12419.49	10.85%
Seedling	21349.20	18.65%
FYM cost	3672.65	3.20%
Irrigation cost	19979.73	17.45%
Intercultural operation cost	19116.38	16.70%
Pesticides Cost	2265.67	1.97%
Harvesting cost	18433.53	16.10%
Total variable cost	97237.84	84.97%
III) Total cost	114460.56	100%
Average cultivated land area for large cardamom (Ha)	0.78	
Average cost of production per farm (NPR)	2975.70	
Average production per hectare (kg)	420	
Average cost of production per kg per hectare	272.52	

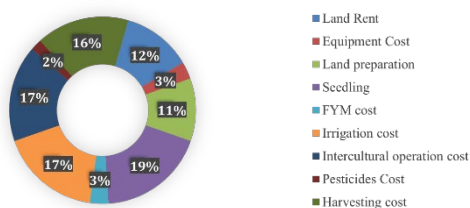


Figure 6. Production cost of large cardamom

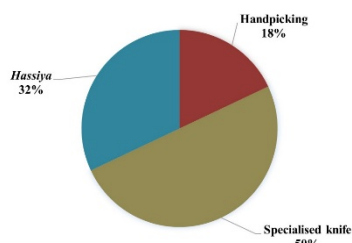


Figure 7. Harvesting technique used in the study area

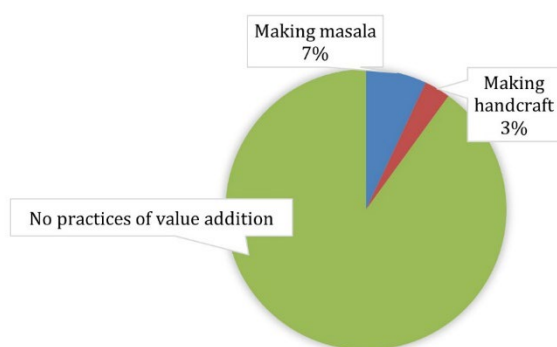


Figure 8. Value-addition practices adopted by farmers in the study area

Table 10. Ranking of Problems in Large Cardamom production in the study area

Problem	IV	R
Increased incidence of disease and insect/pests	0.85	I
Weather uncertainty	0.33	II
Difficulty in marketing	0.25	III
Poor availability of an improved variety	0.13	IV
Poor technical knowledge of farmers	0.11	V

IV: Index Value; R: Rank

Table 11. Prominent diseases of large cardamom in the area

Diseases	Index	Rank
Plant wilt	0.83	I
Furkey	0.73	II
Rhizome rot	0.55	III
Leaves decaying	0.21	IV
Chirkey	0.4	V

Table 12. Important Economic Insects and Pests in the study of the area

Insect/Pest	Index	Rank
Stem Borer	0.8	I
White Grub	0.46	II
Aphids	0.4	III
Caterpillar	0.33	IV

In the study performed by Shrestha et al. (2018), it was found that 90% of the people who responded were using various methods to irrigate their orchards, comprising sprinklers (15%), canal/surface irrigation (39.9%), and both (32.7%), while using the *kulo* system was considered a significant source of fungal disease transmission. However, the present study suggests that most farmers have transitioned to sprinkler irrigation to avoid fungal disease transmission through *kulo* irrigation.

#### Economic Analysis of Large Cardamom

The cost of production per Ha of large cardamom in the study area was found to be NPR. 114460.5

The BC ratio was found to be 1.54 which indicates that large cardamom production is a profitable business in the study area and farmers can earn profit return from large cardamom production in the study area.

From the study of (Baniya et al., 2019) BC ratios of Yangwarak-5, Phidim-14 and Falgunanda- 6 Panchthar were found to be 1.37, 1.21, and 0.66 respectively. This suggests that the BC ratio of Phalelung is comparatively more than adjacent local bodies.

From the field survey conducted at the Panchthar, the average production of large cardamom production was found to be 420kg/Ha.

Table 10 shows the ranking of problems in large cardamom production in the study area which concludes that increased incidence of disease and insect/pests is the most serious problem (0.85) followed by weather uncertainty (0.33). Difficulty in marketing (0.25) is ranked third while poor availability of an improved variety (0.13) is ranked fourth. Poor technical knowledge of farmers is the least serious issue (0.11).

One of the major constraints of large cardamom production in Nepal is that there is still haphazard planting of the crop without proper knowledge of variety and their climatic requirements which is the cause of higher disease, insects and sterility problems (Shrestha, Chapagain & Karna, 2004). *Chirkey* and *Furkey* are the most common viral diseases responsible for lower yield in the crop (Sharma, Sharma & Sharma, 2009). Managing the distribution of quality capsules also stands out as a major challenge (MoAC, 2012).

#### Ranking of Pests and Diseases Occurring in The Cardamom Field

Table 11 shows that Plant wilt (0.83) has the highest severity followed by *Furkey* (0.73), Rhizome rot (0.55), leaves decaying (0.21) and *Chirkey* (0.4) which indicates that currently *Chirkey* disease is under control, and rhizome rot, previously a significant issue, is also managed. However, efforts are needed to control Plant wilt and *Furkey* disease. Various disease pests have been detected while cultivating large cardamom, but major diseases include *Chirkey*, *Furkey* and rhizome rot that has caused severe yield loss to an extent of 68-100%. Likewise, the crop is also attacked by many insect species. Shrestha et al., (2018) reported that rhizome rot and *Chirkey* were severe, while plant wilt was moderate, and *Furkey* disease had the lowest severity in Panchthar.

From Table 12, the most important economic insect and pest in the study area was found to be stem borer (index value 0.8), followed by white grub (index value 0.46), aphids (index value 0.4), and caterpillars (index value 0.33).

Table 13. Drying techniques used by farmers

Drying Techniques	Frequency	Percentage
Improved kilns (Bhatti)	38	63
Traditional kilns (Bhatti)	22	37

Table 14. Subsidy for making *Bhatti*

Subsidies received	Frequency	Percentage
Yes	41	68
No	19	31

Table 15. Calyx cutting techniques used by farmers

Calyx Cutting	Frequency	Percentage
Tail cut	51	85
Shave it off	9	15

Table 16. Grading of Large cardamom

Grading	Frequency	Percentage
Yes	18	30
No	42	70

Table 17. Packing materials used by the farmers in the study area

Packaging Material	Frequency	Percentage
Jute Bag	41	69
Plastic Bag	18	31
Total	60	100

Table 18. Adoption of various practices for value addition

Practices for value addition	Frequency	Percentage
Yes	6	10
No	54	90

Ten years ago, the major pest of Large Cardamom was the leaf caterpillar (*Artona chorista*, Jordan), while at present minor pests like stem borer (*Glyphopteryx* spp.), shoot fly (*Merochlorops dimorphous* Cheria), lacewing bug, and white grub (*Holothrichia* spp.) have emerged as major pests. Along with it, a significant pest of ginger, *Holothrichia* spp., has become a significant pest of large cardamom at present (Gurung et al., 2020). The stem borer, known as *Glyphepteryx* sp., is a pest that specifically targets large cardamom. It can be observed in the fields throughout the year. Its peak abundance occurs during December–January, March–April, May–June, and September–October (Bala & Bala, n.d.).

### Harvesting and Post-Harvesting Techniques

#### Harvesting techniques used in the study area

From figure 7 it was found that half the farmers use specialized knives, while 32% use *hassiya*, and 18% use the handpicking technique. This shows that the farmers are slowly acknowledging and adopting the instruments and methods that are easy and timesaving. The harvesting practice is generally done from September to November. The maturity of the cardamom is assessed by opening the topmost capsule of the spike. After the topmost capsule fully matures, the shoot with spikes is cut at 45 cm and kept for another 10–15 days to ensure the maturity of the capsules (Board, 2001). To separate the capsule easily harvested spikes are stored for 2-3 days (Board, 2001).

Separation and cleaning of capsule is done manually before curing to remove other plant materials. (Board, 2001).

Table 13 shows that the majority i.e., 63% farmers were using the improved kiln (*Bhatti*) while 37% were using the traditional kilns (*Bhatti*) and none of the farmers were found to be using Sun drying for drying of large cardamom for curing of cardamom. The use of improved *Bhatti* directly relates to excellent quality products with a maroon colour and volatile oil content.

Curing cardamom is usually associated with the colour balance, humidity, and maturity. According to Mande et al., (1999), fresh large cardamom capsules at harvest contain about 70 to 80% moisture (on a wet basis). Initially, flue-cured cardamom had a moisture content of 12.5% and 67% RH, but it was found, that a moisture content of 11.0% at 60% RH was optimum for the storage of large cardamom (Naik et al., 2000). Depending on the variety, the colour of these capsules varies from pale pink, and brownish pink to dark pink, which ultimately becomes black after drying. From the study of Deka et al., (2003) curing large cardamom at 45-55°C was found to be ideal. Depending upon curing methods and capsule size, the weight ratio from fresh to cured capsule varies from 4:1 to 5:1 (Bhutia et al., 2017). Some volatile substances that are components of the essential oil of large cardamom are lost during the process of curing (Rout et al., 2003). Curing is done in traditional and improved kilns (*bhattis*).

Data in Table 14 shows that 68% of farmers receive subsidies for making the *Bhatti* for drying large cardamom. Farmers who received the subsidy for making *Bhatti* followed the improved kiln (*bhatti*).

The data in Table 15 shows that 85% of farmers perform tail cut while 31% were found to shave it off. Removing the tail from cardamom capsules is a crucial step undertaken by local traders before selling the product. Capsules without the tail fetch a higher price in the market (Shrestha et al., 2018). The process, known as tail cutting, involves manually removing the outer layer of the capsule using scissors. This step is essential for grading the cardamom, with capsules categorized as either "*kainchi-cut*" (with the tail removed) or "*non-kainchi cut*" (with the tail intact) (Mande et al., 1999). According to local dealers, the additional labour required for tail-cutting costs approximately US\$ 0.41 per kilogram of capsules (Singh & Pothula, 2013).

The data in Table 16 shows that 70% of the farmers had no idea of grading and only 30% farmers graded their large cardamom. Thus, cumulatively it was found farmers got low market prices of the produced items around the study area. The majority of the farmers were found to be not using grading techniques because of a lack of grading technology and a lack of proper knowledge about grading.

There is a variation in the size of large cardamom due to cultivar differences or pre-harvest conditions. Mechanical grading machines are not reported in Nepal so manual screening method is applied for grading the capsules. Large cardamom is graded in the local market as *badadana* (big capsules) or *chotadana* (small capsules) and, as discussed previously, as *kainchi-cut* (capsule tail removed) or *non-kaichi-cut* (capsule tail intact) (Sharma et al., 2009).

Table 17 shows that 69% of farmers were found to use jute bags as a packaging material and 31% were found to use plastic bags. Dried large cardamom capsules are usually packed in polythene-lined jute bags. For storage, a dried capsule with an optimal moisture level of 11% is recommended (Naik et al., 2000). Polypropylene and ethylene terephthalate/polyethylene have been reported to considerably reduce moisture and volatile oil exchange under normal storage conditions (Sulochanamma et al., 2008).

Storage stability has been maintained for large cardamom capsules with up to 11% moisture content. (Gurudutt et al., 2000). The use of fumigants like Methyl bromide ( $0.016 \text{ kg m}^{-3}$ ), Phosphine ( $0.0015 \text{ kg m}^{-3}$ ) and ethyl formate ( $0.30 \text{ kg m}^{-3}$ ) are suggested to control the storage insect pest that affects the quality of large cardamom (Naik et al., 2005).

From Table. 18 it was found that 90% of farmers were found to have no idea about the value addition of large cardamom and only 10% of farmers were found to practice the value addition of large cardamom.

#### **Value-addition practices adopted by farmers in the study area**

Figure 8. shows that 7% of the farmers were found to make masala from the capsule of large cardamom and 3% were making handcraft like *Gundri*, *Chakati* and Bags from the pseudo-stem of large cardamom.

#### **Conclusion**

Large cardamom, locally known as '*Kholsa ko sun*,' is a valuable spice crop that uplifts farmers' rural economy of the Panchthar district and eastern Nepal. This area is emerging as a key producer of large cardamom and holds substantial potential. A significant portion of the economically active population is engaged in its cultivation. While farmers have considerable experience, their cultivation, harvesting, and processing techniques still lack scientific knowledge. This has increased issues with pests, diseases, and particularly plant wilt and stem borer infestations. Many farmers still use traditional kilns (*bhatti*), and large cardamoms are often not graded or packed properly, leading to rapid quality deterioration during storage. Despite these challenges, large cardamom farming remains profitable, with an acceptable BC ratio and an average production cost. With the provision of proper marketing system, IPM and IDM integrated clean cultivation practices and right support from the government, extension services, and other agencies, Panchthar can potentially become a leading centre for large cardamom production in Nepal.

#### **Recommendations**

The assessment of this research findings was informative for farmers, policy implications and for further researchers.

- Large cardamom cultivating farmers of Panchthar district should improve their cultivation practices like selecting agro-ecologically suitable varieties, maintaining recommended planting distance, orchard management to get higher yield.

- Farmers should use specialized large cardamom harvesting knife to minimize plant damage as well as capsule and disease infestation.
- Grading and appropriate drying, packaging and practices of value addition must be done to get higher prices.
- Appropriate interventions should be done to capacitate farmers like training.
- Marketing Information should be made easily available.
- The economic aspect of the traditional and modern method of large cardamom cultivation practices should be analyzed.
- Action research on detailed study of problems must be carried out.

#### **Declarations**

##### **Ethical Approval Certificate**

The procedures of this study were approved by the Faculty of Science and Technology, Nepal Polytechnic Institute (NPI), Purbanchal University

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PU. Regd. No: 113-3-2-08493-2018

##### **Author Contribution Statement**

S. B.: Conceptualization, Conducting survey, Data collection, Formal analysis

A. B.: Interpretation of data, Data analysis, technical assistance, Manuscript writing and designing

S. G.: Conducting survey, Data collection, Formal analysis

S. D.: Conducting survey, Data collection, Formal analysis

S. A.: Assisting, Validation

##### **Conflict of Interest**

The authors declare no conflict of interest.

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## Bio-efficacy of Different Traps and Baits Under Field Condition to Control Fruit Flies (Diptera: Tephritidae) in Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai)

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### ABSTRACT

Tephritid fruit flies (Diptera: Tephritidae) are the major damaging pest for the Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). The quality as well as quantity of watermelon is greatly affected by fruit fly. This study was conducted to test the efficacy of different traps and baits for fruit fly control. The experiment was designed in a randomized complete block design containing eight treatments and three replications. Each treatment, 0.2 ml of cue lure as an attractant and 0.1 ml of malathion was used as a toxicant except control. Treatments were installed when the flowering was started. Data collection and treatment replacement were done simultaneously in every 6-day interval. The findings revealed that the diverse fruit fly species (up to 7) were trapped in all treatments however, the *Zeugodacus cucurbitae* (Coquillett, 1889) followed by *Zeugodacus tau* (Walker, 1849) were dominating over the other species in all treatments. Yellow sticky trapped the highest number of fruit flies (15.01±0.38), followed by brewery wastage (13.65±0.37). The male-female ratio of the trapped fly was (>1) in all treatments. Furthermore, the lowest fruit fly damaged percentage (0.87±0.16) with the highest obtainable yield (32.57±1.31) and benefit-cost ratio (1.63±0.06) was observed in the yellow sticky trap. From this experiment, the yellow sticky trap captured more fruit flies caused the least amount of fruit fly damage, and yielded the highest possible yield than the other treatments.

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## Introduction

Insect pests are a major threat to watermelon production globally (Okrikata et al., 2021). A variety of pest's attack watermelon at different stages throughout its growing period however, the seedling, mid-vegetative, and mid-fruiting stages are more critical than others (Okrikata & Ogunwolu, 2019). The mining insect, leaf miner (*Liriomyza Spp*) (Hamza et al., 2023), piercing and sucking insects melon thrips (*Thrips palmi* Karny) (Whitfield et al., 2005) and leaf feeding pumpkin beetles like red (*Aulacophora africana* Weise) and blue (*Asbecesta nigripennis* Weise, *A. transverse* Allard.), sap-sucking aphids (*Aphis gossypii* L.) & white fly (*Bemisia tabaci* Genn.) attack mainly in vegetative stages. Similarly, melon fly (*Zeugodacus cucurbitae* Coq.) and fruit borer (*Helicoverpa armigera* Hub.) attack in the flowering and fruiting stages (Okrikata & Ogunwolu, 2019). Whereas, two-spotted red spider mites, being devastating leaf-

feeding pests, occur throughout the growing period (Schmidt-Jeffris et al., 2021).

The fruit flies in the Dacine sub-family of the Tephritidae family are a widespread pest of cucurbits (Dhillon et al., 2005; Nair et al., 2021; Sapkota et al., 2010) causing up to 90% fruit damage (Ryckewaert et al., 2010). They are the primary contributor to the decline of both quantity and quality (Sulaeha et al., 2020), resulting in 63.2% production damage in watermelons (Masika et al., 2022). *Zeugodacus*, *Rhagoletis*, *Dacus*, *Certitis*, *Bactrocera*, and *Anastrepha* are the six major economically significant genera (Li & Zhang, 2019). Sex pheromone (Tan et al., 2014) and cue-lure (C-L) [4-(para-acetoxyphenyl)-2-butanone] are used in current fruit fly programs for detection, monitoring, and control (Vargas et al., 2010). Similarly, female fruit flies show good responses in food containing ammonia derivatives and get

more attracted to protein-rich food (Piñero et al., 2020). However, rainfall limits the effectiveness of bait in the open field condition (Delpoux & Deguine, 2015). This encourages the placement of baits in bottles. Furthermore, fruit flies are attracted to those color traps that mimic their natural host indicating their inherent tendency toward those visual stimuli (Singh & Singh, 2018). The direct application of chemical insecticides to control fruit flies is practical but ineffective and unsafe. And microbial pesticides are not widely available in Nepal (Adhikari et al., 2020). To overcome the relevant problems in open field conditions, this study evaluates the effectiveness of different traps and baits, which contain lure and malathion to control fruit flies in watermelon.

## Materials and Methods

### Experimental Site

The study area was located at Bharatpur-15, Chitwan near the Agriculture and Forestry University Figure 1. Geographically, it is situated at 27°39'09''N latitude and 84°21'57'' E longitude with an elevation of 186m above the mean sea level. The experiment was carried on during the spring season from February to May 2023. The soil property was sandy loam having ph-6.8, which is suitable for watermelon production (Shrefler et al., 2015). The average relative humidity and temperature of the site was 64.14%. and 16.75 °C. The average maximum and minimum temperatures were 22.5 °C and 12.5 °C respectively. Additionally, the average maximum and minimum relative humidity were 82.22% and 48.26% respectively.

### Experimental Detail and Treatment Setup

Randomized Completely Block Design (RCBD) with eight treatments and three replications were performed. A total of 24 plots, each 5.4m×2.4 m (12.96 m<sup>2</sup>), were prepared for experimentation. The treatment gap was maintained at 5m with ref. (Akhtaruzzaman et al., 2000) and a 6m distance was kept between the two replications. In each plot, 18 plants were planted with a spacing of 60\*60 cm<sup>2</sup> as in Figure 1. The extra early, vigorous variety of watermelon “Saraswati” (F<sub>1</sub> hybrid) product no. IA087 (<https://shorturl.at/mqrwG>), which was highly popular among the producer and consumers, was cultivated. The seeds were collected from Bharatpur, Chitwan. A plastic tray (of 72-hole type) with peat moss as growing media, was used for growing seedlings. The seedling stage having 3 fully and one half developed leaf, was transplanted on the main field. Table 1 shows the treatment description. The commercial cucurbits grower used cue lure as the fly attractant near the experimental site, and to minimize the biases in the fruit fly attraction to the commercial and experimental field @0.1 ml/ treatment cue lure (Eco-man Biotech Co. Ltd. China) was used as an attractant media in every treatment. Furthermore, @0.2ml malathion 50%EC (Plant Remedies Pvt. Ltd, India)/ treatment was added as a toxicant except control plot.

T<sub>8</sub> was kept as control, 60 grams of black jaggery was mixed with 120 ml of distilled water to prepare 60 ml jaggery bait. Similarly, 60 ml of molasses was used for preparing molasses bait. Watermelon flesh including juice was collected in the beaker and 60 ml of it was used as bait for the next treatment.

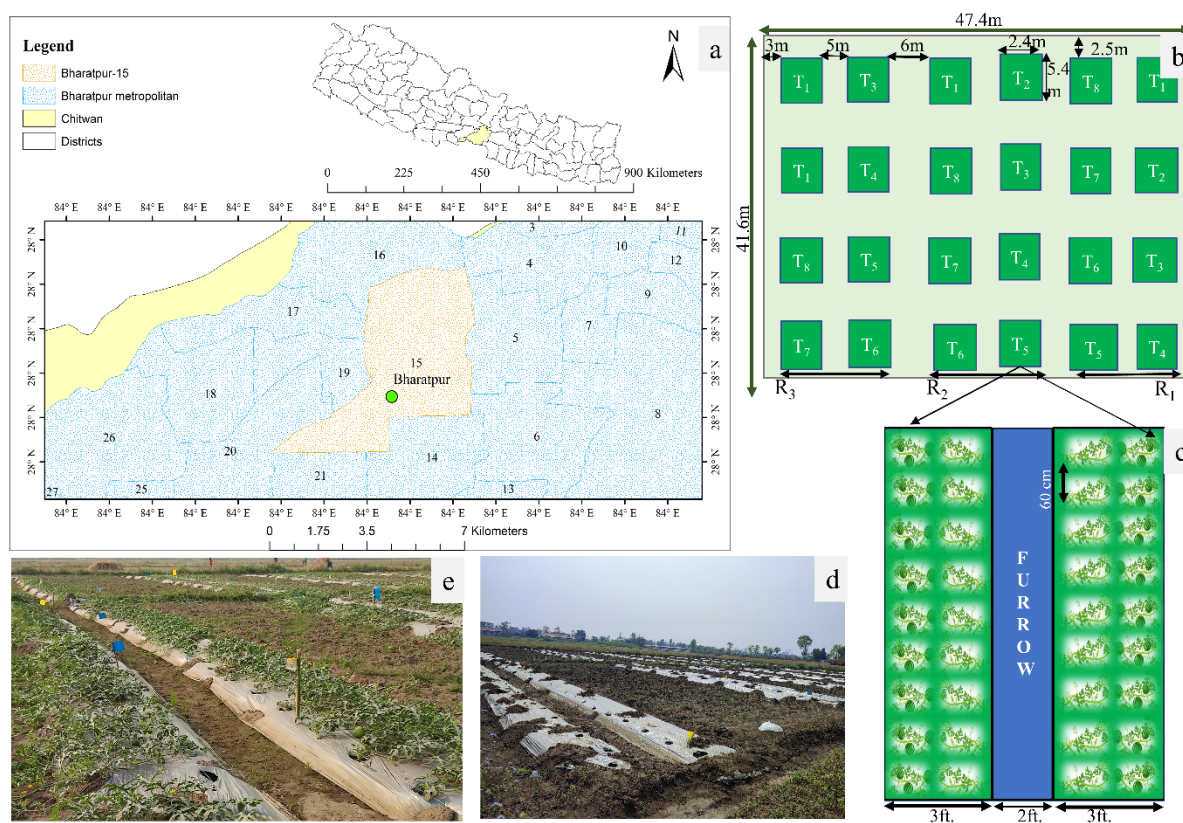


Figure 1. Experimental layout in detail (a. Study area map using Arc. GIS. version 10.8; b. Experimental layout; c. Plot detail; d. Field layout; e. Treatments installment on the field)



Table 1. Detailed explanation of various treatments used in the experiment

Treatments	Attractant <sup>1</sup>	Toxicant <sup>2</sup>	Quantity	Baits and traps media	Source
T <sub>1</sub>	0.2	0.1	60ml	Black Jaggery solution	Local retailer, Mangalpur
T <sub>2</sub>	0.2	0.1	1 piece	Yellow Sticky Trap	Sharma Agrovat, Chanauli
T <sub>3</sub>	0.2	0.1	60ml	Watermelon flesh solution	Local retailer, Mangalpur
T <sub>4</sub>	0.2	0.1	1 piece	Blue sticky trap	Sharma Agrovat, Chanauli
T <sub>5</sub>	0.2	0.1	60ml	Sugarcane Molasses	Local retailer, Mangalpur
T <sub>6</sub>	0.2	0.1	60gm	Brewery solution	Gorkha Brewery Pvt. Ltd
T <sub>7</sub>	0.2	0.1	20ml	Citronella oil	Barali Pvt. Ltd. Mangalpur
T <sub>8</sub>	0.2	-----	60ml	Tap Water	Local tap water

<sup>1</sup>(Lure on ml); <sup>2</sup>(Malathion ml 50%EC)



Figure 2. Bait and trap installed on field; (a.) Black jaggery bait with malathion and cue lure on a separate cotton wick with separately hanging and b. Yellow sticky trap with cue lure and malathion kept on a cotton wick separately

A<sub>4</sub> size (21×29.7 cm<sup>2</sup>) of yellow sticky trap (YST) and blue sticky trap (BST) were used as trapping media for the rest two of the treatments. All the treatments were installed above 45cm height from the ground, which is a good strategy for monitoring as well as control of *B. dorsalis* (Said et al., 2017).

For the bait installment, plastic bottles (7.5 cm diameter & 12.5 cm height) having 3 holes of 7mm diameter each were used according to (Gupta & Regmi, 2022). Inside the bottle, food bait was added to the base, whereas 0.2 ml of malathion and 0.1 ml of cue lure were dropped in each cotton wick separately and hung with the two wires as shown in Figure 2(a). Similarly, in YST and BST, 0.2 ml of malathion and 0.1 ml of cue lure were dropped in each cotton wick and attached separately as shown in Figure 2(b) All the treatments were installed in the early morning of 7<sup>th</sup> April, when 25% of the total plants were reached at the flowering stage. Data collection and treatment replacement were performed every 6-day intervals.

#### Data Collection

Data collection was done on Apr 13<sup>th</sup>, Apr 19<sup>th</sup>, Apr 25<sup>th</sup>, and May 1<sup>st</sup> respectively, when the plants at flowering, fruit softening, fruit hardening, and fruit maturation stages.

The trapped fruit flies and their sex were counted. Additionally, they were taxonomically identified

according to (Adhikari & Joshi, 2018; Australia, 2018; Leblanc et al., 2021; Li & Zhang, 2019) and (Dhillon et al., 2005; Singh et al., 2019) which were visually presented in S1 & S2 respectively. Furthermore, the male-female ratio was calculated according to given formula;

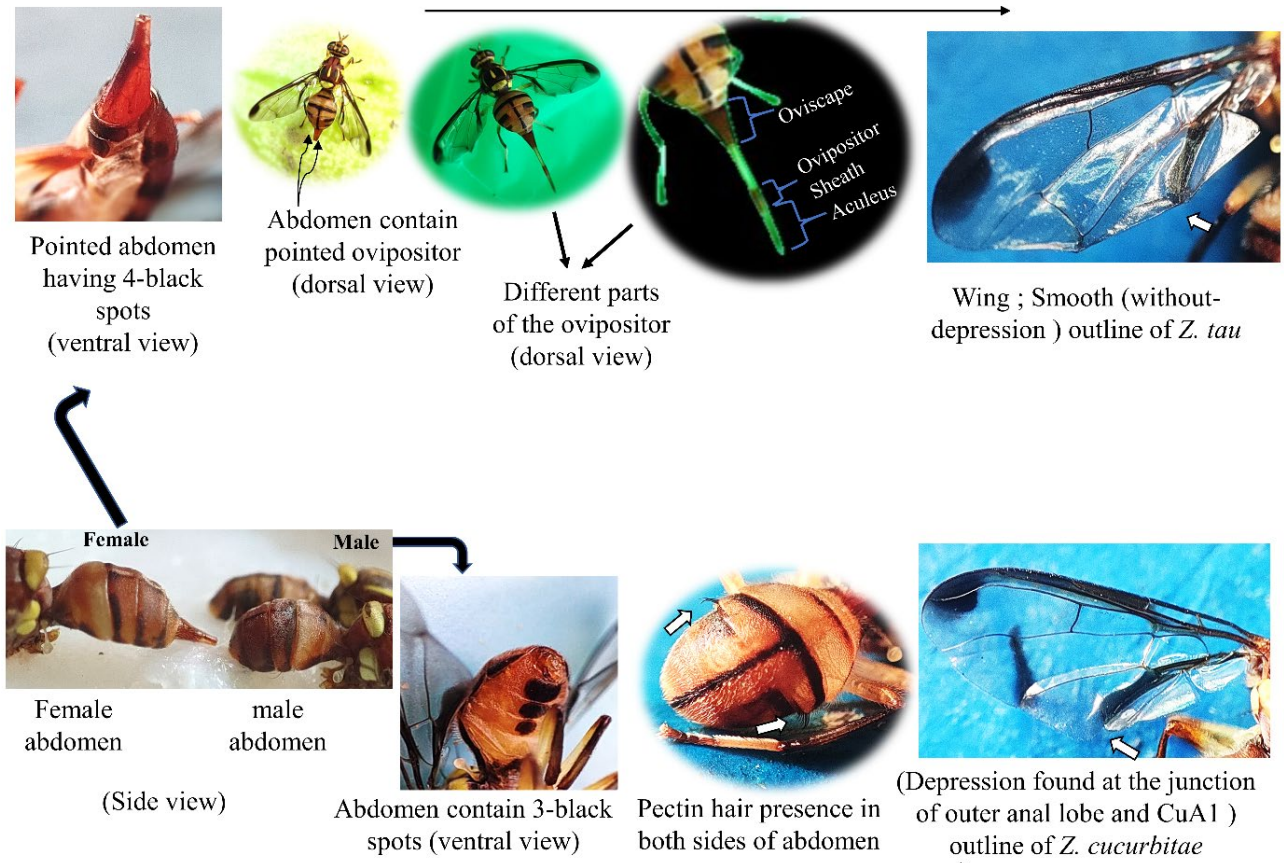
$$\text{Male-female ratio} = \frac{\text{Total number of trapped male}}{\text{Total number of trapped female}} \quad (1)$$

For the economic analysis, five plants were selected as sample plants. which were then used for calculation of total yield (Mtha<sup>-1</sup>), BCR and percentage infected (damaged) fruits. Infested fruits (IDF) were calculated by the sum of total infected fruits is divided by total number of fruits (Healthy and infected fruits/plant).

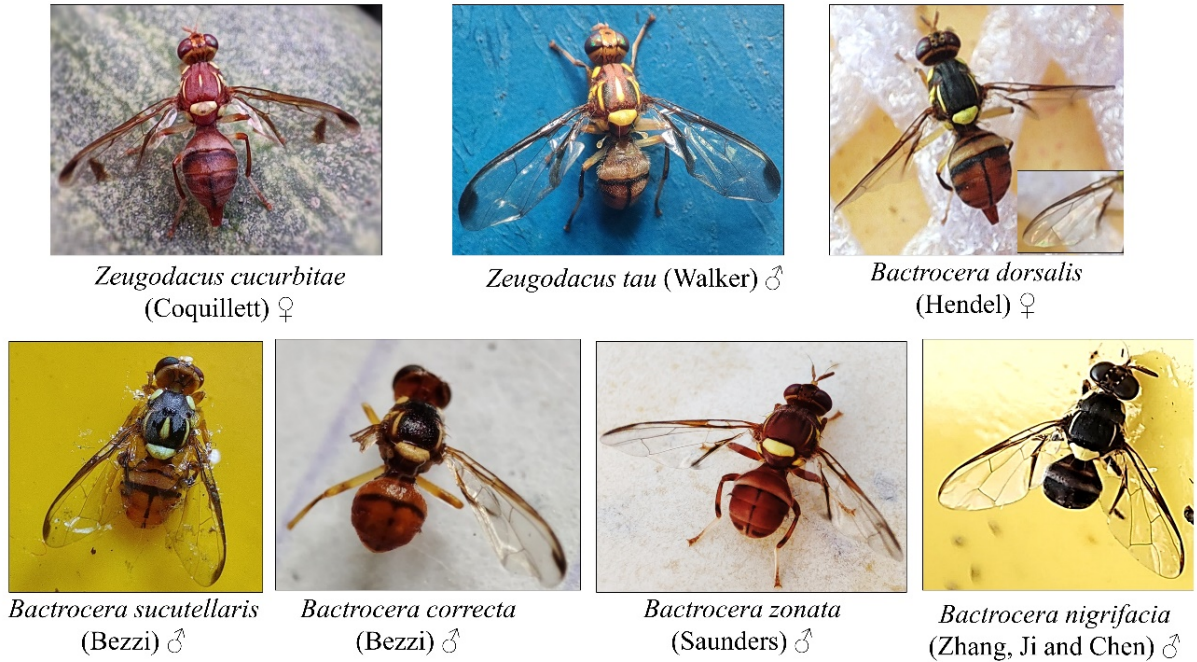
$$\text{IDF (\%)} = \frac{\text{Total no.of infected fruits}}{\text{Total no.of fruits in this plant}} \times 100 \quad (2)$$

Discounting interest is not necessary to calculate for the economic analysis being watermelon as seasonal crops. Hence, only variable cost is included here to calculate

$$\text{benefit cost ratio, BCR} = \frac{\text{Gross return}}{\text{Total variable cost}} \quad (3)$$



S1. Different parts of the trapped male and female fruit flies on watermelon at Chitwan, April-June 2023



S2. Different species of trapped fruit flies in watermelon during the experimental period at Chitwan, April-June 2023

*Data entry and Statistical analysis*

The data was tabulated and compiled in MS Excel 2019. Most of the counted data were skewed and for minimization of variation, square root  $\sqrt{(x + 0.5)}$  transformation was performed (Gomez & Gomez, 1984; Wolda & Marek, 1994). R studio version 4.1.1 was used for data wrangling, inferential analysis and graphical representation. Normal distribution was tested by the gvlma package, and multiple mean comparison of data were performed through agricolae (R Core Team, 2022). Both Fishers and Kruskal-Wallis test were performed by using ggbetweenstats function of ggstatsplot package (Patil, 2021). Furthermore, Fishers test was used for the data which fits for the normality test and Kruskal-Wallis test for non-normal data (Ostertagová et al., 2014). Post

Hoc Tukey’s test at a 0.05 level of significance was used for multiple mean comparisons (Diaz-Fleischer et al., 2009).

**Results and Discussions**

*Effect Of Treatments on Trapped Fruit Flies*

Figure 3 displays the significant differences of treatments on the trapped fruit fly populations at ( $p < 0.001$ ). The highest trapped number (mean per stage) was found in yellow sticky trap (15.01±0.38), followed by brewery wastage (13.65±0.37) which was statistically similar with blue sticky trap (13.26±0.31), black jaggery (13.25±0.39), sugarcane molasses (13.2±0.32), watermelon flesh (12.96±0.21).

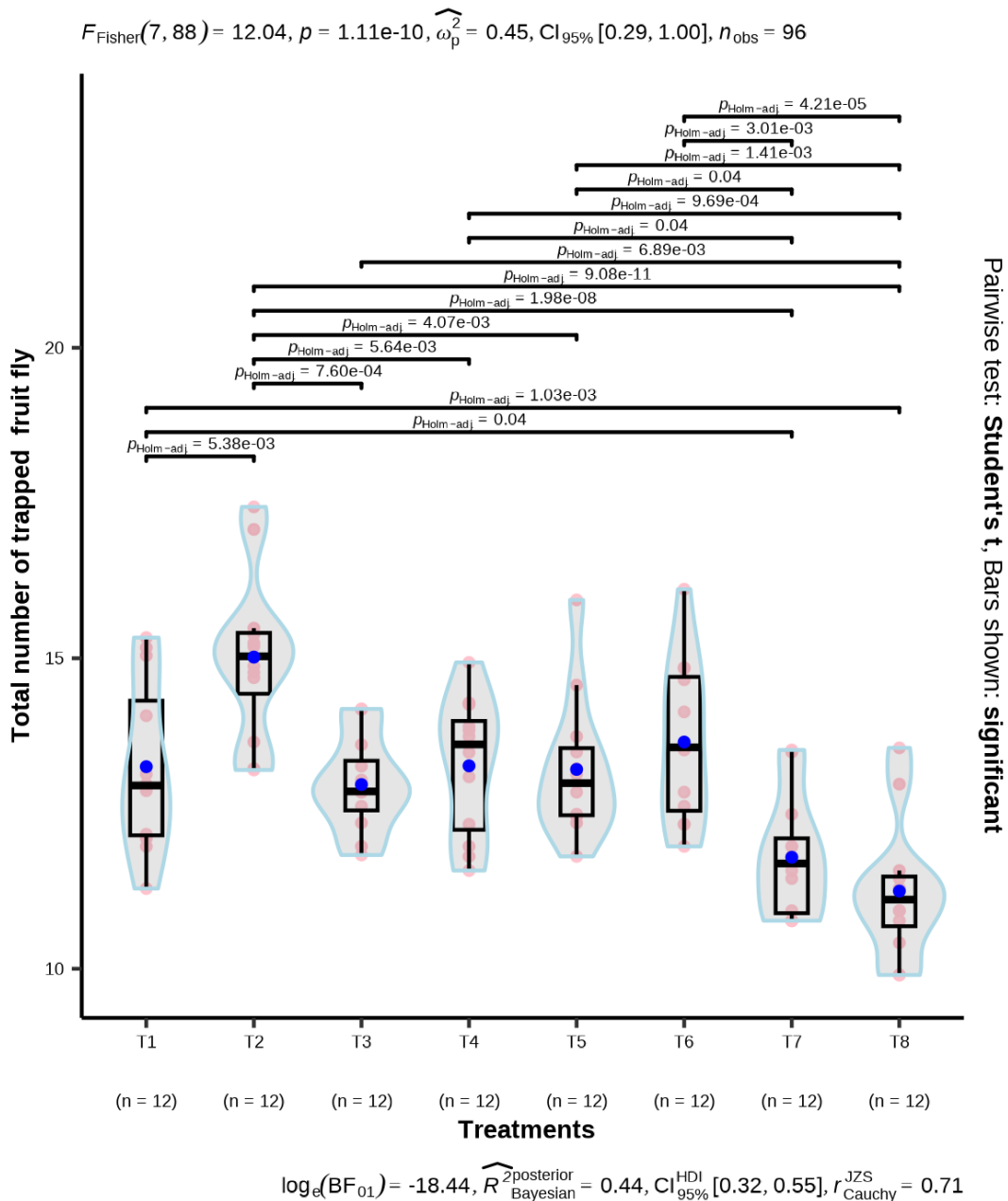


Figure 3. Status of mean trapped fruit fly (per stage) in different treatments installed in watermelon at Chitwan, April-June 2023. Inside the box violin plot, blue dot represents the average mean value, Similarly, n represent the total number of sample (3 replications×4 weeks =12) number was observed in tap water (11.25±0.31)

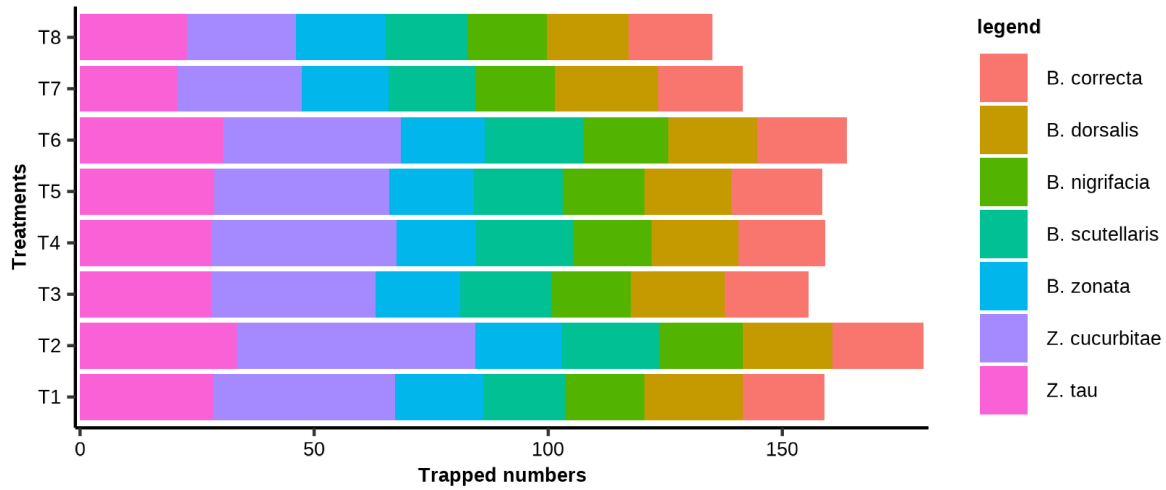


Figure 4. Trapped fruit fly species scenario in different treatments in watermelon at Chitwan, April-June 2023

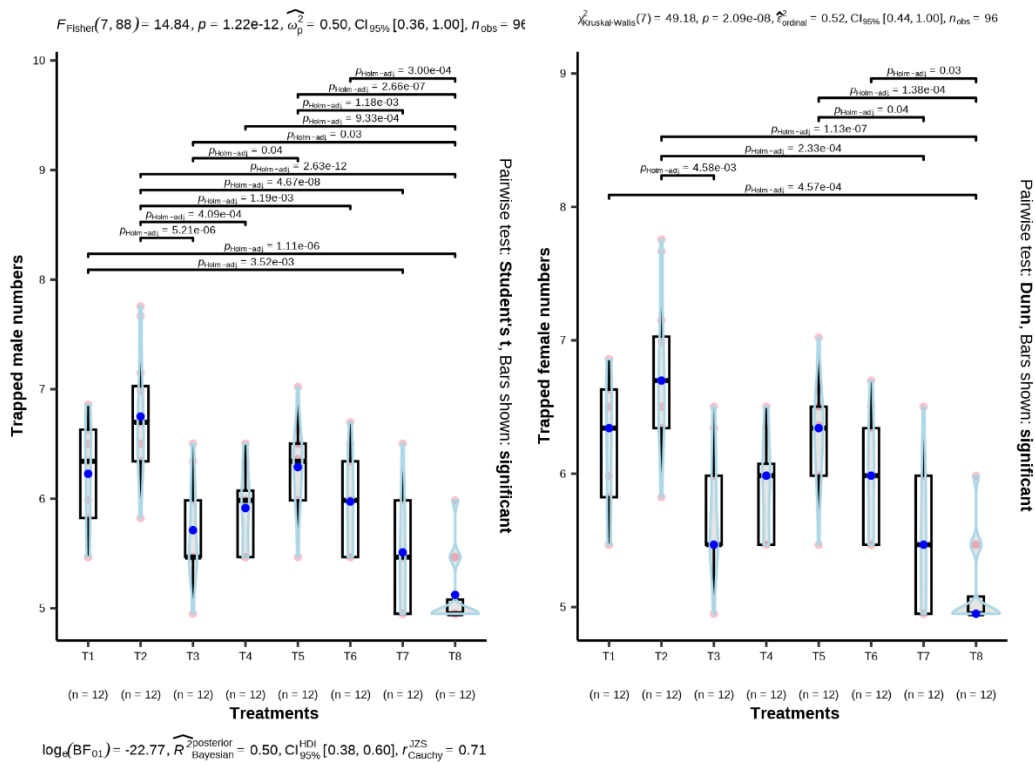


Figure 5. Status of mean male (right) and female (left) trapped fruit fly (per stage) in different treatments installed in watermelon at Chitwan, April-June 2023. Inside the box violin plot, blue dot represents the average mean value, Similarly, n represent the total number of sample (3 replications×4 weeks =12)

Furthermore, the least trapped which was statistically at par with citronella oil (11.79±0.28). Both trapped male and female numbers in each treatment were significantly different (p<0.001). Figure 5. More adult fruit flies were trapped in sticky yellow traps than in other trap colors (Said et al., 2017). Protein bait prepared from brewery wastage is also effective and economical for fruit fly trapping and their control program (Gopaul & Price, 2001). Among eight treatments, the highest number of trapped males (8.26±0.25) and females (6.75±0.16) were observed in the yellow sticky trap. Whereas, the lowest numbers (6.12±0.27) & (5.12±0.09) were found in the control. The male female ratio was >1 in all treatments however they were not significantly different with each other's Figure 6.

**Effect of Different Treatments on Trapped Fruit Fly Species**

A total of seven species of fruit flies were captured during the whole experimental period, which is visually displayed via a Stack bar plot Figure 4. Among them, *Zeugodacus cucurbitae* (Coquillett, 1889) followed by *Zeugodacus tau* (Walker, 1849) dominating over other species, and *Bactrocera nigricifacia* (Zhang, Ji and Chen, 2011) was found in the least number. The proportion of *Bactrocera zonata* (Saunders, 1842), *Bactrocera scutellaris* (Bezzi, 1913), *Bactrocera dorsalis* (Hendel, 1912), *Bactrocera correcta* (Bezzi, 1916), *B. zonata* was similar. According to (Umeh & Garcia, 2008), brewery-containing bait can be used for the fruit fly detection

program since it can easily attract and prefers *Ceratistis spp.*, *Z. cucurbitae* (Coquillett) followed by *Z. tau* (Walker). *Z. cucurbitae* (Coquillett) followed by *B. dorsalis* (Hendel) and *B. tau* (Walker) were the dominating species in Budgam and Srinagar (Ganie et al., 2013).

**Economic Analysis of Different Treatments**

The highest obtainable yield was found in yellow stick trap and citronella oil (32.57±1.31 Mtha<sup>-1</sup>) whereas the lowest was in control (18.14±4.74 Mtha<sup>-1</sup>). All treatments except the control treatment were found profitable for long-

term watermelon farming. Despite of high initial investment, damage due to fruit fly was low in citronella oil considering its repellency nature and hence results in BCR:1.63±0.06. Similarly, the initial installment cost of the yellow sticky trap was also high however it provided a high return (BCR:1.65±0.06). This finding is in line with (Lu et al., 2012), which suggested that the yellow sticky trap can also use for the white fly control in tomato, inside the greenhouse. Further, instead of low initial investment, the damaged percentage due to fruit fly was high (11.1±4.02) in black jaggery bait Table 2.

Table 2. Economic analysis of various treatments for the fruit fly control in watermelon at Chitwan, April-June 2023

Treatments	Obtainable Yield (Mtha <sup>-1</sup> )	Installment cost \$ ha <sup>-1</sup>	Total cost \$ ha <sup>-1</sup>	Total revenue \$ ha <sup>-1</sup>	Benefit Cost Ratio
Black jaggery	28.77±01	168.47	3,134.6	4,597.42	1.46±0
Yellow sticky trap	32.57±1.31	186.41	3,152.33	5,203.9	1.65±0.06
Watermelon flesh	28.01±1.31	166.14	3,132.28	4,476.13	1.42±0.06
Blue sticky trap	31.81±1.31	186.41	3,152.33	5,082.61	1.61±0.06
Sugarcane molasses	31.05±3.94	182.51	3,148.47	4,961.31	1.57±0.2
Brewery waste	31.81±1.31	194.2	3,160.04	5,082.61	1.6±0.06
Citronella oil	32.57±1.31	209.79	3,175.45	5,203.9	1.63±0.06
Tap water	18.14±4.74	153.19	3,119.49	2,899.27	0.92±0.24

Note: Installment cost = Treatment Installment cost, total cost = Installment cost+ other cost, farm sell price of the watermelon was 0.16\$kg<sup>-1</sup>

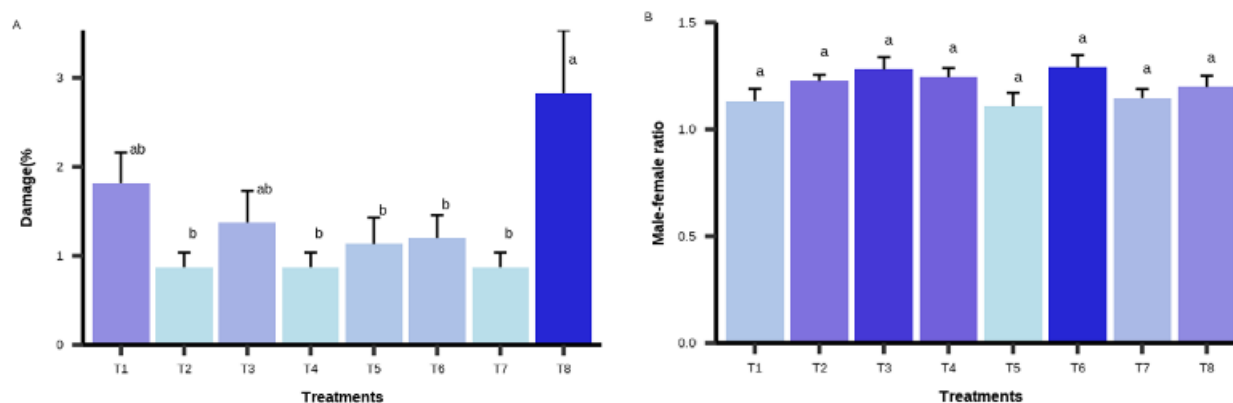


Figure 6. Male female ratio and fruit damage/ infestation percentage in various treatments in watermelon at Chitwan, April-June 2023

**Conclusion**

These findings reflect the result of the experiment carried out on the Saraswati F<sub>1</sub> hybrid variety of watermelon grown in the summer season of the subtropical region (Chitwan). Yellow sticky trap attracts the highest number of male and female fruit flies. The experiment documented seven different species of *Zeugodacus* and *Bactrocera* genera. *Z. cucurbitae* (Coquillett, 1899), followed by *Z. tau* (Walker, 1849), were dominant over the other five species i.e., *B. dorsalis* (Hendel, 1912), *B. scutellaris* (Bezzi, 1913), *B. correcta* (Bezzi, 1916), *B. zonata* (Saunders, 1842), and *Bactrocera nigrifacia* (Zhang, Ji, and Chen, 2011). In overall yellow sticky trap with cue lure and malathion would be efficient solution for fruit fly control.

**Declarations**

**Authors' Contribution**

Nawaraj Pandey: conceptualization, data curation, data visualization & analysis, and writing-original draft,

Priya Karna: data visualization & analysis, methodology preparation and language editing

Nabin Bhusal: Supervision, reviewing & editing and approved the final manuscript

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**Availability of Data and Materials**

Data will be made available upon reasonable request

**Conflict of Interest**

The author declares no conflicting interest.

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Not needed

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## Optimization of Angoumois Grain Moth (*Sitotroga cerealella* Olivier) Infestation in Stored Grains as Influenced by Some Botanical Powders

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### ABSTRACT

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*Sitotroga cerealella*

The Angoumois grain moth, *Sitotroga cerealella* Olivier is predominantly a devastating infested stored grain pest of cereals, whose development proceeds within a single grain of infested cereals. Investigating greener alternatives to widely used chemical control techniques is crucial because synthetic chemicals pose risks to public health and the environment. This investigation was carried out for developing the ecofriendly control management of the Angoumois grain moth in stored cereals through utilizing four botanical powders and one insecticide, wood ash (1 gm), and a single synthetic insecticide (Carbaryl) (0.25mg), neem (1 gm), Korobi (1 gm), Bishkatali (1 gm) and Datura (1 gm) treatments against untreated control in Completely Randomized Design (CRD). It was revealed that Neem (*Azadiracta indica*) powder at 1 g/100 g seed performed excellently, resulting in minimization of adult mortality percent, adult emergence, grain weight loss, and number of holes per ten seeds. The maximum percent of germination was noted in bishkathali (*Persicaria lapathifolia*) powder at 1 g/100 g seed, and moreover, bishkathali powder functions more effectively for limiting infestation percent. Therefore, the botanical neem and bishkathali powder at 2 g/100 g seed rate is the better alternative of carbaryl at 0.5 g/100 g rate, and it could be suggested for Angoumois grain moth management at storage.

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### Introduction

Cereals have been a major source of nutrition for nearly one-third of humanity, with a particular emphasis on developing regions including Sub-Saharan Africa and Southeast Asian countries. Rice, wheat, and maize account for approximately 85% of the global production of cereals (Erenstein et al., 2022). Bangladesh produces 38.09 million metric tons of rice using 26.02 million acres of land, with around 90 percent of people relying on rice for sustenance (BBS, 2023). Over 65% of this rice is stored by farmers for a variety of purposes, such as food, feed, and seed, and the rice is preserved as paddy, brown, or milled rice. Bamboo containers (dole or golas) and earthen jars (motka) are among the standard storage methods for parboiled rice. In addition, grain losses are considerable as a result of improper storage, which is primarily caused through pests such as weevils, beetles, moths, and rodents, despite manufacturing advances. Song et al. (2020) calculated that storage losses in rice can exceed 10% when farm losses are included, and insects alone are to blame for up to 12.61% of the losses (Maziku, 2019).

Along with the most negative pests in stored rice are the grain moth (*Sitotroga cerealella*), rice weevil (*Sitophilus oryzae*), and red flour beetle (*Tribolium castaneum*), via moths and beetles more often attacking raw rice and weevils targeting milled rice (Zote Vaishali & Shukla, 2023a). *Sitotroga cerealella*, also known as the Angoumois grain moth or paddy moth, is one of the most critical parasites in safeguarded rice (Zote Vaishali & Shukhla, 2024). It infests grains in fields and storage conditions, boosting its potential for damage (Sowmya et al., 2023), as well as the moth is especially hazardous in Bangladesh because it is termed "surui" and affects stored wheat, maize, and other agricultural products like joar and bran; therefore, single larvae can cause 13–24% economic losses in grain weight as well as reductions in nutritional value. Furthermore, the larvae bore into the cereal, making it more susceptible to secondary parasites and rendering the rice unsatisfactory for digestion.



Suppression of pests, notably the Angoumois grain moth, has traditionally relied on synthetic pesticides, which, while effective, pose ecological risks and contribute to pest resistance (Zhang et al., 2021). Due to these concerns, there is growing interest in learning alternative, eco-friendly pest control methods. Historically, botanical products have been used as natural insecticides, repellents, and antifeedants (Yesmin et al., 2023). These solutions based on plants are cost-effective, locally available, and less detrimental to the natural world compared to synthetic chemicals; additionally, some of the most effective botanical products include extracts, oils, and granules from leaves like neem (*Azadirachta indica*), karanja (*Millettia pinnata*), and mahogany (*Swietenia macrophylla*). In Bangladesh, the fundamental applications of these botanicals or organic plant materials have proven highly effective against stored insects and pests, as well as increased plant protection mechanisms through improved biochemical synthesis (Karim et al., 2024; Laboni et al., 2024; Howlader et al., 2023). This study aims to investigate the damage caused by the Angoumois grain moth in rice storage and evaluate the effectiveness of various indigenous extracts from plants as a sustainable, eco-friendly pest management solution. The study will assess the extent of grain damage caused by the pest and look at the use of botanicals as an alternative to synthetic insecticides, reducing environmental pollution and minimizing contamination of stored grains. In addition, this technique is intended to provide farmers with a more sustainable solution for pest control while ensuring enhanced storage situations.

## Materials and Methods

### Experimental Setup

The present research assessed the impact of various botanicals on handling of Angoumois grain moth (*S. cerealella*) under laboratory conditions, as well as the scientific study was carried out in the MS Laboratory-II, Department of Entomology, Bangladesh Agricultural University (BAU), from March 2023 to February 2024. Treatments were put together in a completely randomized design (CRD) with five replications; additionally, ten adult moths were introduced into each container holding disinfected, uninfected rice grains.

In the present experiment, the BR-11 rice variety was used, and an examination presents four types of botanical powders, wood ash, and one chemical pesticide, with each treatment replicated five times. The botanical granule dosage was 1 gram per 50 grams of grain, while the pesticide dosage was 0.25 gram per 50 grams of grain (Figure 1c). There were six treatments used in this experiment compared to the control. Among all treatments, there were three types, such as botanical, wood ash, and chemical. Seeds were acquired in March 2023 to carry out the task at hand. The seeds were scrubbed, dried, and salted out from damaged, unhealthy seeds and stored in a large polythe box in an airtight condition to keep them free from insects and microorganisms.

### Statistical Analysis

The research investigations were conducted in a highly randomized manner. The data were analyzed using the SPSS 29 statistics software, version 2022. Mean values are separated by the X-stat test at 5% probability to gauge the significance of individual treatment.

Table 1. Details about the treatments

Type	Treatments Name	Scientific Name	Doses	Form
Botanical	Neem	<i>Azadirachta indica</i>	1gm	Powder
	Korobi	<i>Nerium oleander</i>	1gm	
	Bishkatali	<i>Persicaria lapathifolia</i>	1gm	
	Datura	<i>Datura metel</i>	1gm	
	Wood Ash		1gm	
	Control	No uses	No uses	
Insecticide	Name of the insecticide	Group	Doses	Form
	Abin 85 SP	Carbaryl	0.25gm	Powder



a



b



c

Figure 1. a. Set up of the experiment; b. Mass Rearing of Angoumois Grain Moth Inside of Earthen Pots and c, Preparation of different treatments

**Rearing of Angoumois grain moth (*S. cerealella*) and test insects**

Steps	Details
Moth Placement	50-60 moths placed in 10×10-inch earthen pots with rice grains at 26-27°C for 30-35 days.
Identification	Males and females distinguished via microscope; females (5.5 mm) and males (5.0 mm) separated by aspirator based on abdominal characteristics.
Mass Rearing	Thousands of adults collected and reared in a glass cylinder covered with a 32-mesh net for mating and egg-laying.
Egg Collection	Eggs laid on cylinder walls were brushed, sieved, cleaned, and stored in a refrigerator at 4°C for future studies.
Egg Hatching	Collected eggs placed on white paper in a Petri dish for hatching.
Larvae Transfer	Newly hatched larvae transferred to Petri dishes containing grains for further observation and study.

**Data Collection**

Parameters	Details
Moth Exposure	Ten newly emerged moths were placed in plastic containers with insect-free rice grains treated with various substances, including an untreated control. Moth mortality was monitored every 24 hours for 21 days.
Adult Emergence Observation	After 24–28 days, new adults began emerging from the grains; the numbers of emerged adults were recorded by gently shaking the containers and removing cloth covers.
Mortality Counting	Adult moth mortality was counted at 7, 14, and 21 days after treatment (DAT). The total death count in treated and untreated conditions was compared, and the percentage of adult mortality was calculated using the formula: % Mortality = (Total deaths / Initial population) × 100
Cumulative Mortality	Cumulative mortality was calculated at 5, 10, 15, and 20 DAT as the total number of deaths within the population over time, expressed as a percentage.
Seed Damage Evaluation	After adult emergence, seeds were cleaned, and 10 random seeds were inspected to count holes made by larvae. The percentage infestation was calculated as % Infestation = (Number of infested seeds / Total seeds) × 100.
Adult Emergence Rate	To determine adult emergence rate, larvae or pupae were reared and monitored daily, with emergence rates calculated at 7, 14, 21, and 28 DAT.
Cumulative Adult Emergence	Daily emergence counts were recorded and cumulatively added up to obtain a total. Cumulative emergence was plotted to observe trends, with counts at 7, 14, 21, and 28 DAT.
Seed Hole Count	Ten random seeds were inspected for visible holes, with the total number of holes counted.
Grain Weight Loss Measurement	Initial grain weight and subsequent weights at each recording time were measured. Percentage weight loss was calculated as % Grain Weight Loss = (Initial Weight - Recorded Weight) / Initial Weight × 100.
Seed Viability (Germination Test)	The germination rate was tested by placing 10 seeds on water-soaked blotting paper for 5 days at 27-34°C. After germination, the rate was calculated as % Seed Germination = (Germinated Seeds / Total Tested Seeds) × 100.

**Results**

The mortality rates of adult Angoumois grain moths are significantly different from one another, and the percentage of adult mortality increases each day following treatment. Among all treatments, insecticide had the highest mortality rate (99%) at 21 DAT, followed by Neem (86%), Bishkatali (62%), Korobi (42%), Dhutora (40%), Ash (36%), and Control (34%), respectively (Table 2).

Among the all treatments, the highest cumulative mortality (10%) was observed in insecticide (Carbaryl) after 5 DAT, while among the botanical treatments, it was observed that Neem had the highest cumulative mortality (9%) after 20 DAT. Control treatment demonstrated the lowest cumulative mortality in different days after treatment (Figure 2).

The mean number of adult moths that emerged from grains varied significantly among the various treatments that were applied (Table 2). In terms of all treatments that were executed, the highest adult emergence was observed at 14 DAT, with a value of 50.45 in control (Table 2). This number of adult emergences was followed by wood ash (32.46), korobi (29.80), and dhutora (26.43).

The mean value of the untreated control was 138.11, which is the maximal cumulative adult emergence that was recorded. The second highest emergence was observed in Ash (74.03), followed by Korobi (72.73) and Dhutora (65.41). Bishkathali's mean value was 56.4. The considerably lowest cumulative number of adult emergences observed was 43.77 in Neem (Table 2).

Table 2. Effect of different treatments on adult mortality (%) and numbers of adult emergence of Angoumois grain moths

Treatment	Adults Mortality (%)			Numbers of adult emergence			
	7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT	28 DAT
Neem	56 a	66 b	86 b	9.62 b	16.01 b	14.31 b	3.83 b
Korobi	36 a	38 b	46 d	13.83 d	29.80 d	22.29 d	6.81 d
Dutora	28 d	34 d	40 d	12.81 d	26.43 cd	19.41 cd	6.76 d
Bishkatali	40 c	54 c	62 c	10.26 c	23.89 c	17.63 c	4.62 c
Wood ash	26 d	44 d	36 e	13.81 d	32.46 d	20.49 cd	7.27 d
Insecticide (Carbaryl)	91 a	94 a	99 a	1.40 a	2.60 a	4.00 a	2.01 a
Control	14 e	32 d	34 e	16.44 e	50.45 e	45.00 e	26.22 e
LS	*	*	*	*	**	**	**
CV (%)	16.26	14.76	14.4	14.67	8.94	12.87	18.46
LSD (0.05)	3.23	4.68	2.29	1.15	0.96	1.32	1.67

Here, Means having same letter within a column do not differ significantly at 5% level of probability, LS= level of significance \*= Significant at 5% level of probability and \*\*= significant at 1% level of probability

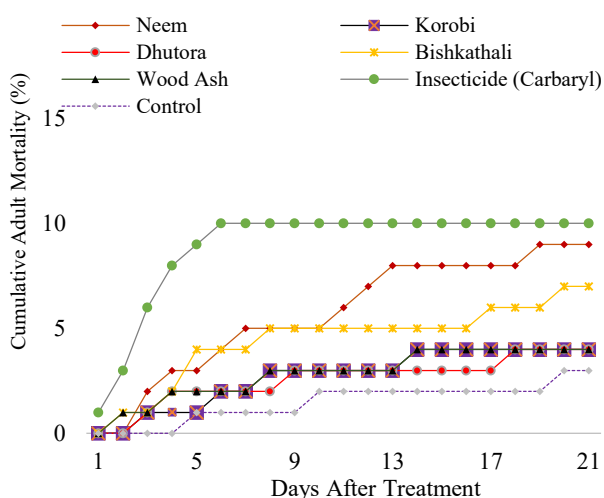


Figure 2. Cumulative mortality of Angoumois grain moth against different Treatments for several days

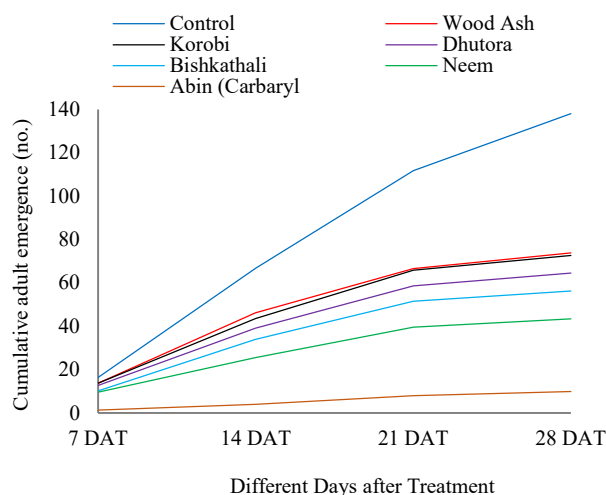


Figure 3. Effects of different treatments on cumulative adult emergence

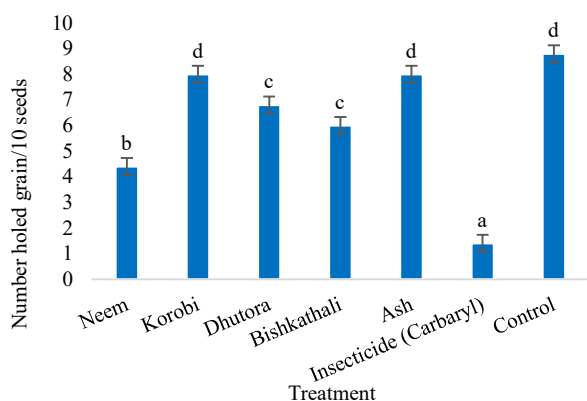


Figure 4. Number of holes grains per 10 seeds for different treatments.

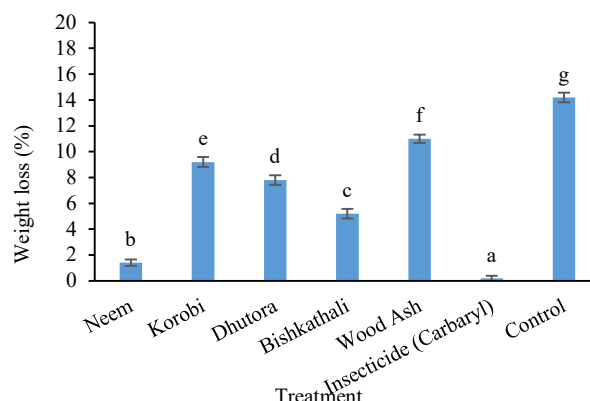


Figure 5. Weight loss of grain (%) for different treatments by AGM.

The number of holes per 10 seeds for different botanicals, ash, and pesticides displays inconsistent results. Neem, pesticide, (dhutora and bishkatali), and (korobi, ash, and control) are substantially distinct from each other, while dhutora and bishkatali do not significantly differ. On the other hand, Korobi, wood ash, and control are considerably dissimilar. It had been observed that the control treatment had the most numbers of hole seeds per ten seeds (8.8), and moreover, it was demonstrated that the wood ash (8) and the korobi both had the exact same

numbers of hole seeds per ten seeds (8), followed by Bishkathali (6). The lowest numbers of holes per grain per 10 seeds were exhibited in insecticide (1.4), and additionally, Neem resulted in better performance with a value of 4.4 (Figure 4).

Untreated control treatment had the highest quantity of weight loss (14.2%), followed by wood ash (11%) and korobi (9.2%), respectively, whereas it was the minimal in insecticide (Carbaryl) (0.2 %) (Figure 5).

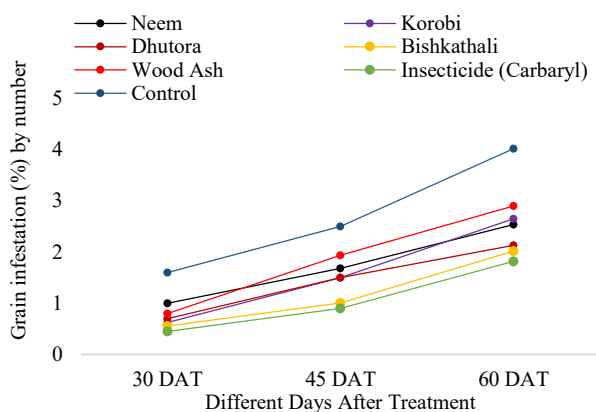


Figure 6. Effect of different treatments on the grain infestation by number of stored grain seeds during the management of AGM

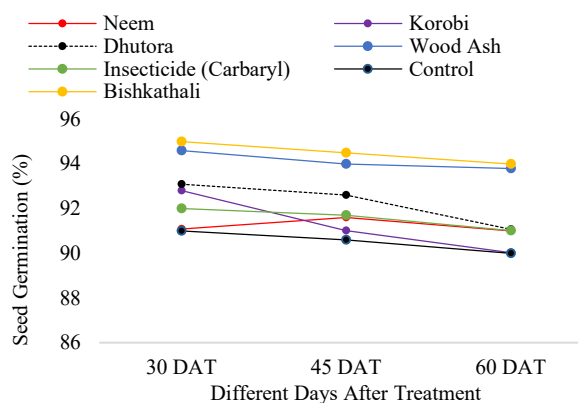


Figure 7. Effect of different treatments on germination of stored seeds during the management of AGM

The diseases infestation was statistically significant at the 1% level of probability, considering the seven promising treatments. Bishkathali demonstrated the minimum percent of infestation to be 0.56, 1.01, and 2.02% among the botanical treatments at 30, 45, and 60 DAT, respectively. The minimum percent of infestation was lowest in insecticide (Carbaryl) at 0.45, 0.905, and 1.82% for 30, 45, and 60 DAT, respectively (Figure 6).

Statistically, percent germination of rice seed was varied significantly at the 1% level of probability among seven promising treatments. At 30, 45, and 60 DAT, the maximum germination percent of the evaluated seven treatments was bishkathali, with values of 95, 94.5, and 94%, respectively. The subsequent maximum germination percent was observed in wood ash, with values of 94.8% at 30 DAT, 94% at 45 DAT, and 93.80% at 60 DAT (Figure 7).

## Discussion

Protecting crops from insects is one of the global problems. Continuous uses of synthetic chemicals lead to toxicity, endangering the health of farm operators, bioagents, animals, farmers, and food consumers, and additionally harming the ecology. Application of botanical extracts and dried powders has an enormous effect on biorational or integrated pest management tactics for insects and pests. This strategy is recognized by many as eco-friendly. From the outcomes of this investigation, the highest mortality and cumulative mortality percent recorded in carbaryl were followed by Neem. In spite of the fact that the mortality time of *Sitophilus granarius* and *Rhizopertha dominica* decreased as concentrations of botanical treatments increased (Hassan et al., 2022). *Azadirachta indica* (neem) was more effective at reducing *S. cerealella* by increasing mortality percent; however, different levels of efficacy may be observed. The adult emergence of the insect was substantially reduced or prevented by the botanical powders and extracts.

Akter & Jahan (2013) found that neem plant extracts had the most deleterious effects, such as the number of dead insects at 72 DAT (14.75), insect mortality (100%), adult mortality (0.00), and germination (96%), and additionally, biskatali, dhutora, korobi, and ash showed inferior performance than neem.

The study appears to be similar to Zote Vaishali & Shukla (2023b), and findings mentioned that the neem leaf powder was found most effective due to causing higher mortality. Naseri et al. (2017) found that *Corcyra cephalonica* (Stainton) in stored rice kernels is used as some selected botanicals, such as neem, tulsi, karanj, and eucalyptus leaf powder. The result revealed the highest mortality (81.67%) after 30 DAT, and it was concluded that the neem leaf powder was most effective. Untreated control treatment had the highest number of adult emergence and cumulative adult emergence; similar observations were recorded by Akter & Amin (2017). The least number of adult emergences was observed in Neem, indicating that the minimum number of newly emerged adult Angoumois grain moths was observed. After that, bishkatali, dhutora, korobi, and ash exhibit a lower number of adult emergences than control. The study's findings indicate that atypical development prevents adult emergence. By using botanicals, the average grain weight loss was 13.63%, and a germination percentage of 68.75% was recorded in *Sitophilus*-infested sorghum. Hossein et al. (2018) observed that bishkatali leaf powder had the effect of minimizing grain infestation (0.58%) at 30 DAT and increasing germination (92.13%) of paddy.

## Conclusion

Considering the findings of this study, it was exhibited that the insecticide (Carbaryl) was performed superiorly in the extent of highest mortality and cumulative mortality, weight loss control, but to protect the ecological environment, which is not suggested for controlling Angoumois Grain Moth. Among the botanical treatments, Neem powders performed better at increasing adult mortality, reducing grain weight loss, adult emergence, and reducing grain hole problems in seeds. Additionally, bishkathali powders are suggested for reducing infestations and subsequently increasing the germination rate. The results of this study indicate that Neem and Bishkathali powders were more effective in suppressing the Angoumois grain insect.

## Declarations

### Acknowledgements

All authors express their gratitude to the laboratory staff of the Department of Entomology at Bangladesh Agricultural University, Mymensingh-2202, for their cooperation during the evaluation period of this investigation.

### Conflicts of interest

All authors declared there was no conflict of interest.

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## Evaluation of the Aquaculture Consumption Habits of Yalova University Students

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 30.09.2024 Accepted : 06.12.2024</p> <p><b>Keywords:</b> Aquaculture Consumption Survey Yalova University Student</p>	<p>This study aims to identify the seafood consumption habits and preferences of students at Yalova University and was conducted using an online survey on a voluntary basis. Among the participants, 67% were women, 33% were men, and 88% were aged between 18 and 25. The results show that 60% of students consume chicken, while 26% consume fish. 29% of the students eat fish once a month, and 54% consider their fish consumption insufficient. 40% of participants find farmed seafood to be healthy, while 16% prefer omega-3 supplements over seafood. Additionally, 40% believe they do not consume enough seafood. The primary sources of information on the benefits of seafood are mobile devices and their close social circles. When purchasing fish, participants prioritize freshness and price, and they mostly buy fish from fishmongers. The most preferred fish species are marine fish, and the majority of participants do not prefer processed seafood. Among cooking methods, frying is the most common. Mussels and shrimp are the most consumed seafood after fish, and seafood consumption is highest during the fishing season in the winter months. The study results indicate that various strategies need to be developed to increase seafood consumption among Yalova University students. To raise awareness about healthy eating, more effective informational campaigns on the benefits of seafood should be conducted. Local fish markets should be supported to ensure seafood is fresh, affordable, and accessible. Additionally, including seafood in student housing menus and incorporating nutrition and seafood consumption education into curricula will be crucial steps to increase consumption.</p>

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## Yalova Üniversitesi Öğrencilerinin Su Ürünleri Tüketim Alışkanlıklarının Değerlendirilmesi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 30.09.2024 Kabul : 06.12.2024</p> <p><b>Anahtar Kelimeler:</b> Su ürünleri Tüketim Anket Yalova Üniversitesi Öğrenci</p>	<p>Bu çalışma, Yalova Üniversitesi'nde eğitim gören öğrencilerin su ürünleri tüketim alışkanlıkları ve tercihlerini belirlemeyi amaçlamakta olup, çevrim içi bir anket yöntemiyle ve gönüllülük esasına dayanarak toplam 315 öğrenciyle gerçekleştirilmiştir. Katılımcıların %67'si kadın, %33'ü erkek olup, %88'i 18-25 yaş aralığındadır. Araştırma sonuçlarına göre, öğrencilerin %60'ı tavuk eti tüketmekte, balık eti tüketiminin ise %26 olduğu tespit edilmiştir. Öğrencilerin %29'u ayda bir kez balık tüketmekte, %54'ü ise tükettikleri balık miktarını yeterli bulmamaktadır. Katılımcıların %40'ı yetiştiriciliği yapılan su ürünlerini sağlıklı olarak değerlendirmekte, %16'sı ise su ürünleri yerine omega-3 gibi takviye edici gıdaların tercih edilebileceğini ifade etmektedir. Ayrıca, %40'ı su ürünlerini yeterince tüketmediklerini düşünmektedir. Öğrencilerin su ürünlerinin faydaları hakkında genel bilgi kaynakları arasında mobil cihazlar ve yakın çevrelerindeki insanlar öne çıkmaktadır. Balık satın alırken katılımcıların öncelikli olarak tazeliğe ve fiyata dikkat ettikleri, balığı genellikle balıkçılardan satın aldıkları belirlenmiştir. En çok tercih edilen balık türü deniz balıkları olup, katılımcıların büyük kısmı işlenmiş su ürünlerini tercih etmemektedir. Balık pişirme yöntemleri arasında ise en yaygın tercih kızartmadır. Balık dışında en çok tüketilen su ürünleri midye ve karides iken, su ürünleri tüketiminin en yoğun olduğu dönem ise balıkçılık sezonunun kapsamadığı kış aylarıdır. Çalışma sonuçları, Yalova Üniversitesi öğrencilerinin su ürünleri tüketimini artırmaya yönelik çeşitli stratejilerin geliştirilmesi gerektiğini göstermektedir. Özellikle öğrenciler arasında sağlıklı beslenme bilincinin artırılması için su ürünlerinin faydaları konusunda daha etkili bilgilendirme çalışmaları yapılmalı ve su ürünlerinin taze, uygun fiyatlı ve ulaşılabilir olmasını sağlamak amacıyla yerel balıkçılık pazarları desteklenmelidir. Ayrıca, öğrenci barınma yerlerinde su ürünlerinin menülere dahil edilmesi, beslenme ve su ürünleri tüketimi konusunda eğitimlerin müfredata eklenmesi de bu tüketimi artırmaya yönelik önemli adımlar olacaktır.</p>

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## Giriş

Dünya nüfusu hızlı bir şekilde artarken, beslenme için kullanılan sağlıklı gıda kaynaklarının yeterli bir düzeye getirilmesi önem arz etmektedir. İnsan sağlığının korunması ve sağlıklı nesiller yetiştirmek için dengeli beslenmenin önemi ve gerekliliği konusunda hassasiyet gösterilmelidir. Bu açıdan, dengeli beslenme konusunda bireylerin tüketim alışkanlıklarının belirlenmesi daha iyi sonuçlar verecektir (Sezgin ve ark., 2023).

Gelişmiş ülkelerde insanlar, beslenmelerine çok dikkat etmekte ve sağlık açısından beslenme alışkanlıklarında uygun gıdaları seçmeye özen göstermektedirler. İnsan sağlığı açısından önemli besin kaynağı olan su ürünleri, insanların protein ihtiyacını karşılamak amacıyla kullanılan kaynakların başında gelmektedir (Karslı ve ark., 2021). Sağlıklı ve dengeli beslenme için günlük alınması gereken protein ihtiyacının %40-50'si hayvansal kaynaklı proteinlerden karşılanmaktadır (Karakuş ve ark., 2008; Saygı ve ark., 2015; Beyter, 2020). Su ürünleri, sağlıklı beslenme öğelerinin vazgeçilmezleri arasında yer alan bir besin maddesidir.

Su ürünleri yapısında bulunan çoklu doymamış yağ asitleri, esansiyel aminoasitler, mineral maddeler, vitaminler ve zengin protein içeriği olan besinler ile sağlıklı ve dengeli beslenmede önemli rol oynamaktadır (Çağlak ve ark., 2016). Su ürünleri insan vücudunun fizyolojisi ve metabolik fonksiyonları üzerinde olumlu etkiler yaparak sağlıklı yaşam sürdürmede önemli bir besin kaynağıdır (Kaya ve ark., 2004). Bu ürünler zengin besin içeriği sebebiyle günlük diyet listelerinde tercih edilmektedir (Gündüz ve ark., 2018). Denize kıyısı olmayan alanlarda yaşayan ve sağlıklı beslenmek isteyen tüketicilerden de giderek daha fazla talep gören bir ürüne dönüşen su ürünlerinin uzmanlar tarafından haftada en az iki öğün tüketilmesi tavsiye edilmektedir (Kayalı, 2022). Dengeli ve sağlıklı beslenme bilincine sahip olan tüketiciler, kolesterolü düşük, bağ dokusu düşük, doymamış yağ ve esansiyel amino asit içeriği yüksek ve sindirimi kolay olan su ürünlerini özellikle tercih etmektedir (Çağlak ve Karşlı, 2017).

Türkiye'de 2020 yılında kişi başına su ürünleri tüketimi yıllık 6,7 kg iken dünya ortalaması 22 kg'dır (TEPGE, 2023). Tüketim miktarı; kişilerin alışkanlıkları, su ürünlerinin üretim miktarı, ürünlerin fiyatları ve tüketicilerin alım gücü gibi faktörlerle ilişkilidir. Türkiye'de su ürünleri tüketimi bölgelere göre farklılık göstermekle birlikte, 2022 yılında kişi başı yıllık su ürünleri tüketimi bir önceki yıla göre %12 oranında artışla 7,3 kg olarak belirlenmiştir (TÜİK, 2023).

Dünya genelinde kişi başı tüketim miktarına bakıldığında, Türkiye'de su ürünleri tüketim alışkanlığının gelişmemiş ülkelerin bile gerisinde olduğu gözlenmektedir (Arslan ve Oğuzhan Yıldız, 2021). Bu bağlamda, su ürünleri tüketiminin artırılmasına yönelik çalışmaların yapılması gerekmektedir.

Çalışmanın yapıldığı Yalova, coğrafi konumu gereği Türkiye'de Marmara denizine en çok kıyısı olan illerden biridir. Bu nedenle Yalova'da balık tedarikinin genelde avcılık yoluyla sağlandığı görülmektedir. Yalova Üniversitesine ülkemizin çeşitli bölgelerinden öğrenciler eğitim görmek için gelmektedir. Öğrencilerin balık tüketimi hem geldikleri bölgenin sosyo-ekonomik ve demografik faktörlerine, hem de hane gelirlerine göre

farklılık göstermektedir. Literatür çalışmalarına bakıldığında su ürünleri tüketim alışkanlıkları ile ilgili (Balık ve ark., 2013; Uzundumlu ve Dinçel, 2015; Arıca, 2017; Bolat ve Cevher, 2018; Bolat ve Telli, 2019; Cengiz ve Özoğul, 2019; Kaplan ve ark., 2019; Karakulak ve ark., 2020; Kuşat ve Şahan, 2021; Selvi ve ark., 2022; Terin ve İnanç, 2023) ve öğrenci esaslı (Yavuzcan ve ark., 2010; Sarı ve ark., 2019; Olgunoğlu ve ark., 2020) çalışmalar mevcuttur. Fakat Yalova'da üniversite okuyan öğrenciler özelinde su ürünleri tüketimine yönelik böyle bir çalışmaya rastlanmamıştır. Çalışmanın temel amacı, Yalova Üniversitesinde öğrenim gören öğrencilerinin balık tüketim tercihlerini etkileyen faktörlerin belirlenmesidir.

## Materyal ve Yöntem

Çalışmamız 2023-2024 eğitim-öğretim yılının Nisan ayında Yalova Üniversitesinde okuyan ve basit rastgele örnekleme yöntemi kullanılarak farklı eğitim seviyelerinde öğrenim gören (Önlisans, Lisans ve Yüksek Lisans) öğrenciler ile yapılmıştır. Gönüllülük esasına göre çalışmaya katılan 315 öğrenciye, kişisel bilgileri ve sosyo-ekonomik durumlarıyla ilgili 6 soru, su ürünleri tüketimi hakkında ise 24 soru olmak üzere toplamda 30 soru yöneltilmiştir. Öğrencilere yöneltilen anket soruları; yapılan literatür taramaları ve araştırmacıların mesleki deneyimleri ile birleştirilerek oluşturulmuştur. Anket çevrim içi olarak öğrencilere gönderilmiş, bu amaçla Google Forms adlı internet uygulaması kullanılmıştır. Uygulamadan elde edilen veriler yüzde oran olarak tanımlanmıştır. Ankette öğrencilerden en fazla beğendikleri ve en fazla tükettikleri et grubu, en beğendiği ve en fazla tükettikleri balık türleri, balık etini ne şekilde pişirdikleri, ne kadar sıklıkla balık eti tükettikleri, balık eti dışında hangi su ürünlerini tükettikleri, balık fiyatları, su ürünlerini nerelerden aldıkları gibi sorular yöneltilmiştir. Elde edilen sonuçlar Microsoft Excel programı kullanılarak değerlendirilmiştir.

## Bulgular ve Tartışma

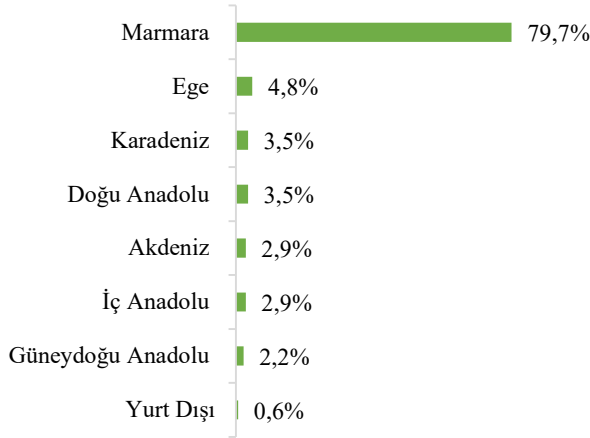
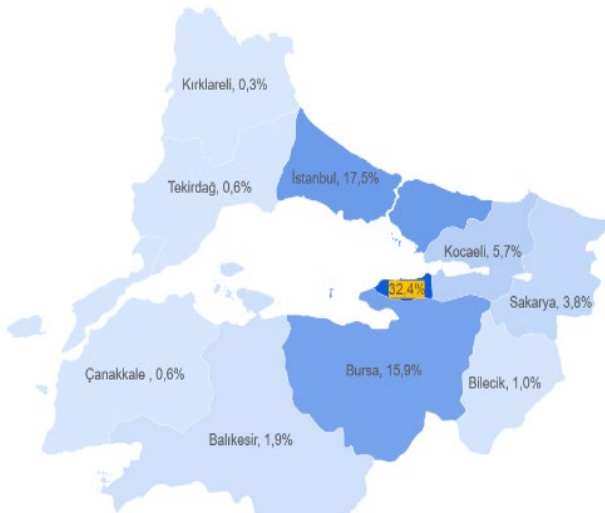
Ankete katılan öğrencilerin %43,2'si kadın, %56,8'i erkek, %83,5'inin 18-25 yaş aralığında olduğu, katılımcıların ailelerinin gelir düzeylerinin kategorik olarak %14,3'ü 15.000₺'nin altında, %18,7'sinin 15.001-20.000₺ arasında, %20,6'sının 20.001₺-30.000₺ arasında, %15,2'sinin 30.001-40.000₺ arasında ve %31,1'inin 40.000₺ üzerinde belirlenmiştir (Çizelge 1). Çalışmamızda Türkiye'nin her bölgesinden ve yurt dışında ikamet eden öğrenciler bulunmaktadır (Şekil 1). Katılımcıların %79,7'sinin Marmara bölgesinde ikamet ettiği görülmektedir (Şekil 1). Marmara Bölgesi'ne ait il bazında dağılımlar ise Şekil 2'de verilmiştir. Çalışmamızda hane reisinin mesleği %30,2 özel sektör çalışanı, %24,4 emekli, %19,7 kamu çalışanı, %14,9 esnaf, %6 işsiz, %2,5 işçi ve %2,5 diğer meslek grupları olarak tespit edilmiştir (Çizelge 1).

Yaptığımız çalışmada ankete katılan öğrencilerin et tüketim tercihleri incelendiğinde katılımcıların %28,4'ü tavuk eti, %24,4'ü kırmızı et ve %11,3'ü balık eti tüketmekte iken %1,3'ünün et tüketmediği (vejetaryen olduğu) belirlenmiştir. Sadece balık etini tüketmeyenlerin oranı ise %34,6 olarak hesaplanmıştır (Şekil 3).

Çizelge 1. Katılımcıların cinsiyet, yaş, aile gelir düzeyi ve hane reisinin meslek grubu dağılımları

Table 1. Distribution of participants by gender, age, family income level and occupational group of the household head

Cinsiyet	N	%
Kadın	179	56,8
Erkek	136	43,2
Yaş		
18-25	263	83,5
26-36	32	10,2
36-45	14	4,4
45+	6	1,9
Ailesinin Gelir Düzeyi		
<15.000₺	45	14,3
15.001-20.000₺	59	18,7
20.001-30.000₺	65	20,6
30.001₺-40.000₺	48	15,2
40.000+	98	31,1
Hane Reisinin Mesleği		
Özel Sektör	95	30,2
Emekli	77	24,4
Kamu Çalışanı	62	19,7
Esnaf	47	14,9
İşsiz	19	6
İşçi	8	2,5
Diğer	7	2,5

Şekil 1. Katılımcıların bölgelere göre dağılımı  
Figure 1. Distribution of participants by regionŞekil 2. Marmara bölgesinde ikamet eden katılımcıların oranı  
Figure 2. Participants residing in the Marmara Region

Sarı ve ark. (2019) “Öğrencilerin Balık Eti Tüketim Düzeyleri ve Tüketim Alışkanlıklarının Belirlenmesinde Çine MYO Örneği” adlı çalışmada öğrencilerin en çok tükettiği et türünün piliç eti olduğunu belirtmişlerdir. Kuşat ve Şahan (2021) “Su Ürünleri Tüketim Tercihleri Üzerine Uşak İlinde Bir Anket Çalışması” adlı çalışmada ankete katılanların en çok kırmızı et tükettiğini ve katılımcıların balık eti fiyatlarını diğer et türleri fiyatlarına göre normal bulduğunu belirlemişlerdir. Karakulak ve ark. (2020) yapmış oldukları “Erzurum İli Merkez İlçelerinin Su Ürünleri Tüketim Davranışları Üzerine Araştırmalar” tüketicilerin büyük bir kısmının fiyatın pahalı olmasından dolayı balık tüketimlerini olumsuz etkilendiğini, ayrıca katılımcıların yarısından fazlasının Erzurum’da balık fiyatlarını pahalı bulduklarını, tüm et türlerinin fiyatlarının aynı olması durumunda tüketicilerin kırmızı eti tüketeceklerini bildirmişlerdir. Bolat ve Telli (2019) yaptıkları “Denizli İli Su Ürünleri Tüketim Alışkanlıklarının Belirlenmesi” adlı çalışmada katılımcıların %63’ünün bütün et çeşitlerini tükettiklerini, fakat et türleri özelinde en çok kırmızı eti tükettiğini, balık eti tüketen katılımcıların %90’ının balığı lezzetli ve besleyici olmasından dolayı tercih ettiğini, katılımcıların %51’inin balık fiyatlarını normal bulduğunu, hamsi tüketmeyi tercih eden katılımcıların ucuz ve lezzetli olmasından dolayı tercih ettiklerini belirtmişlerdir. Bolat ve Cevher (2018) “Konya İli Su Ürünleri Tüketim Alışkanlıkları Üzerine Bir Anket Çalışması”nda katılımcıların en çok kırmızı eti tükettiğini balık fiyatları hakkında %64’ünün normal olarak belirttiğini bildirmişlerdir. Arıcı (2017) “Hatay İlinde, İskenderun Halkının Balıkçılık Ürünleri Tüketim Alışkanlığı ve Tercihlerinin Belirlenmesi” adlı çalışmasında tüketicilerin büyük bir kısmının kırmızı et tükettiğini, katılımcıların %79’unun balık etini sevdiğini, balık etini tüketmeme sebebi olarak en çok kokusu, ardından sırasıyla kılçıklı oluşu, temizleme zorluğu ve tadı/aroması şeklinde tespit ettiklerini belirtirken; tüketicilerin en çok balık fiyatlarının pahalı olması ve taze olmaması nedeniyle olumsuz olarak etkilendiklerini, tüketimin artması için fiyatların azaltılmasının gerektiğini düşündüklerini bildirmişlerdir.

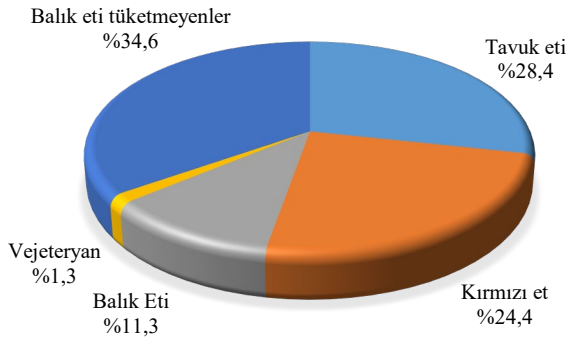
Yaptığımız çalışmada et grubu tüketim tercihinin en çok tavuk eti olması öğrenci merkezli yapılan Sarı ve ark. (2019)’nın çalışmasından elde edilen sonuç ile uyumludur. Burada kendi yemeğini pişiren öğrencilerin daha ucuz ve kolay hazırlanabilen bir et türü olarak tavuk etini tercih ettiği veya yurtlarda çıkan toplu yemeklerde genellikle tavuk etinin kullanılması kaynaklı olduğu söylenebilir.

Çalışmamızda öğrencilerin balık etini tüketmeme sebepleri arasında ilk sırada yaşam alanlarının uygun olmaması (%37,5) gelmektedir. Bunu sırasıyla pişirme sırasında meydana gelen koku (%13,3), fiyatının pahalı gelmesi (%1,3), balık etini sevmeme (%0,6), alışkanlıklar (%0,3) ve diğer sebepler (%0,3) izlemektedir (Çizelge 2). Öğrenciler barınma alanları olarak yurtları, pansiyonları vb. alanları kullanmaktadırlar. Bu alanların yemek pişirilebilecek alanlarının olmaması bu sonucun elde edilmesini sağlamış olabilir. Bunun yanında diğer seçeneklerin öğrenci merkezli olmayan çalışmalarla benzerlik gösterdiği söylenebilir. Öğrencilerin %37,5’i ise balık etini tükettiklerini, %16,5’i ise sebep göstermeden tüketmediklerini belirtmişlerdir. Ayrıca katılımcıların büyük bir kısmı balık fiyatlarını diğer et türleri ile kıyasladıklarında fiyatların normal olduğunu ve fiyatların yarıya inmesi durumunda balık tüketimlerinin artacağını belirtmişlerdir (Çizelge 2).

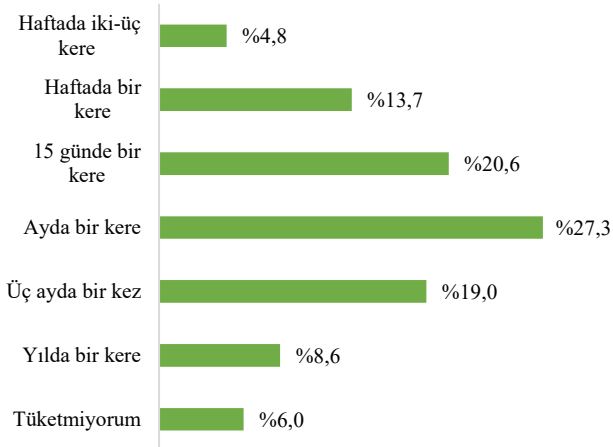


Tablo 2. Ankete katılanların balık etini tüketmeme sebebi  
Table 2. Reasons why respondents do not consume fish

Balık Etini Tüketmeme Sebebiniz Nedir?	N	%
Yaşam Alanım Uygun Değil	118	37,5
Tüketmiyorum	95	30,2
Tüketiyorum	52	16,5
Pişirme sırasında Meydana Gelen Koku	42	13,3
Bütçeme Pahalı	4	1,3
Balık Etini Sevmeme	2	0,6
Alışkanlık	1	0,3
Diğer	1	0,3
Balık Fiyatlarının Diğer Et Türlerine Göre Durumu Nasıldır?		
Normal	149	47,3
Pahalı	101	32,1
Bilgim yok	34	10,8
Ucuz	31	9,8
Balık Fiyatları Yarıya İnse, Tükettiğiniz Balık Miktarı Artar Mı?		
Evet	214	67,9
Değişmez	80	25,4
Hayır	21	6,7



Şekil 3. Et tüketim tercihi  
Figure 3. Meat consumption preference



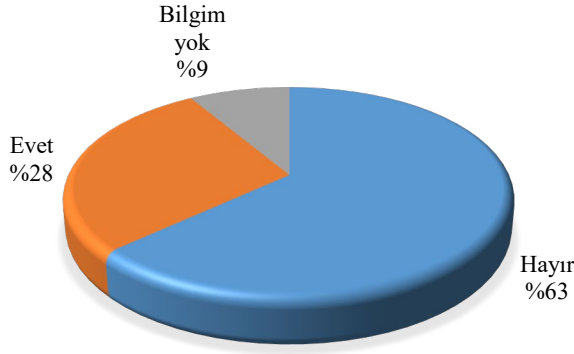
Şekil 4. Ankete katılanların balık tüketim sıklığı  
Figure 4. Frequency of fish consumption of respondents

Yavuzcan ve ark. (2010) tarafından yapılan “Ankara Üniversitesi Ziraat Fakültesi Öğrencilerinin Su Ürünleri Tüketim Alışkanlıklarının İncelenmesi” başlıklı çalışmada öğrencilerin %6’sının kötü koktuğu için balık pişirmeyi tercih etmediği, %3’ünün balık fiyatlarını yüksek bulduğunu, Terin ve İnanç (2023) “Hakkâri İli Yüksekova İlçesinde Balık Tüketim Yapısı ve Tercihleri Üzerine Bir Araştırma” adlı çalışmada katılımcıların %50’sinin alışkanlık olmaması sebebiyle balık tüketmediklerini, Selvi ve ark. (2022) “Çanakkale’nin Yenice ve Bayramiç İlçelerinde Su Ürünleri Tüketiminin Değerlendirilmesi” adlı anket çalışmasında balık tüketmeme nedenini Bayramiç ilçesinde en çok tadı/kokusu, Yenice ilçesinde ise yemesi zor/kılıklı olması olarak tespit ettiklerini, her iki ilçedeki katılımcıların yarısından fazlasının balık fiyatlarını normal bulduklarını bildirmişlerdir. Kaplan ve ark. (2019) “Mardin İlinde Balık Tüketim Alışkanlıklarının Belirlenmesi: Kızıltepe İlçesi Örneği” adlı çalışmada katılımcıların balık fiyatlarını %43,5’i pahalı olarak değerlendirdiğini, Yüksel ve Diler (2019) “Ankara İlinde Su Ürünleri Tüketim Tercihlerinin Belirlenmesi” adlı çalışmasında katılımcıların %57,2’si piyasadaki balık fiyatlarını pahalı bulduğunu ve katılımcıların balık tüketimlerinin az olmasının sebebi olarak katılımcıların ürünü tüketime hazırlamanın zahmetli olması ve balık fiyatlarının pahalı olmasını belirttiklerini bildirmişlerdir. Cengiz ve Özoğul (2019) yaptıkları Adana ve Mersin illerinde su ürünleri tüketimi anket çalışmasında balık tüketmeyen katılımcıların başlıca sebebi olarak tat ve kokunun sevilmemesi olduğunu, alerji ve diğer sağlık problemlerinin çok küçük bir oran oluşturduğunu; Balık ve ark. (2013) “Ordu İli Fatsa ve Aybastı İlçelerinde Balık Tüketim Alışkanlıklarının Karşılaştırmalı Olarak İncelenmesi” başlıklı çalışmada, katılımcıların balık fiyatlarının pahalı bulduğunu belirtmişlerdir.

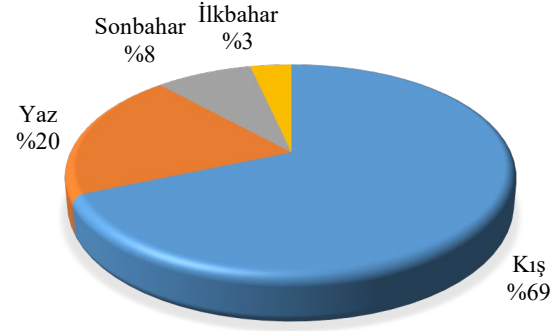
Yapılan çalışmalara bakıldığında elde edilen sonuçlar Kuşat ve Şahan (2021) ve Bolat ve Telli (2019) çalışmaları ile benzerlik göstermektedir. Diğer çalışmalarda katılımcılar balık fiyatlarını pahalı olarak değerlendirmişlerdir. Bu durumun bölgelerin ekonomik özellikleri arasındaki fark ile ilişkili olduğu düşünülmektedir.

Çalışmamızda balık tüketen öğrencilerin %18,5’i en az haftada bir kere balık tüketirken, %20,6’sı 15 günde bir, %27,3’ü ayda bir, %19’u üç ayda bir ve %8,6’sı ise yılda bir kere balık tükettiğini belirtmiştir (Şekil 4).

Sarı ve ark. (2019) yaptıkları çalışmada, öğrencilerin %3,68’inin haftada bir, %43,71’inin ayda bir, %13,95’inin ise yılda bir su ürünleri tükettiklerini belirtmişlerdir. Yavuzcan ve ark. (2010) ise, öğrencilerin %11’inin hiç su ürünleri tüketmediğini, %29’unun haftada bir kez, %26’sının 15 günde bir ve %18’inin ayda bir balık tükettiklerini bildirmiştir. Olgunoğlu ve ark. (2020) “Meslek Yüksekokulu Öğrencilerinin Balık Tüketim Alışkanlıklarının İncelenmesi (Kâhta MYO Örneği)” başlıklı çalışmada, öğrencilerin %70,42’sinin balık tükettiği, %29,58’inin ise tüketmediğini bildirmişlerdir. Aynı çalışmada kız öğrencilerin daha çok balık tükettiği (%77,67), balık tüketen öğrencilerin %14,45’inin haftada bir kez, %14,46’sının on beş günde bir ve %34,34’ünün ayda bir defa balık tükettiğini belirlediklerini ifade etmişlerdir.



Şekil 5. Tükettiğiniz balık miktarı yeterli mi?  
Figure 5. Is the amount of fish you consume enough?



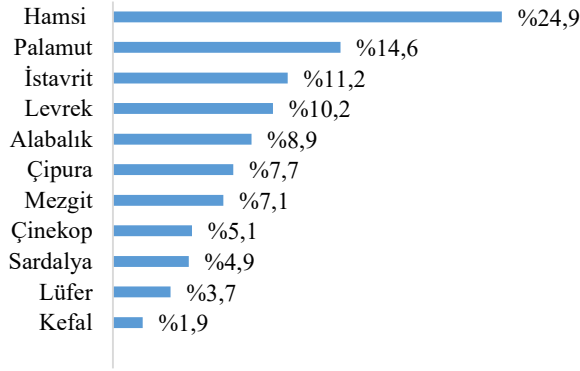
Şekil 6. Su ürünlerinin en çok tüketildiği mevsim  
Figure 6. The most consumed season of aquaculture

Terin ve İnanç (2023) balık tüketmeyenlerin oranını %24,7 olarak belirlerken, balık tüketenlerin %16'sının haftada bir, %18'inin on beş günde bir, %47,7'sinin ayda bir ve %22,7'sinin yılda bir balık tükettiklerini, en fazla balık tüketiminin kış aylarında olduğunu bildirmiştir. Selvi ve ark. (2022) balık eti tüketenlerin oranını Bayramiç ve Yenice ilçelerinde %90'nın üzerinde olduğunu ve balık tüketenlerin oranını sırasıyla haftada birkaç kez %39,4 ve %52,5, ayda birkaç kez %41,9 ve %38,1, yılda birkaç kez %10 ve %3,8, en çok balık tüketilen mevsimi her iki ilçede sonbahar-kış olarak bildirmişlerdir. Kuşat ve Şahan (2021) balığı severek tüketenlerin oranını %15,5, olarak, katılımcıların balık tüketim sıklığını %38,1 ile haftada bir, %3'ü haftada birden fazla, %26,4'ü on beş günde bir, %22,3'ü ayda bir şeklinde belirlediklerini, en çok balık tüketilen mevsimin kış aylarında olduğunu bildirmişlerdir. Karakulak ve ark. (2020) yapmış oldukları çalışmada balık tüketim sıklığının haftada bir %20,7, iki haftada bir %25,6, ayda bir %38 ve yılda bir %15,8 olarak bildirmişlerdir. Kaplan ve ark. (2019) yaptıkları çalışmada katılımcıların balık tüketim sıklığını %18,97'i haftada bir, %29,58'i on beş günde bir, %46,62'si ayda bir ve %21,22'si yılda bir olarak belirlemişlerdir. Yüksel ve Diler (2019) yaptıkları çalışmada katılımcıların %19'unun haftada bir, %74'ünün haftada iki kez, %26,7'sinin on beş günde bir, %23,9'unun ayda bir, %11,1'inin üç ayda bir, %2,3'ünün altı ayda bir ve %3,3'ünün yılda bir kez balık tükettiğini, tüketicilerin en çok kış aylarında balık tükettiklerini belirtmişlerdir. Bolat ve Telli (2019) yaptıkları çalışmada katılımcıların %58'inin ayda 2-4 defa su ürünleri tükettiğini, %32'sinin ayda bir defa tükettiğini ve katılımcıların yine en çok kış aylarında su ürünleri tükettikleri olduğu belirtilmiştir. Bolat ve Cevher (2018) yaptıkları çalışmada ankete katılan bireylerin %37'sinin ayda 2-4 defa, %51'inin ayda bir defa su ürünleri tükettiğini, katılımcıların en çok kış aylarında balık tükettiklerini belirlemişlerdir. Arıca (2017) yaptıkları çalışmada tüketicilerin balık tüketim sıklığını %13'ü haftada bir, %5'i haftada 2 veya üç kez, %22'si on beş günde bir, %31 ayda bir, %24'ü yılda birkaç kez balık tükettiği, tüketimin en fazla kış mevsiminde olduğu, tüketicilerin %35'i yeterli düzeyde, %48'i orta düzeyde tükettiği ve %17'sinin yeterli düzeyde tüketmediği bildirilmiştir. Uzundumlu ve Dinçel (2015) "Trabzon İli Beşikdüzü İlçesinde Balık Eti Tüketim Alışkanlıklarının Belirlenmesi" adlı çalışmada hanelerin %50'si en az

haftada bir, %40,52'si ayda bir veya iki kez, %6,9'u üç ayda bir veya iki kez, %2,59'u yılda bir veya iki kez balık tükettiği en çok balık tüketilen mevsimin kış ayları olduğu ifade edilmiştir. Balık ve ark. (2013) yaptıkları çalışmada Fatsa ilçesinde katılımcıların %4,2'si Aybastı ilçesinde katılımcıların %9,4'ü hiç balık tüketmediği, Fatsa ilçesinde katılımcıların %34,8'i haftada bir, %17,4'ü haftada birden fazla, %36,7'si on beş günde bir ve %11,1'i ayda bir balık tükettiği, Aybastı ilçesinde katılımcıların %34,8'i haftada bir, %10,4'ü haftada birden fazla, %36,5'i on beş günde bir, %18,4'ü ayda bir balık tükettiği bildirilmiştir.

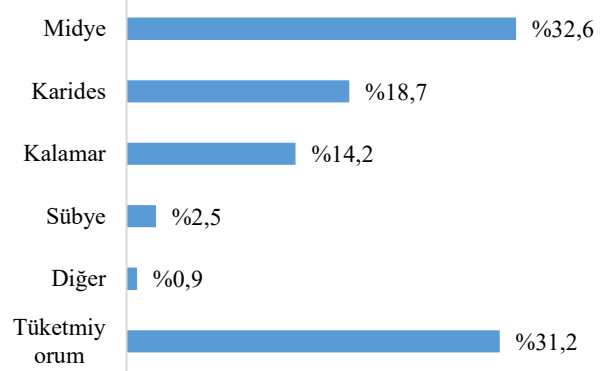
Literatür çalışmalarında Karadeniz ve Marmara bölgelerinde haftada bir ve daha sık aralıklarda yapılan balık tüketimi diğer bölgelere kıyasla daha fazladır. Çalışmamızdan elde edilen sonuçlarda haftada bir balık tüketim oranı diğer bölgelerde öğrenci merkezli yapılan çalışmalara göre yüksek bulunmuştur. Bu durum özellikle Karadeniz ve Marmara bölgelerinde bulunan balık tüketim alışkanlığı ile açıklanabilir. Çünkü çalışmamızdan elde edilen ve Şekil 9'da verilen balık tüketme nedenleri arasında aile kültürü en yüksek orana sahiptir ve Yalova Üniversitesinde okuyan öğrencilerin birçoğu Şekil 2'de belirtildiği gibi Marmara bölgesinden gelmektedir. Bu durum ikamet edilen bölgenin balık tüketme sıklığı ile bir ilişkisi olduğunu yönünde değerlendirilebilir. Çalışmamızda katılımcıların en çok balık tükettiği mevsimin kış ayları olduğu tespit edilmiş (Şekil 6) ve literatür çalışmaları ile uyumlu bulunmuştur. Çalışmamızdan elde edilen sonuçlarda katılımcıların büyük bir çoğunluğu tükettiği balık miktarını yeterli bulmamıştır (Şekil 5). Bu durum Arıca (2017)'nin yaptığı çalışma ile uyumsuzdur. Bu durum bölgelerin farklı tüketim alışkanlığının olmasından kaynaklanıyor olabilir. Nitekim çalışmamız Marmara bölgesinden katılımcıların yoğun olduğu bir çalışma iken Arıca (2017) çalışmasını Hatay ilinde gerçekleştirmiştir.

Çalışmamızda ankete katılan öğrencilerin en çok tükettiği balık türü hamsi (%24,9), en az tüketilen balık kefal (%1,9) olmuştur. Hamsiden sonra en çok tüketilen balıklar palamut (%14,6), istavrit (%11,2), levrek (%10,2), alabalık (%8,9), çipura (%7,7), mezgıt (%7,1), çinekop (%5,1), sardalya (%4,9) ve lüfer (%3,7) şeklindedir (Şekil 7). Katılımcıların tercih ettiği balık türlerinin çoğunluğunu deniz balıkları oluşturmaktadır. Katılımcıların balık dışında en çok tükettiği tür ise %32,6 oranı ile midye olurken bunu sırasıyla %18,7 ile karides, %14,2 ile kalamar ve %2,5 ile sübyenin takip ettiği görülmüştür (Şekil 8).



Şekil 7. Katılımcıların tükettiği balık türleri  
Figure 7. Types of fish consumed by participants

Sarı ve ark. (2019) yaptıkları çalışmada öğrencilerin %87'sinin balık dışında diğer su ürünlerini tüketmediğini, Yavuzcan ve ark. (2010) öğrencilerin en çok tükettikleri su ürünlerinin %76 oranı ile deniz balıkları olduğunu, tatlı su balıklarının %22 oranında olduğu ve kabuklu su ürünlerinin %22 olduğu bildirilmiştir. Olgunoğlu ve ark. (2020) yaptıkları çalışmada öğrencilerin %52,84'ünün tatlı su türlerini deniz balıklarından daha fazla tükettiğini, balık dışı ve işlenmiş su ürünleri tüketimine ilgi duymayanların oranını %94,41 olarak bildirmişlerdir. Terin ve İnanç (2023) tüketicilerin en çok tercih ettikleri balıkları sırasıyla %34,5 ile alabalık, %25,5 ile inci kefali ve %24,8 ile hamsi olduğu görülmüştür. Selvi ve ark. (2022) balık tercihini Bayramiç ve Yenice ilçelerinde sırasıyla %27,5 ve %55 oranı ile deniz balığı, %26,3 ve %7,5 ile tatlı su balığı, %37,5 ve %31,9 ile her ikisi, %8,8 ve %5,6 ile tüketmiyorum şeklinde ifade etmişlerdir. Aynı çalışmada tüketilen diğer su ürünleri türleri ise Yenice ve Bayramiç İlçelerinde sırasıyla, midye %38,8 ve %26,9, karides %18,8 ve %9,4, ahtapot/kalamar %7,5 ve %10 olarak tespit etmişlerdir. Kuşat ve Şahan (2021) tüketicilerin %61,5'inin deniz balıklarını, %5'inin tatlı su balıklarını ve %33,5'inin her iki balık türünü de tükettikleri belirtilirken, en fazla tüketilen balığın %61,2 ile hamsi ve en az tüketilen balığın %1,2 ile sazan olduğu belirtilmiştir. Aynı çalışmada ankete katılanların %70'inin ucuz balıkları (hamsi, istavrit, palamut) tercih ettiği vurgulanmıştır. Karakulak ve ark. (2020) yapmış oldukları çalışmada, balık dışı en çok hangi su ürünlerini tüketmekteyiz sorusuna %30,4 midye olarak belirlemişlerdir. Aynı çalışmada en fazla tükettiğiniz balık sorusuna katılımcıların %55,1'i hamsi, %5,2'si istavrit, %22,1'i alabalık, %4,7'si sazan, %7,7'si çipura/levrek, %2,4'ü somon ve %2,8'i diğer türler belirlemişlerdir. Kaplan ve ark. (2019) yaptıkları çalışmada tüketicilerin %64,95'i deniz balıkları, %62,06'sı tatlı su balıkları, %2,25'i de kabuklu ve yumuşakçalar (midye vb.) tükettiklerini, tercih edilen balık türlerine katılımcıların %56,91'i hamsi, %25,08'i alabalık, %16,40'ı çipura ve %1,61'i lüferi tercih ettiklerini bildirmişlerdir. Yüksel ve Diler (2019) yaptıkları çalışmada katılımcıların %78,5'inin avcılık, %15,1'inin ise yetiştiricilik ürünlerini tercih ettiği, %27,3'ünün hamsi, %3,9'u sardalya, %56,9'u alabalık, %6,7'si yayın balığı olarak belirtilmiştir. Aynı çalışmada katılımcıların %31,2'si kalamar, %2,9'u ahtapot, %4,2'si istakoz, %17,8'i karides, %37,4'ü midye tükettikleri bildirilmiştir. Bolat ve Telli (2019) yaptıkları çalışmada katılımcıların %72'sinin deniz balıklarını tercih ettiğini,



Şekil 8. Katılımcıların tükettiği balık dışı su ürünleri  
Figure 8. Non-fish aquaculture consumed by participants

%5'ünün tatlı su balıklarını tercih ettiğini ve %23'ünün her iki tür balığı da tercih ettiğini belirtmişlerdir. Aynı çalışmada katılımcıların deniz balıklarından %41'i hamsi, %18'i çipura, %12'si levrek, %8'i sardalya, %6'sı istavrit, %5'i palamut, %3'ü somon ve %7'si diğer türleri tercih ettiği, tatlı su balıklarından %88 ile alabalık, %10 ile sazan %2 ile sudak balığının tercih edildiği belirtilmiştir. Bolat ve Cevher (2018) yaptıkları çalışmada katılımcıların %48'i hamsi, %27'si alabalık, %8'i sazan, %8'i levrek, %5'i istavrit, %1 çipura, %1 palamut, %1 sudak ve %1 somon tükettiklerini bildirmişlerdir. Arıca (2017) yaptıkları çalışmada tüketicilerin %48'i deniz balığı, %9'u tatlı su balığı, %38'i her iki tür balığı, %5'lik grup ise hiçbirini şeklinde olduğunu, tüketicilerin %26'sı hamsi, %19'u çipura, %15'i levrek, %14'ü alabalık, %9'u barbunya, %7'si istavrit, %4'ü uskumru, %4'ü lüfer ve %2'si sazan olarak belirtilmiştir. Aynı çalışmada tüketicilerin %53'ü balık dışında da su ürünleri tüketmediği belirtilirken %25 midye, %9 karides, %3'ü de istakoz şeklinde olduğu belirtilmiştir. Uzundumlu ve Dinçel (2015) yaptıkları çalışmada tüketicilerin %96,12'sinin hamsi, %71,38'inin alabalık, %64,64'ünün istavrit, %51,73'ünün palamut, %50,78'inin mezgıt, %16,63'ünün levrek, %13,99'unun somon, %12,31'inin çinakop, %10,53'ünün çupra, %7,01'inin ise sazan tercih ettiği bildirilmiştir. Balık ve ark. (2013) yaptıkları çalışmada Fatsa ilçesindeki katılımcıların %49,1'i hamsi, %28,9'u istavrit, %13,5'i mezgıt, %7,3'ü barbunya ve %1,2'si levrek tercih ettiği, Aybastı ilçesinde ise katılımcıların %45,3'ü hamsi, %28,2'si istavrit, %17,1'i mezgıt, %7,5'i barbunya ve %1,8'i levrek tercih ettiği belirtilmiştir.

Literatür çalışmalarında tüketicilerin tercih sırasının deniz balıkları, tatlı su balıkları ve balık dışı su ürünleri şeklinde olduğu görülmüştür. En çok tüketilen balıklar deniz balığı olarak hamsi, tatlı su balıklarından alabalık, balık dışı su ürünlerinden ise midye olduğu görülmektedir. Elde edilen bulgular, mevcut literatür ile tutarlılık göstermektedir.

Çalışmadan elde edilen sonuçlara göre ankete katılanların büyük çoğunluğu yetiştiriciliği yapılan su ürünlerinin sağlıklı olup olmadığı, satılan su ürünlerinin beslenme açısından sağlıklı olup olmadığı ve su ürünlerinin uygun (insancıl, ekolojik vb.) yöntemlerle avlanıp avlanmadığı sorusuna orta derecede katılıyorum cevabını vermişlerdir. Bu oranlar sırasıyla %41,6, %36,2 ve %39,7 olarak belirlenmiştir (Çizelge 3).

Çizelge 3. Katılımcıların yetiştiriciliği yapılan su ürünlerinin sağlıklı olup olmadığı, satılan su ürünlerinin beslenme açısından sağlıklı olup olmadığı ve su ürünlerinin uygun (insancıl, ekolojik vb.) yöntemlerle avlanıp avlanmadığı sorularına verilen cevaplar

Table 3. The answers given to the questions whether the aquaculture are healthy, whether the aquaculture sold are healthy in terms of nutrition and whether the aquaculture are fished with appropriate (humane, ecological, etc.) methods

	1	2	3	4	5
Yetiştiriciliği yapılan su ürünleri daha sağlıklıdır	%7,9	%10,8	%41,6	%22,2	%17,5
Satılan su ürünleri beslenme açısından sağlıklıdır	%5,7	%10,5	%36,2	%29,8	%17,8
Su ürünler uygun (insancıl, ekolojik vb.) yöntemlerle avlanmaktadır	%7,9	%17,5	%39,7	%22,9	%12,1

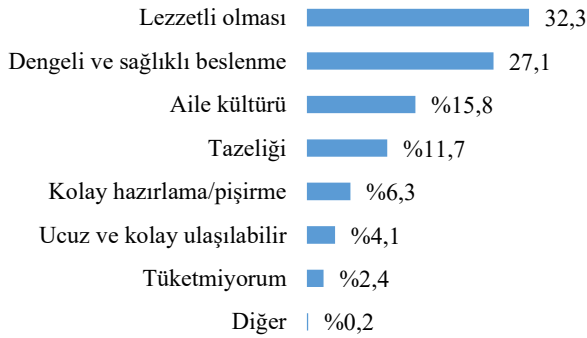
1: Kesinlikle Katılmıyorum; 2: Katılmıyorum; 3: Orta derecede katılıyorum; 4: Katılıyorum; 5: Kesinlikle Katılıyorum

Çizelge 4. Katılımcıların su ürünlerini tüketme ile ilgili sorulara verdiği cevapların dağılımı

Table 4. Distribution of participants' answers to questions about consuming aquaculture

	1	2	3	4	5
Su ürünleri tüketmek yerine Omega-3 gibi takviye edici gıdalar tercih edilebilir	%30,8	%27,0	%24,8	%10,8	%6,7
Su ürünleri tüketimi beslenme alışkanlığı ile ilgilidir	%4,8	%7,6	%17,8	%40	%29,8
Su ürünleri tüketimi inançlarımız ile ilgilidir	%31,1	%28,6	%20,6	%10,8	%8,9
Su ürünleri tüketimin artması için fiyatlarda indirim yapılmalıdır	%4,8	%5,7	%20,6	%23,5	%45,4

1: Kesinlikle Katılmıyorum; 2: Katılmıyorum; 3: Orta derecede katılıyorum; 4: Katılıyorum; 5: Kesinlikle Katılıyorum

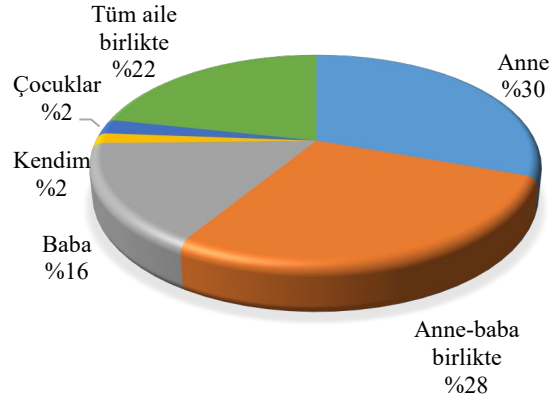


Şekil 9. Katılımcıların balık tüketme nedenleri  
Figure 9. Reasons why participants consume fish

Yavuzcan ve ark. (2010), öğrencilerin %84'ünün su ürünlerini sağlık açısından önemli bulduklarını belirtmişlerdir. Yüksel ve Diler (2019), katılımcıların %1,8'inin balık tüketirken yetiştiricilik ürünü olmasına dikkat ettiğini, Cengiz ve Özoğul (2019) ise katılımcıların %95,6'sının balık tüketiminin sağlık açısından faydalı olduğu fikrine katıldığını ifade etmişlerdir. Bolat ve Cevher (2018), katılımcıların %62'sinin balığın besin değeri hakkında yeterli bilgiye sahip olduklarını, Arıca (2017) ise piyasada satılan su ürünlerinin sağlıklı olduğu fikrine tamamen katılanların oranının %16, su ürünlerinin besleyici özelliğini bildiğini belirtenlerin oranının %77 olduğunu belirtmişlerdir. Ayrıca, Yavuzcan ve ark. (2010), katılımcıların %9'unun balık satın alırken çiftlik ürünü olup olmadığına dikkat ettiklerini ortaya koymuşlardır.

Literatür çalışmalarına bakıldığında su ürünleri tüketiminin sağlık açısından önemli görüldüğüne dair sonuçlar mevcuttur. Fakat yetiştiriciliği yapılan su ürünlerinin sağlıklı olup olmadığı ve avcılığının nasıl gerçekleştiğine dair kesin net bir bilgi sahibi olmadığı görülmektedir. Çalışmamızdan elde edilen sonuçlar bu durum ile uyumludur.

Çalışmamızda katılımcılara neden balık tükettikleri sorulduğunda tüketenlerin %15,8'i aile kültürünü, %11,7'si tazeliği, %6,3'ü kolay hazırlama/pişirme, %4,1'i



Şekil 10. Hanede mutfak alışverişini yapan kişi  
Figure 10. The person in the household who does the grocery shopping

ucuz/kolay ulaşılabilirliği ve %0,2'si diğer nedenlerden dolayı tükettiklerini belirtmişlerdir (Şekil 9). Ayrıca hanede alışverişini yapan kişilere bakıldığında katılımcıların %30'unda anne, %28 anne-baba birlikte, %22 tüm aile, %16 baba, %2 diğer şeklinde belirlenmiştir (Şekil 10).

Literatürde yapılan çalışmalara bakıldığında, Karakulak ve ark. (2020) katılımcıların balık tüketmesinin en önemli sebebinin sağlıklı olduğu düşüncesine dayandığını, Kuşat ve Şahan (2021) ise katılımcıların balığı lezzetli ve besleyici olduğu için tükettiklerini bildirmişlerdir. Diğer çalışmalarda ise katılımcıların çoktan aza doğru balık tüketme sebeplerini Sarı ve ark. (2019) lezzet, besin değeri, Selvi ve ark. (2022) sağlıklı olması, lezzetli olması ve aile alışkanlığı, Kaplan ve ark. (2019) sağlıklı, lezzetli ve dengeli beslenme, Yüksel ve Diler (2019) dengeli ve sağlıklı beslenme, lezzet, fiyat, alışkanlık ve ulaşılabilirlik, Bolat ve Cevher (2018) lezzetli ve besleyici olması, tazelik, alışkanlık, ucuz ve ulaşılabilir olması, Arıca (2017) balıkçılığının sağlıklı olması, damak zevki, aile kültürü, fiyat ve kolay hazırlama, şeklinde ifade etmişlerdir.

Yapılan çalışmalarda balık tüketme sebebi olarak genellikle sağlıklı olduğu düşüncesi, ardından lezzetli olması sebebinin geldiği görülmektedir. Katılımcıların balık tüketme sebebi olarak aile kültürü seçeneğinin

yüksek çıkması, hanede alışverişi yapan veya mutfak işlerini yapan kişinin genellikle anne, baba veya diğer bir aile büyüğünün yapmasından dolayı olduğu ve sonuçlara yansdığı düşünülmektedir.

Çalışmamızda katılımcıların büyük bir kısmı su ürünleri tüketmek yerine Omega-3 gibi takviye edici gıdaları tüketmeyi tercih edilebilir bulmadığını, su ürünleri tüketiminin beslenme alışkanlığı ile alakalı olduğunu, düşündüğü, su ürünleri tüketiminin inanca/dini görüş tarafından etkilenmediğini ve su ürünleri tüketiminin artması için fiyatlarda indirim yapılması gerektiğini düşündükleri tespit edilmiştir (Çizelge 4).

Yavuzcan ve ark. (2010) yaptıkları çalışmada öğrencilerin %3'ünün su ürünleri tüketiminin çok da gerekli olmadığını düşündüklerini, Olgunoğlu ve ark. (2020) yaptıkları çalışmada katılımcıların %84,05'inin balık tüketimini dengeli beslenme ve sağlık açısından önemli bulmaları sebebiyle tükettiklerini ifade etmişlerdir.

Yaptığımız çalışmadan elde edilen sonuçlar Yavuzcan ve ark. (2010) ve Olgunoğlu ve ark. (2020) tarafından yapılan çalışmalar ile uyumlu olarak görülmüştür.

Balık etinin önemi ile ilgili bilgileri nereden öğrendiniz sorusuna en çok verilen cevabın mobil cihaz (%21,3) ve bunu sırasıyla tanıdıklar/çevrem (%20,3), kitap, dergi, gazete vb. (%20), bilgisayar (%16,4), televizyon (%13,4), okul (%3,1) ve aile (%0,3) şeklinde olduğu tespit edilmiştir (Şekil 11).

Yavuzcan ve ark. (2010) yaptıkları çalışmada öğrencilerin %27'sinin su ürünleri ile ilgili reklamları olumlu bulduğunu, Kaplan ve ark. (2019) tüketicilerin %88,6'sının reklam ve tanıtımları yeterli görmediği, Bolat ve Telli (2019) katılımcıların %92'sinin besin değeri hakkında yeterli bilgiye sahip olduklarını, Balık ve ark. (2013) yaptıkları çalışmada Fatsa ilçesindeki katılımcıların %60'ı ve Aybastı ilçesindeki katılımcıların %20'si balık etini diğer etlerden ayıran besinsel özellikler hakkında bilgi sahibi oldukları ifade etmişlerdir.

Tüketicilerin özellikle de öğrencilerin mevcut koşullarının, yapılan reklam ve tanıtımlara ulaşabilecek düzeyde olduğu anlaşılmaktadır. Fakat bu reklam ve tanıtımların yeterli düzeyde olmadığı veya tüketicinin dikkatini çekemediği literatür çalışmalarına bakılarak söylenebilir (Yavuzcan ve ark., 2010; Çiçek ve ark., 2014; Gürel ve ark., 2017; Kuzgun ve Demirbağ, 2018; Kaplan ve ark., 2019; Karakaluk ve ark., 2020).

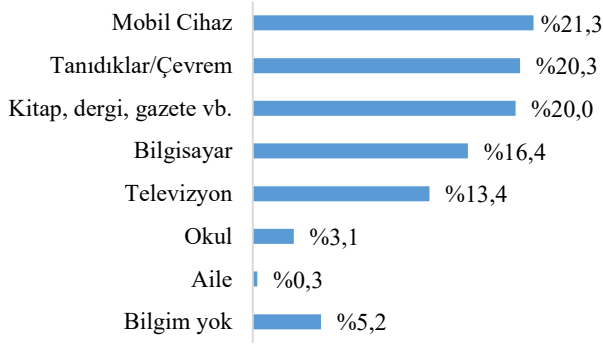
Yürütülen bu çalışmada öğrencilerin pişirme yöntemi tercihinde en çok %64,1 ile kızartma yöntemini, ikinci olarak %21,3 ile ızgara/mangal ve ardından %12,4 ile

buğulama olduğu görülmektedir. Ayrıca katılımcıların büyük bir çoğunluğu işlenmiş balık (konserve, tütsülenmiş vb.) tüketmediklerini bildirmişlerdir (Çizelge 5).

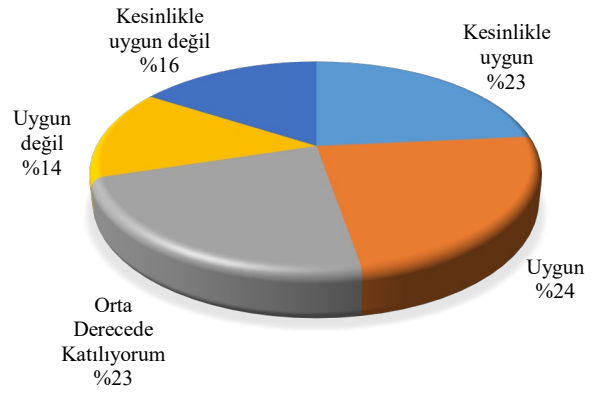
Sarı ve ark. (2019), katılımcıların %81'inin balığı kızartarak tükettiklerini, konserve balık tüketenlerin oranını ise %8 olarak belirtmişlerdir. Yavuzcan ve ark. (2010), balık tüketen öğrencilerin %93'ünün ızgara ya da kızartma yöntemini tercih ettiğini, konserve tüketenlerin oranının %3, dondurulmuş ürün tüketenlerin ise %4 olduğunu bildirmiştir. Olgunoğlu ve ark. (2020), katılımcıların %92,77'sinin taze balık tükettiğini, %6,03'ünün dondurulmuş, %1,20'sinin ise konserve tükettiğini, taze balık tüketenlerin %97,07'sinin ızgara ya da kızartmayı tercih ettiğini belirtmişlerdir. Terin ve İnanç (2023), katılımcıların %94,5'inin taze balığı tercih ettiğini, %3,2'sinin dondurulmuş, %2,3'ünün ise konserve balık tükettiğini ve balığı en çok kızartarak pişirenlerin oranının %40,9 olduğunu ifade etmişlerdir. Selvi ve ark. (2022), Bayramiç ve Yenice ilçelerinde en yaygın balık pişirme yönteminin sırasıyla kızartma, ızgara ve fırın olduğunu belirtmiş, aynı ilçelerde balık tüketim biçimlerinin ise sırasıyla taze, dondurulmuş ve konserve olduğunu bildirmişlerdir. Kuşat ve Şahan (2021), katılımcıların %95,9'unun taze balık, %1,8'inin konserve, %1,6'sının tuzlanmış ve %0,7'sinin salamura olarak tükettiklerini, Kaplan ve ark. (2019) ise katılımcıların %96,78'inin taze balık tükettiğini belirtmişlerdir. Yüksel ve Diler (2019), %81,7'sinin taze, %5,2'sinin dondurulmuş ve %3,5'inin konserve balık tükettiklerini, %43,6'sının ise balığı kızartarak pişirdiğini bildirmişlerdir. Cengiz ve Özoğul (2019), katılımcıların %67'sinin balığı kızartarak, %62'sinin ızgarada pişirdiğini ifade etmişlerdir. Bolat ve Telli (2019), katılımcıların %66'sinin kızartma, %24'ünün ızgara, %5'inin buğulama ve %4'ünün diğer yöntemlerle balık pişirdiğini belirtmişlerdir. Bolat ve Cevher (2018) ise katılımcıların %95,5'inin taze balık tükettiklerini, %60'ının balığı kızartarak pişirdiğini ifade etmişlerdir. Arıca (2017), katılımcıların %90'ının balığı taze, %5'inin dondurulmuş, %3'ünün konserve, %2'sinin ise işlenmiş olarak satın aldıklarını ve en çok kızartma yönteminin tercih edildiğini belirtmiştir. Uzundumlu ve Dinçel (2015), %81,22'sinin balığı kızartarak, %79,04'ünün ızgarada, %69,44'ünün buğulama ile pişirdiğini ifade etmişlerdir. Balık ve ark. (2013), Fatsa ilçesinde katılımcıların %97,2'sinin taze balık tükettiklerini ve en yaygın pişirme yöntemlerinin buğulama, kızartma ve ızgara olduğunu belirtmişlerdir.

Tablo 5. Su Ürünlerini Pişirme ve İşlenmiş ürün tüketim şekli  
Table 5. How to cook aquaculture and consume processed products

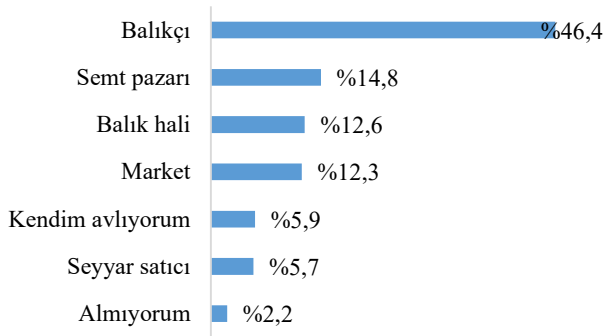
Su Ürünlerini Pişirme Şekli	N	%
Kızarma	202	64,1
Izgara/Mangal	67	21,3
Buğulama	39	12,4
Fırlama	2	0,6
Diğer	1	0,3
Tüketmiyorum	4	1,3
İşlenmiş balık (Konserve, tütsülenmiş vb.) Tüketimi		
Hayır	183	58,1
Evet	132	41,9



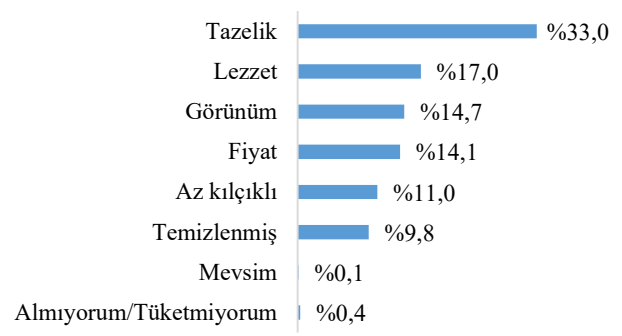
Şekil 11. Katılımcıların su ürünlerinin faydalarını nereden öğrendikleri  
Figure 11. Where participants learned about the benefits of aquaculture



Şekil 12. Katılımcıların yaşam alanlarının su ürünlerini pişirmeye uygunluk durumu  
Figure 12. The suitability of the living spaces of the participants for cooking aquaculture



Şekil 13. Su ürünlerinin satın alındığı yerler  
Figure 13. Where aquaculture are purchased



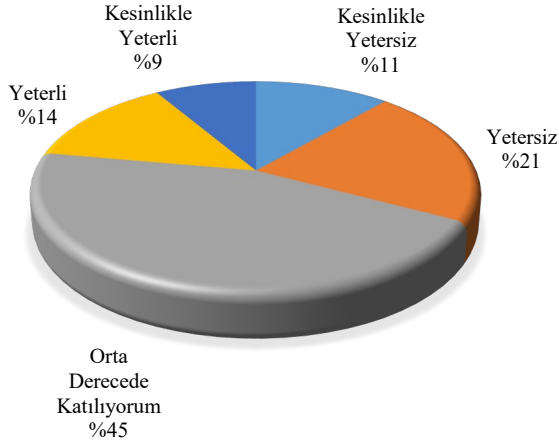
Şekil 14. Katılımcıların balık satın alırken dikkat ettiği hususlar  
Figure 14. What respondents pay attention to when buying fish

Çalışmamızdan elde edilen sonuçlara bakıldığında kızartma yönteminin en çok balık pişirme yöntemi olarak tercih edilmesi ve işlenmiş balık tercihinin düşük olması yukarıda belirtilen çalışmalar ile uyumlu olduğu görülmüştür.

Ankete katılan öğrenciler büyük oranda (%47) yaşam alanlarının su ürünlerini pişirmek için uygun ortamlar olduğunu, %23'ü ise bu duruma orta derecede katıldıklarını belirtmişlerdir (Şekil 12). Çalışmamızda elde edilen sonuçlara göre su ürünlerinin satın alınmasında en çok tercih edilen yer olarak öğrencilerin hanelerinde alışverişini yapan kişinin/kişilerin %46,4'ünün balıkçılardan, %14,8'inin semt pazarından, %12,6'sının balıkçı halinden, %12,3'ünün marketlerden ve %5,7'sinin seyyar satıcılardan aldığı belirlenmiştir. Katılımcıların %5,9'u ise balığı kendilerinin avladığını belirtmişlerdir (Şekil 13).

Literatürde yapılan çalışmalara göre, balık satın alma yerleri katılımcılar arasında farklılık göstermektedir. Sarı ve ark. (2019), öğrencilerin balığı genellikle marketlerden (%43,9) ve balık halinden (%42,2) aldıklarını belirtmişlerdir. Yavuzcan ve ark. (2010), öğrencilerin %57'sinin balığı balıkçıdan, %17'sinin marketten, %16'sının balık halinden ve %9'unun kendisinin avladığını bildirmiştir. Terin ve İnanç (2023), katılımcıların %71,1'inin balığı seyyar satıcılardan, %28,9'unun ise balıkçılardan satın aldıklarını ifade etmişlerdir. Selvi ve ark. (2022), Bayramiç ve Yenice ilçelerinde balık satın alma yerlerini sırasıyla %72,5 ve %91,3 ile balık hali veya pazarlardan, %11,3 ve %0,6 ile marketlerden ve %7,5 ile %2,5 ile kendilerinin avladığını

belirtmişlerdir. Kuşat ve Şahan (2021), katılımcıların %58'inin pazaryerini, %33,1'inin balık halini ve %8,8'inin süpermarketleri tercih ettiğini bildirmiştir. Kaplan ve ark. (2019), katılımcıların %31,51'inin pazaryerinden, %34,08'inin balık halinden ve %8,36'sının seyyar satıcılardan balık satın aldığını ifade etmiştir. Yüksel ve Diler (2019), katılımcıların %42,7'sinin balığı süpermarketten satın aldığını ve %3,1'inin balığı kendisinin avladığını belirtmişlerdir. Bolat ve Telli (2019), katılımcıların %67'sinin balığı balıkçılardan, %20'sinin pazaryerinden aldığını bildirmiştir. Bolat ve Cevher (2018), katılımcıların %53'ünün balığı balık halinden, %24'ünün marketlerden/AVM'lerden, %19'unun pazaryerinden ve %4'ünün seyyar satıcılardan aldıklarını ifade etmişlerdir. Arica (2017), katılımcıların %68'inin balığı balıkçı tezgahından, %13'ünün balıkçı barınaklarından, %9'unun süpermarketlerden ve %4'ünün seyyar satıcılardan aldıklarını belirtmiştir. Uzundumlu ve Dinçel (2015), katılımcıların %92,9'unun balığı balıkçılardan, %83,68'inin balık halinden, %51,65'inin balık tesislerinden ve %29,76'sının süpermarketlerden aldığını ifade etmişlerdir. Balık ve ark. (2013), Fatsa ilçesindeki katılımcıların %47,2'sinin balığı balık marketlerinden, %29,9'unun seyyar satıcılardan ve %5,5'inin kendisinin avladığını, Aybastı ilçesindeki katılımcıların ise %48,2'sinin balık marketlerinden, %39,5'inin seyyar satıcılardan ve %2,5'inin kendisinin avladığını bildirmişlerdir.



Şekil 15. Katılımcıların su ürünlerinin işlenmesinde uygulanan gıda güvenliği kontrol ve denetimlerini yeterli bulup bulmama oranları

Figure 14. Rates of whether the participants find the food safety controls and inspections applied in the processing of aquaculture sufficient or not

Literatür çalışmalarına bakıldığında tüketicilerin balığı genellikle balıkçı veya balık hali gibi mesleği balıkçılık olan satış yerlerini tercih ettiği, marketler veya AVM'lerden daha az talep ettikleri görülmektedir. Çalışmamızdan elde edilen sonuçlar literatürde bahsedilen satış yerleri tanımı ile uyuşmasa da benzerlik göstermektedir. Nitekim balık satış yerleri yerleşim alanlarına veya kişilere göre Pazar, balık hali, balıkçı, seyyar satıcı, tablacı gibi farklı isimlerle nitelendirilebilmektedir. Bu konuda bir söylem bütünlüğünün olmaması bu farklılığın sebebi olarak düşünülmektedir.

Çalışmamızda balık satın alırken dikkat ettiğiniz hususlar nelerdir sorusuna öğrencilerin %33'ü tazeliğe, %17'si lezzetine, %14,7'si görünüme, %14,1'i fiyatına, %11'i az kılçıklı olmasına ve %9,8'i mevsime dikkat ettikleri tespit edilmiştir (Şekil 14).

Yapılan çalışmalarda, tüketicilerin taze balık satın alırken dikkat ettikleri hususlar incelendiğinde, Sarı ve ark. (2019) öğrencilerin %83,78'inin tazeliğe, %23,89'unun az kılçıklı olmasına ve %15,7'sinin ise balığın temizlenmiş olmasına özen gösterdiklerini belirtmişlerdir. Yavuzcan ve ark. (2010) ise öğrencilerin %51'inin balığın ucuz ve taze olmasına, %40'ının lezzetine ve %9'unun çiftlik ürünü olup olmadığına dikkat ettiğini, ayrıca %62'sinin tazelik ölçütlerini bildiğini ifade etmişlerdir. Olgunoğlu ve ark. (2020) ise öğrencilerin %34,54'ünün taze balık alırken nelere dikkat etmeleri gerektiğini bilmediklerini, Terin ve İnanç (2023) bireylerin %76,6'sının tazeliğe, %18'inin fiyata ve %5,4'ünün görünüme dikkat ettiğini tespit etmişlerdir. Kuşat ve Şahan (2021) ise tüketicilerin %68,6'sının tazeliğe, %11,7'sinin türe, %11,3'ünün fiyata ve %8,4'ünün satıcıya güvenmeye öncelik verdiğini belirtmişlerdir. Karakulak ve ark. (2020) katılımcıların %46,7'sinin tazeliğe, %24,4'ünün hepsi kriterine, %15,4'ünün fiyata, %5,3'ünün lezzete, %6,1'inin az kılçıklı olmasına ve %2,2'sinin ise diğer faktörlere dikkat ettiklerini ifade etmişlerdir. Kaplan ve ark. (2019) tüketicilerin %50,48'inin lezzetli balığa, %33,76'sının az kılçıklı olmasına, %31,51'inin ekonomik olmasına ve

%13,18'inin diğer kriterlere dikkat ettiklerini, Yüksel ve Diler (2019) ise katılımcıların %35,7'sinin mevsime göre seçim yaptığını, %20,4'ünün balığın gözlerine, %10,5'inin ise satıcının tavsiyelerine önem verdiklerini tespit etmişlerdir. Bolat ve Telli (2019) katılımcıların %82'sinin tazeliğe, %6'sının satıcıya güvenmeye, %6'sının balık türüne dikkat ettiklerini, Bolat ve Cevher (2018) ise %71'inin tazeliğe, %11'inin türüne, %9'unun fiyata ve %9'unun satıcıya güvenmeye odaklandıklarını belirtmişlerdir. Arıca (2017) ise tüketicilerin %42'sinin tazeliğine, %7'sinin balığın türüne, %3'ünün kalite ve hijyenine, %3'ünün fiyata, %1'inin satış yeri temizliğine ve %44'ünün ise her şeye dikkat ettiklerini ifade etmiştir. Uzundumlu ve Dinçel (2015) ise balık satın alan bireylerin %99'unun tazeliğe, %75,29'unun fiyata, %51,08'inin satış yerine, %38,46'sının görünüme, %33,47'sinin deniz ürünü olmasına dikkat ettiğini bildirmişlerdir.

Yapılan çalışmalara bakıldığında tüketicilerin genellikle tazelik kriterine önem verdiği, bunu lezzet, fiyat ve az kılçıklı olup olmaması ve diğer seçeneklerin izlediği görülmektedir. Çalışmamızdan elde edilen sonuçlar yukarıda belirtilen çalışmalar ile benzerlik göstermektedir.

Anketimize katılanların büyük bir kısmı su ürünlerinin işlenmesinde uygulanan gıda güvenliği kontrol ve denetimlerinin yapıp yapılmadığı sorusuna orta derecede katıldıklarını belirtirken, yeterli görenlerin oranı %23 ve yetersiz görenlerin oranı ise %32 olarak belirlenmiştir (Şekil 15). Arıca (2017) yaptıkları çalışmada tüketicilerin sadece %13'ü piyasada satılan su ürünlerinin yeterince denetlendiğini düşünürken, %57'si ürünlerin kısmen denetlendiğini ve %30 ise yeterince denetlenmediğini düşündüğü bildirilmiştir. Bu hususta daha çok çalışma yaparak tüketicilerin bu konudaki eğilimi belirlenmelidir. Ayrıca bu konuda yeterince tanıtım ve bilgilendirme yapılarak tüketicilerin su ürünlerine olan güvenini arttıracak çalışmaların yapılması tarafımızca tavsiye edilmektedir.

## Sonuç

Türkiye'de su ürünleri tüketimi, gelişmiş ülkelerle karşılaştırıldığında oldukça düşük seviyelerdedir. Bir toplumun sosyal ve ekonomik kalkınması için bireylerin zihinsel ve fiziksel sağlıklarının güçlü olması gerektiği göz önünde bulundurulduğunda (Onurlubaş ve ark., 2015), su ürünlerinin tüketimi sağlık açısından büyük bir öneme sahiptir. Üç tarafı denizlerle çevrili bir ülke olmasına rağmen, su ürünleri tüketiminin artırılabilmesi için halkın, özellikle de tüketicilerin bu ürünlerin faydaları konusunda bilinçlendirilmesi gerekmektedir. Geçmişte medya aracılığıyla yapılan tanıtımlar olsa da (Çiçek ve ark., 2014), son yıllarda bu tür tanıtımların yetersiz kaldığı gözlemlenmektedir. Araştırmamızın bulgularına göre, Yalova Üniversitesi öğrencileri, sağlıklı beslenme adına su ürünleri ve balık tüketiminin gerekli olduğunu kabul etmekte ancak tüketim düzeyleri istenilen seviyenin altındadır. Bu durumu etkileyen başlıca etkenler arasında sosyo-ekonomik faktörler öne çıkmakta, ikinci sırada ise barınma alanlarının su ürünleri tüketimine elverişsiz olması yer almaktadır. Su ürünlerinin fiyatlarının düşürülmesi ve özellikle üniversite öğrencilerinin sağlıklı beslenmeye yönelik stratejilerle desteklenmesi gerektiği vurgulanmalıdır. Bu doğrultuda, öğrenci yurtlarındaki

yemek menülerinde su ürünlerine daha fazla yer verilmesi, ayrıca eğitim müfredatlarında beslenme konusunun daha fazla işlenmesi, gelecek nesillerin bilinçli bir şekilde su ürünleri tüketimi konusunda eğitilmesini sağlayacaktır. Ayrıca, bu tür araştırmaların devam etmesi, su ürünleri sektörünün geliştirilmesine yönelik temel hedeflerin ve stratejilerin oluşturulmasına yardımcı olacaktır.

## Beyanlar

### Etik Kurul Onayı

Bu makalede yer alan anket çalışması için “Yalova Üniversitesi Rektörlüğü Etik Kurulu”nun 03.04.2024 tarihli ve 2024/8 protokol numaralı kararı ile Etik Kurul Onayı alınmıştır.

### Yazar Katkısı

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

### Çıkar Çatışması

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

### Teşekkür

Bu çalışmaya göstermiş oldukları ilgi ve katılımlarından dolayı Yalova Üniversitesi öğrencilerine teşekkür ederiz.

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## The Impact of Exchange Rate and Inflation Rate Fluctuations on Türkiye's Agricultural Exports: A Statistical Analysis

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### ABSTRACT

Examining agricultural exports from the specific perspective of the Turkish economy is essential, as it facilitates the development of foreign trade policies, strengthens the agricultural sector, enhances global market competitiveness, and optimizes productivity. This study employs multiple regression analysis to assess the impact of exchange rate volatility and inflation on Türkiye's agricultural exports from 2016 to 2023. The results indicate that these two variables have a moderate effect on the value of Türkiye's agricultural exports. Additionally, the analysis highlights that exchange rate volatility and inflation rates significantly affect these exports. Particularly, the positive impact of exchange rate volatility on exports suggests that firms may benefit from increasing their export share.

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### Introduction

Export expansion plays a pivotal role in fostering national economic development, particularly within emerging economies. Notably, since the 1980s, Türkiye has shown a strong commitment to outward-oriented growth strategies within the global economy, a shift marked by the January 24, 1980 Decisions. As a result of these resolutions, Türkiye has experienced a substantial increase in exports and a significant transformation in its economic structure. The growth in exports has benefited the economy by attracting a skilled labor force and enhancing product diversity across multiple sectors. (Genç et al., 2010). Following the January 24 Decisions, trade with both European Union nations and Middle Eastern and Islamic countries experienced a revival, leading to an average 14.7% increase in foreign trade volume from 1980 to 1990 (Özkardeş, 2015). Moreover, this evolving global structure introduces the risk of foreign exchange fluctuations for nations. Türkiye's 2001 economic crisis prompted a shift from a fixed to a flexible exchange rate regime, supporting an export-driven economic growth model. Consequently, the Turkish government aimed to reduce the foreign trade deficit by boosting exports within this framework (Altın and Suslu, 2017).

It can be argued that growth in a country's overall exports, as well as in its agricultural exports, contributes positively to the growth of its GDP (Gilbert et al., 2013; Njimanted and Aqlias, 2015; Ben-Amor et al., 2015; Hyunso, 2015; Verter and Becvár). Since the analysis in this study covers the years 2016-2023, the data representing agricultural exports for these years is displayed in Figure 1. It becomes evident that export values show a general upward trend over time. As illustrated in Figure 1, the average annual value of agricultural exports was approximately USD 1.5 billion in 2013, with projections indicating an increase to USD 3.5 billion by 2023. In 2020, a marked decrease in exports occurred due to the global impact of the SARS-CoV-2 pandemic. The seasonal decline in agricultural exports during winter and summer months may be attributed to these being the primary periods for sowing and harvesting, respectively.

Adopting a distinct approach to studying agricultural product exports within the Turkish economy is essential. Such an approach aims to inform foreign trade policy, support agricultural sector development, enhance international market competitiveness, and boost productivity.

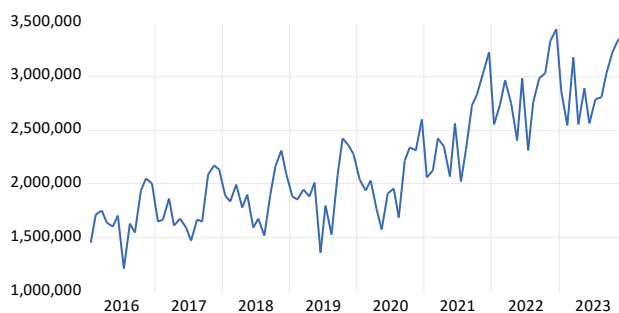


Figure 1. Türkiye's Agricultural Product Exports (Billion USD) Source: TIM

This study's objective is to analyze the relationship between agricultural product exports, inflation, and exchange rates through multiple regression analysis. The study utilizes agricultural export data from the Turkish Exporters Assembly (TIM) and monthly inflation and dollar exchange rate data for the years 2016-2023, sourced from the Central Bank of the Republic of Türkiye's Electronic Data Distribution Centre (EVDS). The importance of agriculture and agricultural production in Türkiye, historically and currently, has attracted substantial interest. The study's significance and originality lie in its examination of agricultural exports in relation to inflation and exchange rates over this period. The observation interval selected is expected to offer valuable insights regarding both dependent and independent variables, contributing unique value to the research.

## Literature review

The impact of exchange rate fluctuations and inflation on a country's agricultural exports is a topic of significant interest in the field of economics. Various studies have been conducted to analyse the relationship between exchange rate movements, inflation, and agricultural exports.

In a 2002 study, Sevela examined the impact of agricultural exports on economic growth in the Czech Republic, incorporating variables such as gross national product (GNP), GNP per capita, agricultural exports, and a gravity model accounting for trade flows between exporting countries. The econometric method applied was weighted least squares (WLS). The findings demonstrated a statistically significant positive correlation between agricultural exports and GNP, while a statistically significant negative correlation was identified between agricultural exports and both GNP per capita and geographical distance.

In their study spanning the years 1990 to 2001, Demirel et al. (2004) utilized the Engle-Granger method to investigate the impact of real exchange rate volatility on exports in the mining and agriculture sectors. Their findings indicated that real exchange rate volatility has a negative effect on exports in these sectors.

In the study by Dawson (2005), the contribution of agricultural exports on economic growth was measured for 62 countries for the period 1974-1995. In the study, fixed effects and random effects models were used within the framework of panel data analysis. As a result of the analysis, there are significant structural differences in economic growth between low, lower-middle, and upper-

income LDCs. Investment in the agricultural export subsector has a statistically identical impact on economic growth as investment in the nonagricultural export subsector. The marginal productivities in nonexport subsectors are over 30% lower than those in respective export subsectors. From a policy perspective, the results suggest that export-promotion policies should be balanced.

The study by Hatab et al. (2010) aimed to analyze the determinants of agricultural exports in Egypt using the gravity model method. The analysis results indicated that a 1% increase in GDP corresponded to a 5.42% rise in Egypt's agricultural exports. Additionally, the findings suggested that an increase in GNP per capita led to a decrease in trade flows as predicted by the gravity model.

Erdal et al. (2011) investigated the impact of real effective exchange rate volatility on Turkish agricultural exports and imports, using monthly data from January 1995 to November 2007. The study employed the GARCH (1,1) model to assess exchange rate volatility and Johansen cointegration to identify the long-term relationship between the variables. Additionally, Granger causality analysis was applied to determine the direction of these relationships. The findings indicate a positive long-term relationship between exchange rate volatility and agricultural exports, while a negative relationship was observed between volatility and agricultural imports.

Yanıkaya et al. (2013) and Peker (2014) used panel data analysis to examine the relationship between exchange rates and the agricultural sector. Their findings indicated a positive correlation between agricultural product exports and exchange rate volatility. Specifically, as the exchange rate increases and the Turkish lira depreciates, agricultural product exports tend to rise.

In a 2015 thesis, Karataş examined the effect of the real effective exchange rate (REER) and REER volatility on Türkiye's agricultural foreign trade. Panel data analysis was conducted for the period 1990-2012, focusing on 25 key countries involved in Türkiye's agricultural trade. The FMOLS model was used to assess the relationship between IGARCH variables and volatility. The results showed a 7.61% increase in agricultural imports and a 2.24% increase in agricultural exports.

Toktaş (2017) conducted a statistical and cointegration analysis covering the period from 1997 to 2015. Using the Bound Test Approach, the findings indicated that agricultural product exports are affected by exchange rate volatility, with hazelnuts identified as the most susceptible export product.

Yetiz and Ozden (2017) conducted an investigation into the causal relationship between Türkiye's GDP and the agriculture, industry and services sectors, utilising 1968-2015 annual data. Accordingly, Engle-Granger causality/Block Exogeneity Wald tests, Impulse-Response and Variance Decomposition analyses were employed. Upon evaluation of the results, it was found that although there is a unidirectional Granger causality relationship from the agricultural sector to the GDP and industry and services sectors, the agricultural sector has a stronger relationship with the other sectors.

The United States Department of Agriculture (USDA) published a report titled "The Competitiveness of the Brazilian Agricultural Sector in 2020: Recent Growth and Future Implications under the Impact of Changing

Macroeconomic Conditions and Currency Weakness.”\* The report highlights that macroeconomic reforms and policies have played a key role in positioning Brazil as a major competitive agricultural exporter. Despite challenges such as declining international commodity prices, slowed demand, and a severe recession from 2014 to 2016, Brazil’s agricultural sector has continued to maintain its export levels, establishing itself as one of the world’s most significant and resilient agricultural exporters.

Aslan (2024) utilized various analytical techniques—including stationarity tests, impulse-response analysis, variance decomposition, and causality analyses—to clarify the impact of exchange rate shocks and fluctuations on the value of Türkiye’s agricultural exports. Findings indicated that in the months following dollar exchange rate shocks, the error variance of agricultural exports increased within a range of 0.01% to 1.76%. This outcome suggests a significant interaction between open exchange rate movements and the value of agricultural exports.

## Materials and Methods

### Data Set

This study employs a dataset covering monthly agricultural export values, exchange rate volatility, and inflation from 2016 to 2023. Given that there are two independent variables, the dataset is considered sufficiently large (Akbulut and Çapık, 2022, p. 133). Data on Türkiye’s agricultural exports were obtained from the Turkish Exporters Assembly (TIM), while exchange rate and inflation data, used as independent variables, were sourced from the Central Bank of the Republic of Türkiye’s Electronic Data Distribution System (EDDS).

Figure 2 illustrates changes in the USD/TL exchange rate in relation to fluctuations in agricultural exports. Analysis of the relationship between these two variables indicates that increases in the dollar exchange rate are associated with a moderate rise in export values.

In general, exchange rate shocks positively influence exports in the short term. To assess the resilience of exports to exchange rate fluctuations, the daily standard deviation of the USD/TL exchange rate within each month was used as an indicator of monthly dollar volatility. Analysis of the relationship between agricultural exports and the monthly standard deviation of the dollar exchange rate revealed that higher exchange rate volatility, particularly after 2021, had a positive effect on agricultural exports.

Figure 4 illustrates changes in the inflation rate alongside agricultural exports. An examination of the relationship between these variables shows that during periods of rising inflation, export values generally increase as well, evidenced by the parallel upward trends in both inflation and exports in Türkiye post-2018. Notably, in 2023, even as inflation declined, export values continued to follow an upward trajectory.

### Research method

The objective of this study is to analyse the effect of exchange rate volatility in Türkiye’s agricultural sector on agricultural exports in monthly periods, taking inflation into account. To achieve this, a multiple regression model was employed as the research method.

## Multiple regression

Regression analysis is a technique used to investigate the relationship between a dependent variable and one or more independent variables. When only one independent variable is present, univariate regression analysis is applied. In contrast, when multiple independent variables are involved, multivariate regression—also referred to as multiple regression—is the primary analytical approach. In economic research, a variable may be influenced by multiple factors simultaneously, and these factors may interact with each other as well. In this study, considering the potential influence of multiple variables on investments, multiple regression analyses were conducted.

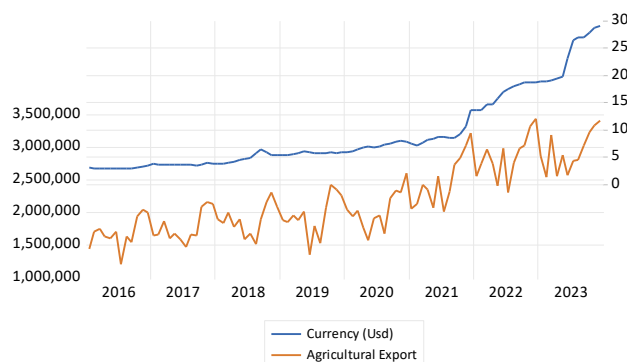


Figure 2. US Dollar/TL Exchange Rate and Agricultural Products Exports (Billion \$)  
Source: The data Organized by author using the data from TIM

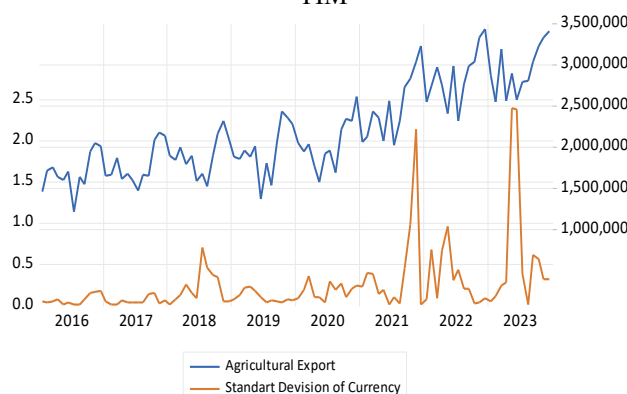


Figure 3. Standard Deviation of US Dollar/TL Exchange Rate and Exports of Agricultural Products (Billion)  
Source: The data were organized by the author using outputs from TIM

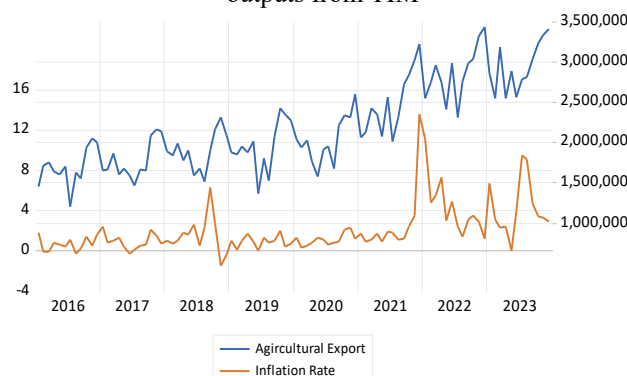


Figure 4. Türkiye's Agricultural Product Exports and Inflation Rate (Billion USD)

In regression analysis, independent variables are represented as model inputs, while the dependent variable serves as the model output. This approach allows for the examination of the relationship between the dependent variable and independent variable(s). In the current study, the dependent variable is defined as the “export values of agricultural products,” with “exchange rate volatility” and “inflation” serving as the independent variables.

Following this methodology, the econometric model to be estimated is structured as follows:

$$\text{export}_t = \alpha + \beta_1(e)_t + \beta_2(\text{inf})_t + \varepsilon$$

## Findings

### Descriptive Statistics

In this study, the Eviews 12 software package was used to perform all statistical and econometric analyses. First, the descriptive statistical values of the data were calculated and are presented in Table 1. As shown, logarithmic transformations of the variables were applied in the descriptive statistical analyses. Given the substantial differences in the numerical magnitudes of the variables at their level values, regression analyses were conducted using logarithmic transformations. This approach minimized differences in magnitude across variables, allowing them to be analyzed on a comparable scale in logarithmic form.

A fundamental assumption in linear regression models is that the independent variables should follow a normal distribution. In this study, descriptive statistics were used to determine whether the variables exhibit normal distribution characteristics. The skewness and kurtosis values indicate the degree to which the variables align with a normal distribution. As noted by Günlük-Şenesen (2007), a skewness value close to zero suggests symmetry around the mean, while a kurtosis value of 3 indicates a mesokurtic (normal) distribution. A kurtosis value below 3 suggests that the series is less peaked, or more platykurtic, than a normal distribution. Conversely, a kurtosis value of exactly

3 indicates that the series is mesokurtic, aligning with a normal distribution. If the skewness value is less than zero, it suggests the distribution is left-skewed, meaning the tail on the left side is longer. Positive skewness values indicate right skewness, where the distribution tail extends more on the right. Typically, skewness values between -1.5 and 5 are considered within acceptable limits for normality (George and Mallery, 2010). Values within this range suggest the dataset is moderately skewed to the right. Based on these criteria, all variables, including the logarithmic transformation of the foreign exchange variable, were found to be normally distributed.

### Stationarity Tests

The stationarity test is a fundamental requirement in time series analysis, as non-stationary series may fail to accurately represent the relationships between variables (McKinnon, 1991). Therefore, it is essential to conduct stationarity tests on the series and transform any non-stationary series into a stationary form.

### Data Stationarity Tests

The test results indicated that the Agricultural Export series was non-stationary at level, exhibiting a unit root. However, after taking the first difference of the series, it became stationary in the first-lagged form without a constant or trend. Additionally, the critical absolute value (4.31) exceeded the thresholds of 3.51, 2.89, and 2.58 at the 1%, 5%, and 10% significance levels, respectively, thereby rejecting the  $(H_0)$  hypothesis. The results of the corresponding ADF test are displayed in Table 2.

The test results indicated that the inflation series was stationary at level. Moreover, the critical absolute value of the series (4.51) exceeded the thresholds of 3.50, 2.89, and 2.58 at the 1%, 5%, and 10% significance levels, respectively, leading to the rejection of the  $(H_0)$  hypothesis. The results of the corresponding ADF test are shown in Table 3.

Table 1. Descriptive Statistics

	Currency (e)	Exp	Inf	LogCur	LogExp	LogInf
Mean	9.169697	2198144	2.059063	1.969647	14.57378	0.334790
Median	6.057865	2064513	1.33	1.801357	14.54040	0.336064
Maximum	29.07486	3435761	13.58	3.369874	15.04975	2.608598
Minimum	2.839848	1206270	-1.44	1.043750	14.00304	-3.506558
Std.Dev.	7.068800	542995.7	2.415763	0.680845	0.242496	1.053424
Skewness	1.356458	0.550534	2.438306	0.529054	0.159906	-0.813778
Kurtosis	3.780544	2.338479	10.02326	2.143405	2.177245	5.201467
Jarque-Bera	31.87664	6.599848	292.4301	7.413387	3.116822	28.10773
Probability	0.00000	0.036886	0.00000	0.024559	0.210470	0.000001
Sum	880.2910	2.11E+08	196.6700	189.0861	1399.083	30.13113
Sum Sq.Dev.	4746.954	2.80E+13	554.4114	44.03725	5.586392	98.76352
Observations	96	96	96	96	96	96

Source: The data were organized by the author using outputs from Eviews.

Table 2. Export Data ADF Unit Root Test

Augmented Dickey-Fuller test statistic		T-Statistic	Prob.*
		-4.311371	0.00008
Test critical values	1% level	-3.511262	
	5% level	-2.896779	
	10% level	-2.585626	

Source: The data were organized by the author using outputs from Eviews.

Table 3. Inflation ADF Unit Root Test

Augmented Dickey-Fuller test statistic		T-Statistic	Prob.*
		-4.512039	0.0004
Test critical values	1% level	-3.500669	
	5% level	-2.892200	
	10% level	-2.583192	

Source: The data were organized by the author using outputs from Eviews.

Table 4. Currency ADF Unit Root Test

Augmented Dickey-Fuller test statistic		T-Statistic	Prob.*
		-5.682593	0.0000
Test critical values	1% level	-3.501445	
	5% level	-2.892536	
	10% level	-2.583371	

Source: The data were organized by the author using outputs from Eviews.

Table 5. Estimation Results of the Model

		Dependent Variable export
e	$\beta_1$	(0.280214) (0.0000)
inf	$\beta_2$	(0.030565) (0.0488)
C	a	(14.00951) (0.0000)
R2		0,72
F-statistic		114.978
Prob(F-statistic)		0.000000
Durbin-Watson		127

Source: The data were organized by the author using outputs from Eviews.

Table 6. White Variable Variance Test Results

F-statistic	0.71	Prob. F(5,84)	0.6171
Obs*R-squared	3.652589	Prob. Chi-Square(5)	0.6004
Scaled explained SS	2.888743	Prob. Chi-Square(9)	0.7171

Source: The data were organized by the author using outputs from Eviews.

The test results indicated that the Currency series was non-stationary at level, exhibiting a unit root. However, after taking the first difference of the series, it became stationary in the first-lagged form without a constant or trend. The critical absolute value (5.68) exceeded the thresholds of 3.50, 2.89, and 2.58 at the 1%, 5%, and 10% significance levels, respectively, leading to the rejection of the  $(H_0)$  hypothesis. The results of the corresponding ADF test are shown in Table 4.

### Model

In this study, model (1) was utilized to determine the proportional change in the dependent variable (xu30) in response to proportional changes in the independent variables. Thus, the proportional change in “exports” in response to a 1% change in the independent variables was estimated. The resulting estimates were then analyzed.

Assumptions regarding the error term are crucial for ensuring the validity and significance of the analyses. Therefore, it is essential that the error term remains free of autocorrelation (Çakmak and Yılmaz, 2018, p. 280).

The R<sup>2</sup> value, representing the explanatory power of the independent variables on the dependent variable, is 0.72, indicating a satisfactory level of explanatory capability for the model. The F-test, which evaluates the statistical significance of a regression model, relies on the model's associated probability value. If this probability is less than 0.05 at a 95% confidence level, the regression model is

considered statistically significant. In other words, the collective explanatory power of the independent variables on the dependent variable is deemed meaningful. In this case, the F-value is 114.978, with a probability of 0.00, thus confirming the overall significance of the model through the statistical relevance of the joint effect of the independent variables, as verified by the F-test.

### White Variable Variance

If the variance of the error terms depends on the values of the independent variables, this issue is known as heteroskedasticity. Table 6 presents the results of the White Heteroskedasticity test, which is used to detect the presence of heteroskedasticity. The F-statistic values shown in the table are used to determine whether the error term variance remains constant. If the F-statistic is significant ( $p < 0.05$ ), it indicates the presence of a heteroskedasticity problem. Conversely, if the p-value is greater than 0.05, it suggests that heteroskedasticity is not an issue.

### Descriptive Statistics

Table 7 presents the descriptive statistics and Jarque-Bera test statistics for the error terms. Based on the results, the skewness and kurtosis values fall within the acceptable range for normal distribution, and the significance probabilities of the Jarque-Bera statistic exceed 0.05. This indicates that the error terms are normally distributed

Table 7. Descriptive Statistics for Error Terms

	E
Mean	3.05-15
Median	0.010155
Maximum	0.215112
Minimum	-0.31536
Std.Dev.	0.128154
Skewness	-0.346434
Kurtosis	2.692718
Jarque-Bera	2.154330
Probability	0.340560
Observations	96

Source: The data were organized by the author using outputs from Eviews.

Table 8. Ramsey Rest Test

	Value	Df	Prob.
T-statistic	0.809239	86	0.4206
F-statistic	0.654867	(1,86)	0.4206
Likelihood ratio	0.682730	1	0.4086

Source: The data were organized by the author using outputs from Eviews.

### Ramsey Reset Analysis

The Ramsey RESET (Regression Specification Error Test) was employed to assess whether the regression model is correctly specified. This test is a specialized diagnostic tool used to determine if additional explanatory variables should be included to improve model accuracy.

The results of the Ramsey RESET test are presented in Table 8. The t-statistic is used to determine whether the coefficients of the added square terms are zero. Here, the t-statistic value is 0.80 ( $p > 0.05$ ), indicating that including these terms does not significantly enhance the model's predictive performance. Additionally, the F-value assesses the model's overall significance. An F-statistic value of 0.65 ( $p > 0.05$ ) suggests that the additional terms do not improve the model's overall significance. The likelihood value, representing the likelihood ratio of the added terms, is 0.68 ( $p > 0.05$ ), further indicating that the added terms do not significantly increase the model's likelihood.

Contrary to expectations, the test results show that the additional terms do not significantly enhance the explanatory power of the model. Thus, the Ramsey RESET test suggests that further variables should \*not\* be incorporated into the existing model to augment its explanatory capacity.

The estimation results revealed a 0.28% decrease in the dependent variable for a 1% change in the independent variable, currency. The findings suggested that fluctuations in foreign exchange rates had a significant impact on exports. Furthermore, the estimation results for inflation, another independent variable, indicated that a 1% change in inflation would lead to a 0.03% increase in agricultural exports. An increase in inflation was found to have a positive and significant effect on agricultural exports.

### Results

This study utilizes multiple regression analysis to investigate the impact of exchange rate volatility and inflation on Türkiye's agricultural exports. The findings

suggest that the effects of these two variables on Türkiye's agricultural export values are moderate.

It has been demonstrated that an increase in exchange rate volatility is correlated with a rise in export values. A higher exchange rate lowers unit costs in TL, enabling firms to offer more competitive export prices.

Another key finding of the study is the discovery that an increase in the inflation rate is associated with a rise in agricultural export values. While conventional economic theory suggests that inflation typically has a negative impact on exports, the results of this study indicate that it may actually have a positive effect.

The results of the analyses presented in this study indicate that exchange rate volatility and inflation have a significant impact on Türkiye's agricultural exports. Specifically, the positive effect of exchange rate volatility on exports suggests that companies should adopt strategies aimed at increasing their export share.

### Discussion

The study demonstrates that increased exchange rate volatility is linked to higher export values. A rise in the exchange rate reduces unit costs for expenses paid in TL, allowing companies to offer more competitive prices as their costs in dollar terms decrease. Another key finding is that higher inflation is associated with an increase in agricultural export values. While economic theory typically suggests that inflation negatively impacts exports, this study finds a positive effect. The analysis indicates that both exchange rate volatility and inflation significantly influence Türkiye's agricultural exports. Notably, the positive effect of exchange rate volatility suggests that companies should aim to expand their export shares.

### Conclusion

The estimation results indicated a 0.28% decrease in the dependent variable for every 1% change in the independent variable, Currency. The findings demonstrated that fluctuations in foreign exchange rates had a favorable impact on exports. Additionally, the estimation results for inflation, another independent variable, suggest that a 1% change in inflation will lead to a 0.03% increase in agricultural exports. An increase in inflation has a positive and statistically significant effect on agricultural exports.

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## Effects of Ethephon and Pruning Practices on Sex Expression and Yield of Cucumber (*Cucumis sativus* L.) in Rupandehi, Nepal

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### ABSTRACT

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Enhancing crop productivity is essential for increasing farmer incomes, and application of ethephon along with proper pruning practices could provide effective approaches for improving yield of cucurbit crops. A field experiment was conducted to study the effect of ethephon and pruning practices on sex expression and yield of cucumber cv long green at Rupandehi, Nepal from April to July 2022. The experiment used a two-factor factorial RCBD, with 2 doses of ethephon (300 ppm & control spray) as one factor and pruning practices (3G, 2G & no pruning) as the other, resulting six treatments which were replicated four times. Morphological and phenological parameters such as plant height, days to flowering, number of male and female flowers per plant, M: F ratio, fruit length, fruit weight, fruit number per plant, and yield were recorded. It was observed that ethephon @ 300 ppm produced the highest yield (65.59 t/ha) with increased fruits per plant (13.19) and individual fruit weight (497.31 g). Highest fruit yield (66.97 t/ha), fruit number (13.47 per plant), and individual fruit weight (497.20 g) was observed with 3G pruning. Ethephon @ 300 ppm delayed male flowers, but female flowers were observed significantly earlier (34.21 DAT), with a similar effect observed in 3G pruning. Both ethephon @ 300 ppm (39.89) and 3G pruning (41.99) significantly increased the total number of female flowers in comparison with other treatments. Control spray of ethephon resulted in highest fruit length and application of ethephon @ 300 ppm resulted to highest fruit width. Pruning did not significantly influence fruit length but increased fruit width. The study revealed that a spray of 300 ppm ethephon and 3G pruning can enhance femaleness and productivity of cucumber.

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### Introduction

Cucumber, a widely cultivated vine crop belonging to the family Cucurbitaceae, originated from southern Asia (Adams et al., 1992). It is typically monoecious, where female flowers appear around a week after the appearance of the male flower. Early male flowers appear on lower nodes and female flowers on upper having small fruit at the base (Bantoc, 1964). Sex expression is a crucial factor affecting the yield of cucumber by influencing number of fruits in a plant. Genetics, chemical and environmental conditions influence the sex of a flower (Arpan, 1974). Seshadri (1990) reported that long days and higher temperature promote male flower production in cucumber. Heavy male flowering and reduced number of female flowers ultimately decrease the economic yield of crop (Chaurasiya et al., 2020).

Plant growth regulators (PGRs) are chemical compounds that influence the morphological and physiological attributes including yield attributing characters. They significantly affect sex expression and flowering in many cucurbits and reduces the number of male flowers or increases the number of female flowers (Al-Masoum & Al-Masri, 1999). Exogenous PGR application promotes femaleness in cucurbits, increasing pistillate flowers, fruit number, and yield (Mia et al., 2014). Application of PGR at two and four true leaf stage is critical for manipulating the sex ratio (Hossain et al., 2006). Chemically ethephon is 2-chloroethylphosphonic acid which releases ethylene when metabolized by plants (Arpan, 1974; Szyjewicz et al., 1984).

Exogenous application of PGRs like ethylene has demonstrated effective alteration of sex expression in cucumber through increment in the number of female flowers and suppression on the production of male flowers. Ethephon was useful in increasing the no. of female flowers, reducing male: female ratio and increasing the yield of cucumber (Dhakal et al., 2019).

Pruning cuts off unnecessary growth, conserving the plant's energy for fruit development, and improving harvest quality and quantity (Kumar et al., 2019), increases air circulation and CO<sub>2</sub> level thereby which increases the photosynthesis rate and increased accumulation of nutrients to economic parts (Mardhiana et al., 2017). Pruning has a positive correlation with vegetative growth and quality of fruit without any adverse effects (Usenik et al., 2008). Performing 2G and 3G pruning helps in growth of secondary and tertiary branches leading to better vegetative growth, higher fruit weight, a greater number of fruits per plant and a greater overall yield (Baral et al., 2022).

Although the previous studies have focused on effect of ethephon application and pruning practices on cucurbit crops, the combined effect of the interaction between level of pruning and application of ethephon are yet to be studied. Thus, this research aims to study the effect of ethephon, level of pruning and their interaction on sex expression and yield in cucumbers, with a goal to provide evidence-based recommendations for enhancing productivity and net returns for the farmers.

## Materials and Methods

### Site Details

The experiment was conducted during the spring-summer season of 2022 at a farmer's field in Siddharthanagar-9, Rupandehi, Nepal. It is located 25 km south of Butwal city and 25 km east of Lumbini. The coordinates of this area are 27°30' N Latitude and 83°27' E

Longitude. The topography of the area is plain terai and there was proper provision of irrigation facility. The Siddharthanagar municipality is located at an altitude of 110 meters above sea level and has a tropical climate. The average spring summer temperature is above 30°C and maximum temperature sometimes exceeds 40°C.

The Meteorological data during the research is depicted as follows

### Land Preparation and Seedling Transplanting

This research was conducted on a long green variety of cucumber. One deep ploughing and 2 light harrowing was done after broadcasting the FYM. The field was levelled, and clods were broken to prevent the interference to root development. Full dose of FYM was applied at the time of land preparation. Recommended dose of chemical fertilizers was applied (i.e. Urea: 100 kg/ha; Di ammonium phosphate (DAP): 40 kg/ha; Muriate of potash (MOP): 100 kg/ha) before transplanting (Krishi diary, 2021). The remaining dose of urea (i.e. 40 kg/ha) was side dressed during flowering stage. The plots of the required size were made, and plastic mulching was carried out. Holes were made on plastic mulch at a distance of 1m and seedlings from the nursery polybags were transplanted in the main field at two true leaf's stages after 15 days of sowing. As frequent irrigation is important in summer season it was carried out at the interval of 2-3 days in early stage and 5-6 days interval in the later stage of plant growth.

### Design of Experiment

The research was conducted in two factor factorial randomized complete block design including a total of six treatments replicated 4 times each. There was a total of 24 treatment plots with 16 plants on each plot. There was 1m plant to plant spacing and 1m row to row spacing. The gross plot size was 25m<sup>2</sup> (5m×5m) and net plot size was 16 m<sup>2</sup> (4m×4m).

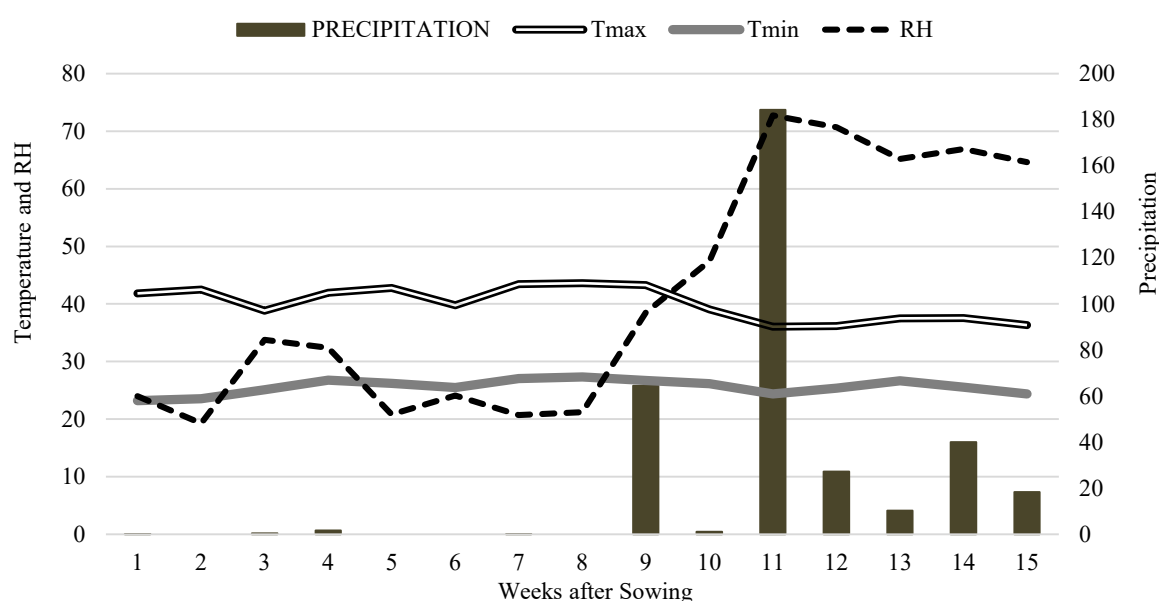


Figure 1. The temperature, RH and Precipitation data of experimental site during research period

Table 1. Details of experimental treatments with Ethephon and Pruning practices

Treatment	Treatment Designation
Ethephon dosages	
300 ppm	E1
Control spray	E2
Pruning practices	
3G Pruning	P1
2G Pruning	P2
No Pruning	P3
Interaction	
Ethephon 300 ppm with 3G pruning	T1
Ethephon 300 ppm with 2G pruning	T2
Ethephon 300 ppm with no pruning	T3
Control spray with 3G pruning	T4
Control spray with 2G pruning	T5
Control spray with no pruning	T6

### ***Spraying of Ethephon and Pruning***

At first, the commercial form of 39 % SL (Soluble Liquid) was diluted to 300 ppm. It was prepared by mixing of 0.7 ml of ethephon per liter of water and was stirred to ensure uniform mixing. The freshly prepared solution was used to maintain effectiveness. Two sprays of the prepared solution of ethephon were sprayed at two true leaf stage and four true leaf stage after transplanting using a hand pump sprayer. Pruning was conducted up to second generation (2G) and third generation (3G) in the experimental plots depending upon the treatment design to balance the vegetative and reproductive characters. In case of 2G pruning the main branch was allowed to grow 5-6 ft tall and pinched to allow growth of secondary branches. Similarly, the secondary branches were also pruned again after reaching 3-4 ft height to promote growth of tertiary branches in case of 3G pruning.

Four plants were chosen as sample plants from each experimental plot which remain after the border plants are excluded. The required parameters were observed from the sample plants at an interval of 15 days.

### ***Growth, Fruit, Flower, and Yield parameters***

Plant height was measured by using a meter scale. The height was measured from soil surface to the tip of the plant. Plant height was recorded after 30 DAT at 10 days interval up to 50 DAT. Days to first male flower, female flower and first harvest of the selected sample plants was recorded from each of the treatment plots and mean was calculated. Total number of male and female flowers of the experimental plots were manually counted, recorded, and averaged to calculate the number of male and female flowers per plant. The length and width of the randomly selected four fruits were measured using a standard measuring tape at the time of harvesting from each of the sample plants. The length and width of fruit was measured. At the time of harvesting three randomly selected fruits were weighed by weighing balance. The average fruit weight was calculated and subjected to analysis. Similarly, the number of fruits harvested from each sample plant was also recorded. The weight of harvested fruits from a sample plot was measured and recorded in each harvest. The weight from each recording was summed up to calculate the yield per plot and averaged to calculate the yield per plant. Fruit yield per hectare was calculated by multiplying

yield per plant with number of plants (NP) per hectare and no of plants per hectare is calculated by the formula below.

$$NP = \frac{A}{DBRR \times DBPP}$$

A : Area

DBRR: Distance between row to row

DBPP : Distance between plant to plant

### ***Statistical Analysis***

The recorded data was entered into MS- Excel and proceeded to analysis of variance (ANOVA). R-Studio was used for data analysis. Duncan's Multiple Range Test (DMRT) was used for mean comparison at 5 % level of significance.

## **Results and Discussion**

### ***Plant Height***

The study revealed that the plant height was significantly influenced by the application of ethephon as well as pruning at 30 DAT, 40 DAT and 50 DAT (Table 2). The highest height was observed in control spray (167 cm) and lowest height was observed in spray of ethephon @ 300 ppm (139.65 cm) at 50 DAT. In all the three dates of data collection for plant height, control spray had significantly higher plant height than spray of ethephon 300 ppm. Ethylene tends to inhibit IAA transport in plant systems elongation of tissue (Morgan & Gaussman, 1966). Hayashi et al. (2001) stated that the anti-gibberellic property of ethylene causes cessation of mitotic process in meristem of root and shoot leading to decreased length which could be the reason for decreased plant height with application of ethylene @ 300 ppm.

Similarly, 3G pruning had significantly higher plant height at 30 DAT but lower at 40 DAT and 50 DAT than no pruning. The highest plant height (180.62 cm) was observed on no pruning at 50 DAT followed by 3G (147.53 cm) and 2G (131.84) respectively. At 30 DAT plant height on 3G pruning was 39.85 cm which was at par with 2G (37.85 cm) but significantly higher than no pruning (31.36 cm). At 30 DAT the higher plant height in 3G pruning and 2G pruning might be due to diversion of nutrients to main shoot after removal of lateral branches.

Comparable results were also reported by Baral et al. (2022) and Shivaraj et al. (2018). Reduced crop height in cucumber enhances light penetration, air circulation, boosting photosynthesis and facilitates tasks such as harvesting and pest control easier eventually promoting higher fruit development and yield.

#### Days to Flowering and First Fruit Harvest

Emergence of 1<sup>st</sup> male and female flower was significantly influenced by application of ethephon as well as pruning (Table 3). The Duncan Multiple Range Test (DMRT) test for the days to first harvest found that there was no significant difference between the use of ethephon @ 300 ppm and control spray. Also, no significant effect of pruning was seen on the first fruit harvest which was in line with the research of Baral et al. (2022).

Days to first male flower was observed significantly higher in control spray (30.65 DAT) over application of ethephon (26.63 DAT). In case of female flower, 300 ppm ethephon spray had early appearance of female flower than control spray. First female flower for control spray was

observed at 38.13 DAT which was significantly higher than application of ethephon (34.21 DAT). The ethylene releasing chemicals enhances the growth of pistillate flowers and delays growth of staminate flowers of monoecious cucurbits (Bhandary et al., 1974; Sheshadri, 1990). This might be the reason for early appearance of male flowers in control spray and female flowers in ethephon @ 300 ppm. Ito et al. (1954) mentioned that application of certain chemicals at primordial stage can transform male flowers into female flowers.

Days to first male flower was significantly higher for 3G pruning (30.25 DAT) which was at par with 2G pruning (29.49 DAT) and lowest number of days for male flower was observed with no pruning treatment (26.18 DAT). First appearance of female flower was observed highest in no pruning treatment (39.41 DAT), and it was significantly higher than 2G (34.75 DAT) and 3G (34.34 DAT). Shivaraj et al. (2018) and Suthar et al. (2007) also reported similar findings. Early flowering in cucumber may be due to increased plant height and increased leaf area which supplemented assimilates required to promote early flowering.

Table 2. Plant height as influenced by ethephon and pruning intensities of cucumber at Rupandehi, 2022

Treatments	Plant height (cm)		
	30 DAT	40 DAT	50 DAT
Ethephon			
300 ppm	32.46 <sup>b</sup>	79.17 <sup>b</sup>	139.65 <sup>b</sup>
Control spray	40.25 <sup>a</sup>	106.35 <sup>a</sup>	167 <sup>a</sup>
SEm (±)	0.83	2.71	3.99
F probability	7.99e <sup>-06***</sup>	3.67e <sup>-06***</sup>	0.00021 <sup>***</sup>
Pruning			
3G	39.85 <sup>a</sup>	87.95 <sup>b</sup>	147.53 <sup>b</sup>
2G	37.85 <sup>a</sup>	82.11 <sup>b</sup>	131.84 <sup>c</sup>
No pruning	31.36 <sup>b</sup>	108.21 <sup>a</sup>	180.62 <sup>a</sup>
SEm (±)	1.10	3.32	4.88
F probability	7.73e <sup>-05***</sup>	0.00014 <sup>***</sup>	1.33e <sup>-05***</sup>
CV %,	7.93	10.11	9.01
Grand mean	36.35	92.76	153.33
Interaction (Ethephon × Pruning)	Ns	Ns	Ns

Note: SEm: Standard error of means; CV: Coefficient of Variation; Means followed by same letter in a column are not significantly different by DMRT at 5 % level of significance, \*\*\*=significant at 0.1 % probability level; Ns: Non significance; DAT: Days After Transplanting.

Table 3. Days to first flowering and first harvest as influenced by ethephon and pruning intensities of cucumber in Rupandehi, 2022

Treatments	Days after transplanting		
	Male flower	Female flower	First Harvest
Ethephon			
300 ppm	30.65 <sup>a</sup>	34.21 <sup>b</sup>	47.23 <sup>a</sup>
Control spray	26.63 <sup>b</sup>	38.13 <sup>a</sup>	50.9 <sup>a</sup>
SEm (±)	0.76	1.02	1.37
F probability	0.0018 <sup>**</sup>	0.02 <sup>*</sup>	Ns
Pruning			
3G	30.25 <sup>a</sup>	34.34 <sup>b</sup>	48.38 <sup>a</sup>
2G	29.49 <sup>a</sup>	34.75 <sup>b</sup>	48.19 <sup>a</sup>
No pruning	26.18 <sup>b</sup>	39.41 <sup>a</sup>	50.63 <sup>a</sup>
SEm (±)	0.93	1.25	1.68
F probability	0.016 <sup>*</sup>	0.02 <sup>*</sup>	Ns
CV %,	9.15	9.76	9.69
Grand mean	28.64	36.17	49.06
Interaction (Ethephon × Pruning)	Ns	Ns	Ns

Note: SEm: Standard error of means; CV: Coefficient of Variation; Means followed by same letter in a column are not significantly different by DMRT at 5 % level of significance, \*=significant at 5 % probability level, \*\*=significant at 1 % probability level; Ns: Non significance; DAT: Days After Transplanting.

Table 4. Number of flowers as influenced by ethephon and pruning intensities of cucumber in Rupandehi, 2022

Treatments	Number of flowers		
	Male	Female	M:F ratio
ethephon			
300 ppm	46.98 <sup>b</sup>	39.89 <sup>a</sup>	1.31 <sup>b</sup>
Control spray	68.14 <sup>a</sup>	27.93 <sup>b</sup>	2.55 <sup>a</sup>
SEm ( $\pm$ )	2.06	1.27	0.11
F probability	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>
pruning			
3G	45.86 <sup>c</sup>	41.99 <sup>a</sup>	1.21 <sup>c</sup>
2G	59.45 <sup>b</sup>	33.48 <sup>b</sup>	1.94 <sup>b</sup>
No pruning	67.39 <sup>a</sup>	26.27 <sup>c</sup>	2.65 <sup>a</sup>
SEm ( $\pm$ )	2.53	1.55	0.135
F probability	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>
Interaction			
T1	33.80 <sup>d</sup>	50.51 <sup>a</sup>	0.68 <sup>e</sup>
T2	50.29 <sup>c</sup>	40.39 <sup>b</sup>	1.27 <sup>d</sup>
T3	56.85 <sup>c</sup>	33.40 <sup>c</sup>	2.00 <sup>c</sup>
T4	57.92 <sup>bc</sup>	28.78 <sup>cd</sup>	1.74 <sup>cd</sup>
T5	68.59 <sup>ab</sup>	26.57 <sup>cd</sup>	2.60 <sup>b</sup>
T6	77.92 <sup>a</sup>	23.77 <sup>d</sup>	3.31 <sup>a</sup>
F probability	Ns	0.40 <sup>*</sup>	Ns
CV %,	12.41	12.97	19.69
Grand mean	57.57	33.91	1.93

Note: SEm: Standard error of means; CV: Coefficient of Variation; Means followed by same letter in a column are not significantly different by DMRT at 5 % level of significance, \*=significant at 5 % probability level, \*\*\*=significant at 0.1 % probability level; Ns: Non significance; DAT: Days After Transplanting.

#### Male Flowers, Female Flowers, and M:F Ratio

It was revealed that there are significant differences in the data recorded for total number of male flowers, female flowers, and M:F ratio due to spray of ethephon as well as pruning of the plant.

Control spray had significantly higher (68.14) number of male flowers than ethephon @ 300 ppm (46.98), but the number of female flowers observed in ethephon @ 300 ppm (39.89) was significantly higher than in control spray (27.93). Thus, higher M:F ratio was observed in control spray, as it had significantly higher no. of male flowers and lower no. of female flowers in comparison to ethephon @ 300 ppm. Application of ethephon decreases internodal distance and increases the number of leaves (Chaudhary & Singh, 1970; Baral et al., 2022). As ethylene increased the number of leaves, there was increased photosynthate accumulation, increased level of carbohydrate and starch (Singh, 1984) which might have promoted the number of female flowers.

Similarly, significantly highest number of male flowers was reported in no pruning (67.39) followed by 2G (59.45) and least was observed in 3G (45.86), but in case of female flower 3G (41.99) was superior to 2G (33.48) and no pruning had lowest value (26.27). As a result, the highest M:F ratio was obtained in no pruning (2.65) followed by 2G (1.94) and least in 3G (1.21). Pruning increases the hormone level which stimulates the cell division, increases stem potential, induces canopy transpiration, and improves water status which might have affected flowers in plants (Saifuddin et al., 2010). Pruning activity removed the lower branches which were likely to bear greater number of male flower and less female flower bearing nodes it might also be the reason for decrease in the total no. of male flowers and M:F ratio.

The interaction effect was observed significant in case of no. of female flowers. Significantly higher number of female flowers were obtained with combination of ethephon @ 300ppm with 3G pruning (50.51) and significantly lowest number of female flowers were obtained with control spray with no pruning (23.77) which was at par with control spray with 2G running and control spray with 3G pruning. This might be due to promotion of female flowers due to hormonal regulation of ethephon for female flowers and optimum allocation of resources because of 3G pruning.

#### Yield Parameters

As illustrated in Table 5, both factors i.e., ethephon and pruning had significant differences in the yield of cucumber.

The fruit length was significantly higher in control spray than ethephon @ 300 ppm, whereas fruit width was observed highest in ethephon @ 300 ppm (20.82 cm) than control spray (18.68 cm). The reduced fruit length with the spray of ethephon is similar to the results observed by Rafeekher et al. (2002) & Dhakal et al. (2019). The length of fruit was not statistically different with the variation in pruning, but higher length of fruit was obtained with no pruning. In the case of fruit width, pruning had a significant effect. The highest fruit width was obtained with 3G pruning (21.20 cm) which was statistically higher than with 2G and lowest width was measured in no pruning (19.26 cm). Thakur et al. (2018) also reported that fruit length was not affected by pruning. The increase in width might be due to increased photosynthetic activity and assimilation of nutrients in fruits.

Table 5. Fruit quality parameters as influenced by ethephon and pruning intensities of cucumber in Rupandehi, 2022

Treatments	Fruit metrics				
	Length (cm)	Width (cm)	Weight (g)	Number/plant	Yield (ton/ha)
ethephon					
300 ppm	23.03 <sup>b</sup>	20.82 <sup>a</sup>	497.31 <sup>a</sup>	13.19 <sup>a</sup>	65.59 <sup>a</sup>
Control spray	25.58 <sup>a</sup>	18.68 <sup>b</sup>	426.79 <sup>b</sup>	11.02 <sup>b</sup>	47.00 <sup>b</sup>
SEm(±)	0.62	0.343	10.24	0.27	1.28
F probability	0.01*	0.000***	0.000***	0.000***	1.25e <sup>-08***</sup>
pruning					
3G	23.74 <sup>a</sup>	21.20 <sup>a</sup>	497.20 <sup>a</sup>	13.47 <sup>a</sup>	66.97 <sup>a</sup>
2G	23.84 <sup>a</sup>	19.44 <sup>b</sup>	459.95 <sup>ab</sup>	11.72 <sup>b</sup>	53.90 <sup>b</sup>
No pruning	25.34 <sup>a</sup>	18.62 <sup>b</sup>	429.00 <sup>b</sup>	11.12 <sup>b</sup>	47.68 <sup>c</sup>
SEm(±)	0.76	0.42	12.54	0.33	1.57
F probability	Ns	0.001**	0.005**	0.0005***	1.20e <sup>-06***</sup>
Interaction					
T1	22.55 <sup>b</sup>	23.21 <sup>a</sup>	558.84 <sup>a</sup>	14.68 <sup>a</sup>	77.25 <sup>a</sup>
T2	22.36 <sup>b</sup>	19.84 <sup>b</sup>	490.31 <sup>b</sup>	12.50 <sup>b</sup>	69.46 <sup>b</sup>
T3	24.17 <sup>ab</sup>	19.42 <sup>bc</sup>	442.79 <sup>bc</sup>	12.37 <sup>bc</sup>	54.64 <sup>c</sup>
T4	24.93 <sup>ab</sup>	19.20 <sup>bc</sup>	435.56 <sup>bc</sup>	12.25 <sup>bc</sup>	52.96 <sup>c</sup>
T5	25.30 <sup>ab</sup>	19.04 <sup>bc</sup>	429.60 <sup>c</sup>	10.93 <sup>cd</sup>	44.59 <sup>d</sup>
T6	26.50 <sup>a</sup>	17.82 <sup>c</sup>	415.21 <sup>c</sup>	9.8 <sup>d</sup>	39.73 <sup>d</sup>
F probability	Ns	0.041*	0.047*	ns	0.045*
CV %,	8.80	6.01	7.67	7.75	7.90
Grand mean	24.30	19.75	462.05	12.10	56.44

Note: SEm: Standard error of means; CV: Coefficient of Variation; Means followed by same letter in a column are not significantly different by DMRT at 5 % level of significance, \*=significant at 5 % probability level, \*\*=significant at 1 % probability level, \*\*\*=significant at 0.1 % probability level; Ns: Non significance; DAT: Days After Transplanting.

A significantly higher weight per fruit was obtained in ethephon @ 300 ppm than control. Also, significantly higher number of fruits per plant was observed in ethephon spray than control spray. As a result, fruit yield was observed higher in ethephon @ 300 ppm spray (65.59 t/ha) than control spray (47.00 t/ha). Ethephon at 200 or 300 ppm promotes appearance of female flowers and number of fruits, reduces the number of male flowers, fruit setting and increases the productivity of cucumber (Li, 1983). Thus, increased leaf number and area might have directed the assimilates to the sink leading to increased fruit weight. Similarly, pruning also had a major influence on fruit weight, fruit number and yield of cucumber. Significantly the highest fruit weight was obtained in 3G (497.20 g). Also, in case of pruning 3G (13.47) had significantly higher fruit number per plant than 2G and no pruning. The higher number of fruits per plant and weight per fruit contributed to highest yield per hectare with 3G (66.97 t/ha) and lowest was obtained with no pruning. Mardhina et al. (2017) mentioned that pruning helps to increase light interception to whole canopy, improves air circulation and CO<sub>2</sub> increased photosynthesis and yield.

The interaction between level of ethephon and pruning was also observed significant for fruit width and fruit weight. A significantly higher fruit width was observed in T1 (Ethephon @ 300ppm with 3G pruning). Similarly, in case of fruit weight, the highest fruit weight was also observed with T1, and lowest fruit weight was observed in T6 (control spray with no pruning) which was at par with T5 (control spray with 2G pruning), T3 (control spray with no pruning) and T4 (control spray with 3G pruning). The higher fruit weight in case of treatment combination of ethephon @ 300ppm with 3G pruning might be promotion of physiological processes like cell expansion, and metabolic changes during fruit growth due to ethylene and

pruning might have directed more resources toward fruit development. This synergistic effect could be the major reason behind higher fruit width and weight of fruit with combination of ethephon with 3G pruning.

## Conclusion

Foliar spray of ethephon 300ppm is better for promoting plant height, earliness in female flowers, number of female flowers, number of fruits harvested per plant and yield of cucumber. Among different types of pruning, 3G pruning is better in improving the performance of cucumber through increased number of female flowers, number of fruits and yield. Also, the combination of ethephon with 3G pruning is found to be beneficial for optimum yield of cucumber. Future research could be conducted investigating interactions on different growing seasons, different genotypes of cucumber and also on other cucurbit crops to improve the robustness and applicability of the findings.

## Declarations

### Author Contribution Statement

**K.G.:** Research design, Data collection, methodology, investigation, formal analysis, and writing the original draft

**P.R.D.:** Research design, supervision, conceptualization, methodology, review

**K.B.:** Data collection and investigation, Data analysis, review, and editing

**S.B.R.:** Data analysis, review, and editing

### Conflict of Interest

The authors declare no conflict of interest.

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## Effects of Different Mulching Practices on Garlic (*Allium sativum* L.) Growth and Production

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### ABSTRACT

Garlic (*Allium sativum* L.) is a shallow-rooted crop mostly grown for cloves, which are used as a food flavoring condiment. Mulching helps to keep soil moisture by lowering energy loss through evaporation and preventing vapor transfer. A field study investigated how different mulches affect the garlic growth metrics. The experiment was conducted in the Chitwan District of Nepal from December to April 2022 under a randomized complete block design with three replications and five treatments (control, polythene mulch, straw mulch, banana leaf mulch, and sawdust mulch). The results reveal that the type of mulching materials employed substantially impacts on garlic growth and clove yield. Rice straw mulch exhibited the highest plant height (70.69cm) at 120 DAP followed by sawdust (64.44cm) and banana leaves (62.34cm). At 120 days after planting, leaf length was found to be statistically similar under rice straw (43.36) and plastic mulch (41.56 cm). Plastic mulch showed the highest results in number of leaves per plant (7.6), Stem diameter (1.58cm), Bulb weight (44.61gm), Bulb diameter (5.11cm), Root length (8.48cm) and Total yield (15.99t/ha). On the other hand, saw dust had a greater impact on bulb length (6.05cm). Notably, plastic mulch regularly outperformed other treatments in most criteria, with rice straw following closely. Based on these findings, plastic mulch appears to be the best option for garlic production.

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## Introduction

Garlic (*Allium sativum* L.) belongs to the family Alliaceae and genus *Allium* and is a shallow-rooted crop (Karaye & Yakubu, 2006). It ranks as the second most widely used *Allium* after onion with a characteristic pungent smell (Kabir et al., 2013). Garlic is mainly cultivated for its cloves; however, its leaves and flower stalks are also consumable. Garlic was produced 74,763 Metric tons in 9943-hectare area in Nepal in 2021/22 (MoALD, 2023).

The probable reason for the decrease in yield is due to the reduction of soil moisture during the early growth phase of garlic. During this period, garlic requires supplemental irrigation, but irrigation facilities are often unavailable in almost all parts of Nepal. Mulching helps to retain soil moisture by reducing the energy lost through evaporation and preventing vapor transport. Garlic, being a thermo-photo-sensitive crop, has its vegetative growth and bulb development significantly influenced by the growing environment. Manipulating growing conditions through cultural practices like mulching has the potential to improve yield (El-Beltagi et al., 2022).

Mulches reduce the quantity of energy consumed in evaporation by blocking the transport of vapor (Farzi et al., 2017). Mulches made from paper, straw, weed or trash form an insulating layer on the soil surface, helping to conserve moisture and lower soil temperature. Using plant residues and synthetic materials as mulch is a well-proven technique for increasing the profitability of various horticultural crops (Gimenez et al., 2002). The primary aim is to enhance crop growth by regulating soil temperatures and maintaining soil moisture (Mutetwa & Mtaita, 2014). Mulches can also control weeds and protect crops from insect pests or diseases (Ngouajio et al., 2008). Mulching helps in a significant increase in N, P and K uptake compare to un-mulched condition. The result obtained from the use of mulching led to the increase in plant growth, yields and bulb size in the garlic (Seifu et al., 2017). Mulch may be permanent (e.g. plastic sheeting) or temporary (e.g. bark chips) which can be applied to bare soil, or around existing plants.

Organic mulches will gradually break down and become part of the soil over time (Yimer, 2020). This is a great advantage, because this decomposition adds organic matter to soil, helping the soil to better retain water and



nutrients and produce healthier plants. Inorganic mulch such as plastic mulch constitutes the largest volume of mulch used in commercial garlic production. Plastic mulches typically consist of polyvinyl chloride or polyethylene films. Due to their higher permeability to long-wave radiation, they can increase the temperature around plants during the night in winter. Hence, polyethylene film mulch is preferred as a mulching material to produce horticultural crops (Bhardwaj, 2013). Additional factors to consider when selecting mulch include cost and availability.

Each mulching technique has its benefits and effects on garlic growth parameters. Straw mulch can provide a more favorable microclimate for plants than plastic mulch (Atif et al., 2019). Rice straw provides long-term benefits in obtaining better plant height through the addition of organic matter and recycling of nutrients. Organic mulches reduce nitrate leaching, improve soil physical properties, prevent erosion, provide organic matter, regulate temperature and water retention, improve nitrogen balance, participate in nutrient cycling and increase biological activity (Yimer, 2020). The exceptional effectiveness of straw mulch in increasing plant height demonstrates its usefulness as a sustainable solution for improving garlic production (Seifu et al., 2017).

Therefore, a deeper knowledge of mulching in garlic cultivation is crucial for creating management strategies that maximize moisture retention and improve garlic yield. Thus, the study was conducted to assess how different mulches affect garlic growth parameters.

## Materials and Methods

This research was carried out in the Chitwan district of Nepal during the growing season from December to April 2022. The experiment field was at the geographical location of 27° 39' N latitude and 84° 20' E longitude at an altitude of 182 masl and has a subtropical climate. Garlic grows best in a cool climate, with temperatures between 10–20°C during its early growth stage and around 20–25°C for proper bulb formation. It thrives in well-drained soil and does not tolerate excessive moisture. The majority of soils found in this region are in pH range of 6.2 (slightly acidic) to 7.8 (alkaline). The experiment was conducted using a randomized complete block design which consists of 5 treatments (control, polythene mulch, straw mulch, banana leaves mulch and saw dust mulch) with three replications. Mulch materials were obtained from the local market. Polythene mulch was 25 microns and other mulches were chopped in small pieces so that they breakdown easily. The plot covered an area of 4.2 square meters with clove planted 15 cm apart between rows and 10cm apart within rows. The spacing between plots was 0.5m, while the distance between blocks was 1m.

The field was prepared on December 15th, 2022, by 2-3 deep plowing followed by leveling, one week before plantation. Weed debris was cleared and soil was finely tilled to ensure uniform moisture distribution, optimal temperature, and proper root penetration as well as consistent leveling was done to prevent waterlogging and promote effective seed germination, emergence, and establishment. The plot was carefully marked out with sufficient spacing between and within rows using common farm tools, tape, hoe, rope and rake. The detailed layout

was arranged before sowing. Cloves of local cultivars of the same size obtained from research center were used for planting. Mulching was done immediately after planting the cloves. Chemical fertilizer was delivered via urea, DAP, and MOP at a rate of 60:40:50 kg NPK/ha-1. Complete doses of potassium and phosphorus, together with half dose of nitrogen were applied as a basal dose. The half left over nitrogen was side dressed in two applications, half at three weeks after plant emergence and remaining half was applied at five weeks later.

## Observation

### Data collection

The growth parameters data were gathered during the field experiment by randomly sampling plants from the two central rows in each plot. The following parameter was recorded on five randomly taken plants from each plot.

### Growth parameters

#### Plant height (cm)

Height of the plants was measured from selected plants from each plot. It was measured from the soil surface to the tip of mature leaf at 30, 60, 90, 120 days after planting.

#### Leaf Length (cm)

It was measured using the ruler from the base of the leaf to the tip of the leaf at 30, 60, 90 and 120 days after planting.

#### No. of leaves per plant

It was the mean number of leaves excluding senescent and newly developing leaves produced by sampled plants at 30, 60, 90 and 120 days after planting.

### Yield parameters

#### Stem diameter

Stem diameter of plants was measured at the time of harvest using a vernier caliper. It is expressed in centimeters (cm).

#### Bulb weight

The fresh weight of garlic bulbs was measured using a digital balance for higher accuracy.

#### Bulb length

Bulb length was measured using a Vernier caliper after harvesting the garlic bulbs to show greater accuracy. Bulb length was measured from the tagged plants.

#### Bulb diameter

The bulb diameter was measured using a Vernier caliper after harvesting the garlic bulbs.

#### Root length

Root length was measured by using measuring scale from the base of the bulb (root plate) to the tip of the longest root.

#### Total yield

The total weight of the entire garlic population for each treatment in each plot was measured using a digital weighing balance and expressed in kilograms (kg).

### Statistical analysis

Data entry and processing were performed using Microsoft Office Excel and R-studio 4.2.2 was used for statistical analysis of the traits in a Randomized Complete Block Design (RCBD) design. Duncan's multiple range test (DMRT) was employed to detect variations in treatment means at the 5% level of significance.

## Results and Discussion

### Plant Height

A significant effect was observed on plant height under different mulching except at 30 days after planting (DAP) (Table 1). After 30 DAP, the maximum height of garlic was observed in the plastic mulch (24.58 cm) and the minimum in plants treated with banana leaves mulch (20.47 cm). After 60 DAP, the maximum height was observed in plants treated with rice straw mulch (49.83 cm) and the minimum in plants treated with control (42.61 cm). The plant heights of plants in plastic mulch treatment (48.47 cm) were found to be statistically similar to plant heights of plants treated with rice straw mulch, whereas the plant heights of plants treated with sawdust (44.64 cm) were statistically similar to plants under control after 60 days. The maximum height in garlic after 90 DAP was observed in plants at plastic mulch treatment (73.58 cm) and the minimum in plants treated with control (62.19 cm). After 120 DAP, the plant heights of garlic in every treatment showed decreasing trends with the maximum in the rice straw treatment (70.69 cm) and a minimum in the control treatment (58.56 cm).

The drop in plant height at 120 DAP is caused by senescence, which occurs when older leaves yellow, dry,

and die. This natural aging process can cause a decline in plant height when the leaves wilt and lose turgidity (Bresson et al., 2018). Furthermore, minerals and carbohydrates begin to translocate from the leaves to the developing bulbs as the plant becomes older; this reallocation of resources reduces leaf growth and may result in a drop in plant height (Schippers et al., 2015).

### Number of Leaves

At 30 DAP, the highest and lowest numbers of leaves were found in plants with plastic mulch (3.83) and sawdust mulch (3.27) (Table 2). The leaves number in plants in control (3.40) and banana leaves mulch (3.40) and rice straw mulch (3.77) was statistically similar. After 60 DAP, the same trend was seen with the highest number of leaves on plants treated with plastic mulch (5.10) and the lowest in plants treated with sawdust mulch (4.40). Similarly, the highest numbers of leaves were observed in plastic mulch after 90 DAP (7.93) and 120 DAP (7.6). After 90 DAP, the lowest number of leaves was found in plants of the control treatment (6.83) and plants with banana leaves mulch (6.83), whereas after 120 DAP, the lowest number of leaves were found in plants treated with sawdust mulch (6.13).

Table 1. Effect of different mulching on Plant height

Treatments	Plant height			
	30 DAP	60 DAP	90 DAP	120 DAP
Control	23.68ab	42.61b	62.19d	58.56c
Banana leaves	20.47b	43.87b	66.89c	62.34bc
Saw dust	20.84b	44.64b	68.59bc	64.44b
Rice straw	23.83ab	49.83a	73.58a	70.69a
Plastic	24.58a	48.47a	71.24ab	61.39bc
LSD (p<0.05)	3.24	3.24**	4.23**	4.06**
CV%	7.57	3.75	3.28	3.39
Grand mean	22.68	45.88	68.51	63.48

DAP: Days after planting, LSD: Least significant difference, CV: Coefficient of Variation.

Table 2. Effect of different mulching on the number of leaves per plant

Treatments	No. of leaves/plant			
	30 DAP	60 DAP	90 DAP	120 DAP
Control	3.4ab	4.83 ab	6.83 b	5.37c
Banana leaves	3.4ab	4.63bc	6.83b	5.47 bc
Saw dust	3.27b	4.4c	6.57c	6.13b
Rice straw	3.77ab	4.9ab	7.2ab	6.2b
Plastic	3.83a	5.1a	7.93a	7.6a
LSD (p<0.05)	0.51	0.26**	0.81**	0.75***
CV%	7.76	2.92	6.18	6.83
Grand mean	3.53	4.77	6.9	6.15

DAP: Days after planting, LSD: Least significant difference, CV: Coefficient of Variation.

Table 3: Effect of different mulching on Length of leaves.

Treatments	Length of leaves (cm)			
	30 DAP	60 DAP	90 DAP	120 DAP
Control	14.67ab	32.76b	40.99c	29.24c
Banana leaves	12.83b	31.84b	43.02bc	35.47b
Saw dust	12.53b	34.03ab	45.32b	33.89b
Rice straw	16.37a	37.12a	49.73a	43.36a
Plastic	15.47a	36.29a	48.73a	41.56a
LSD (p<0.05)	2.28*	3.19*	2.48***	4.05***
CV%	8.45	4.94	2.89	5.86
Grand mean	14.37	34.41	45.56	36.71

DAP: Days after planting, LSD: Least significant difference, CV: Coefficient of Variation.

The ability of plastic mulch to retain a larger number of leaves throughout the growing period demonstrates its usefulness in maintaining favorable growing conditions. According to Kumar Rai & Negi, (2021) maximum number of leaves is found in the treatment that could provide favorable moisture conditions and temperature. Applying organic mulches like rice straw, and banana leaves also contributes positively to the number of leaves, but their benefits may be less noticeable than those of plastic mulches. Organic mulches show positive results at later growth stages when the nutrient needs of plants increase. Leaves number rises to a certain point in plant development and then decreases. This finding aligns with results obtained by (El-Magd et al., 2013)

#### **Leaf Length**

At 30 DAP, the maximum leaf length of garlic was found in plants treated with rice straw mulch (16.37 cm) and the minimum leaf length was found in plants treated with sawdust mulch (12.53) (Table 3). The leaf length for garlic plants treated with banana leaves mulch (12.83 cm) was statistically par with plants grown with sawdust mulch. After 60 DAP, the maximum length of garlic leaves was observed in rice straw mulch (37.12 cm) and the minimum length was observed in banana leaf mulch (31.84 cm). Similarly, the longest leaf length was observed in plants treated with rice straw mulch at 90 DAP (49.73 cm) and 120 DAP (43.36 cm) respectively. The leaf lengths of garlic plants treated with plastic mulch were statistically similar to rice straw mulch in all the observations.

The number of leaves at 30, 60, 90, and 120 DAP were positively influenced by the effect of mulching. Similar findings for plastic mulch were also reported by (Jamil et al., 2005). Mulch retains moisture and improves nutrient availability to plants, resulting in overall plant growth and development, including number of leaves (Awasthi et al., 2022). Mulch produced higher leaf pigments than control plants. Mulched plants produced higher levels of chlorophyll-a, chlorophyll-b, and total chlorophyll, as well as longer leaves (Baten et al., 1995).

#### **Stem Diameter**

The wider stem diameter was recorded in plastic mulch (1.58 cm) and the narrow in sawdust mulch (1.18 cm). The stem diameter of the control treatment (1.15 cm) was statistically similar to sawdust mulch and the stem diameter of banana leaves mulch (1.36 cm) was found statistically at par with rice straw mulch (1.45 cm) (Table 4).

These results were consistent with the conclusions of Jamil et al., (2005). A study by Chen et al., (2024) also highlighted the effectiveness of plastic mulch on stem diameter, due to improved soil conditions and reduced weed competition. Mulch helps conserve soil moisture by reducing evaporation and maintaining more stable soil temperatures. Plastic mulch, in particular, is very effective in this regard and can help plants grow better and have larger stem diameter (Ahmad et al., 2022).

#### **Bulb Weight**

The highest bulb weight was found in plants treated with plastic mulch (44.61 gm) followed by rice straw (39.56 gm) and lowest in plants treated with control (28.41 gm).

Jamil et al., (2005) documented similar results for the bulb weight where they observed higher bulb weight in plastic mulch followed by rice straw. Seifu et al., (2017) studied only polyethylene mulch and therefore reported that this type of mulch significantly enhanced bulb weight of garlic. The fate of stem expansion and bulb formation is regulated by competition for resources between developing bulb and inflorescences (Atif et al., 2019).

#### **Bulb Length**

The maximum bulb length was observed in sawdust mulch (6.05 cm) and the minimum in the control treatment (5.16 cm). The bulb length of garlic grown with rice straw mulch (5.51 cm) was statistically at par with plants treated with banana leaves mulch (5.41 cm) and plastic mulch (5.73 cm).

The slow decomposition of sawdust mulch adds organic matter to the soil, improving soil structure and enhancing the availability of important nutrients like carbon and nitrogen (Haque et al., 2003). Because of their slower breakdown, garlic bulbs keep moisture for extended periods, maintain warmth, and grow longer (Iqbal et al., 2020).

#### **Bulb Diameter**

The longest bulb diameter was observed in plastic mulch (5.11 cm), whereas the shortest bulb diameter was observed in the control treatment (4.38 cm). The bulb diameter of garlic in rice straw mulch (4.99 cm) was statistically equivalent to that observed in the plastic mulch. In contrast, the bulb diameter of plants treated with banana leaves mulch (4.82 cm) was statistically at par with sawdust mulch (4.73 cm).

Plastic and rice straw mulches tend to give the best circumstances for bulb development, whereas banana leaves and sawdust offer minor advantages. Islamet al., (2007) recorded the highest bulb diameter for plastic mulch. As reported by Yimer, (2020) garlic grown on black plastic produced larger bulb diameters and marketable weights than garlic grown in bare soil.

#### **Root Length**

Similar figures for root length were observed in all mulch treatment, with the longest in plants grown with plastic mulch (5.11 cm) followed by rice straw (8.36). Root lengths were statistically similar in all treatments observed.

Garlic being a cold-season crop requires low temperatures for proper growth. High temperatures throughout the growing period can limit root elongation and density, and reduce water and nutrient intake (Larkin, 2020). Rice straw boosted soil water content and lowered soil penetration resistance, which enhanced root growth (Paul et al., 2021).

#### **Total Yield**

A statistically significant effect of mulches was observed on the yield of garlic (Table 4). The highest yield was observed in plastic-treated mulch (15.99 t/ha) followed by rice straw (13.1 t/ha) and the lowest in the control treatment (9.49 t/ha). The performance of garlic yields under different treatments is shown in Figure 1.

Jamil et al., (2005) reported plants grown for the whole season under any mulch produced the highest yield than those grown without mulch.

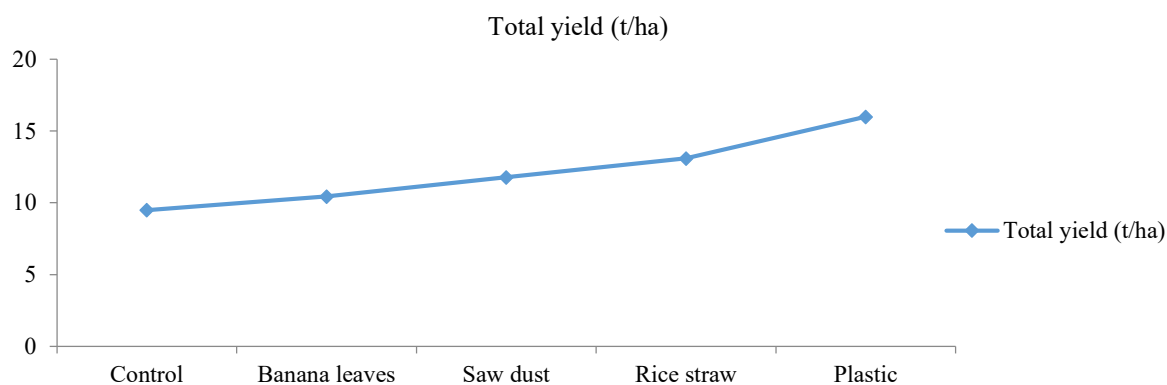


Figure 1. Performance of garlic yield under different treatments

Table 4. Effect of different mulching on stem diameter, bulb weight, bulb length, bulb diameter, root length, and total yield.

Treatments	Stem diameter (cm)	Bulb weight (gm)	Bulb length	Bulb diameter	Root length	Total yield (t/ha)
Control	1.15c	28.41d	5.16d	4.38b	8.14a	9.49d
Banana leaves	1.36b	37.46bc	5.41c	4.82ab	7.43a	10.44cd
Saw dust	1.18c	34.58c	6.05a	4.73ab	7.94a	11.78bc
Rice straw	1.45b	39.56b	5.51bc	4.99a	8.36a	13.1b
Plastic	1.58a	44.61a	5.73b	5.11a	8.48a	15.99a
LSD (p<0.05)	0.13***	3.9***	0.24***	0.46*	1.67	1.6***
CV%	4.95	5.74	2.33	5.07	11.68	7.01
Grand mean	1.34	36.93	5.57	4.81	8.07	12.16

LSD: Least significant difference, CV: Coefficient of Variation.

Each mulch maintained ideal soil moisture, regulated temperatures for plant growth, suppressed weed growth, and increased garlic yields compared to normal control conditions (Baten et al., 1995). According to Najafabadi et al., (2012), mulching increases bulb yield and improves quality indices such as ash percent, TSS, and vitamin C in garlic. Islam et al., (2007) conclude garlic produced in black polyethylene mulch was suitable for increasing garlic production.

## Conclusion

The findings show that the growth and yield of garlic are significantly impacted by the types of mulching materials used. Notably, plastic mulch consistently outperformed other treatments on most parameters; followed closely by rice straw. Plastic mulch offered better circumstances for overall garlic development, but rice straw showed improved plant height because it could produce a better growing environment. Additionally, bulb length was found to be promoted by sawdust mulch.

However, the long-term effects of using plastic mulch must be taken into consideration. Studies have shown that long-term use of plastic mulch can lead to a decline in soil quality, putting risk to sustainable agricultural practices. In contrast, rice straw emerges as a better option for the farmer as it is cost-effective, organic and easily available, making it an ideal choice for garlic production. Considering these results, straw mulch is recommended for garlic cultivation in different agro-climatic conditions, especially for farmers looking for a long-term solution to improve soil health and productivity. Otherwise, plastic mulch is the most effective for garlic production.

## Declarations

### Ethical Approval Certificate

Not Applicable

### Author Contribution Statement

A.K.B. and S.S.: Conceptualization, methodology, project administration, supervision, data collection, investigation, formal analysis, writing the original draft.

A.P. and S.S.: Methodology, investigation, data collection, formal analysis, review and editing

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### Conflict of Interest

The authors declare no conflict of interest.

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## Phenotypic and Molecular Characterization of *Streptomyces enissocaesilis* and *Streptomyces caviscabies* Induced Potato Common Scab in Egypt

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### ABSTRACT

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Potato common scab incited by pathogenic *Streptomyces* spp. is a significant soil-borne disease leading to serious economic losses in potato tubers worldwide. However, there is limited information available in Egypt regarding the pathogenicity, prevalence, and variety of *Streptomyces* spp. inciting common scab. Therefore, this study aims to clarify the aspects of identifying and characterizing *Streptomyces* spp. obtained from scabby tubers as well as to evaluate their pathogenicity. In the present investigation, nine isolates of *Streptomyces* spp. were obtained from various scab lesion symptoms. Of these, the Ag2 and Man strains exhibited pathogenic traits. The pathogenicity assays demonstrated that the strains induced necrotic lesions on tuber slices and abnormal growth of radish seedlings. In potato pot trail, The Ag2 isolates caused deep-pitted lesions with a disease index of 73.30%. Additionally, tubers inoculated with the Man isolate exhibited visible brown raised lesions, resulting in a disease index of 63.97%. Subsequently, the strains were characterized based on morphological, physiological, biochemical and phylogenetic levels. Phylogenetic tree derived from 16S rRNA gene sequences revealed that Ag2 and Man strains share 100% sequence similarity with *Streptomyces caviscabies* ATCC 51928 and *Streptomyces enissocaesilis* NRRL B-16365, respectively. The results of this study demonstrate that *S. caviscabies* and *S. enissocaesilis* are capable of causing CS disease in potatoes and may pose a potential threat to potato cultivation in Egypt.

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## Introduction

The potato (*Solanum tuberosum*) is the world's third-largest crop and a critical component of global food security. Potato tubers provide essential carbohydrates, protein, antioxidants, minerals, and vitamins. They also have various industrial processes and serve as animal feed (Dongyu, 2022). In Egypt, potatoes are a major export commodity, with production reaching approximately 6.9 million metric tons (FAO, 2020).

Among the numerous diseases affecting potato crops, potato common scab (CS) is a significant disease affecting most potato-growing regions worldwide (Braun et al., 2017). Symptoms of CS disease appear as scab-like surface, shallow-pitted, netted, raised, or russet lesions on the tuber (Gong et al., 2017; Li et al., 2019; Loria et al., 1997; Natsume et al., 2005). Recently, deep longitudinal cracks with scabby lesions (fissure scab) have also been produced (Cruywagen et al., 2021). These symptoms cause economic losses for farmers, decrease the quality of the potatoes, and impact potato marketability (Lankau et al., 2020; Loria et al., 1995). The CS disease of potatoes is

induced by gram-positive bacteria in the genus *Streptomyces* which comprises over 900 species (Parte, 2018). Although *Streptomyces* exhibits a wide range of morphological, ecological, and molecular genetic variations, only a specific closely related group is responsible for the widespread occurrence of this disease worldwide (Braun et al., 2017). To date, many studies have reported that potato scab is caused by more than 27 species of pathogenic *Streptomyces*, including *S. scabies*, *S. acidiscabies*, *S. europaeiscabiei*, *S. luridiscabiei*, *S. reticuliscabiei*, *S. stelliscabiei*, *S. turgidiscabies*, *S. puniscabiei*, and *S. niveiscabiei* (Aallam et al., 2021; Braun et al., 2017; Cruywagen et al., 2021; Vincent & Bignell, 2024). Other species that are also associated with CS include *S. caviscabies*, (found in Canada, China, Iran, and Brazil) and *S. enissocaesilis*, (found in China) (Corrêa et al., 2015; Gong et al., 2017; Goyer et al., 1996; Khodakaramian & Khodakaramian, 2013; Yang et al., 2018).

In Egypt, the prevalence of potato scab disease has recently become a significant concern for potato growers, and the incidence of CS across three governorates ranges from 22.5% to 60% (El-Sheikh, 2010; El-Sheikh et al., 2012). Several reports indicate that *S. scabies* is the most frequent pathogen associated with CS in potato cultivation (Abd El-Hafez & El Shishtawy, 2021; Eid et al., 2022; El-Sayed, 2000; Hosny et al., 2016; Hussein et al., 2019). Potato CS is also associated with *S. acidiscabies* (El-Sayed, 2000) and *S. alkaliscabies*, (Abdel-Rahman et al., 2012).

The current investigation aims to identify and characterize the *Streptomyces* species that have the ability to cause scab disease in potatoes under Egyptian conditions.

## Materials and Methods

### *Streptomyces* Isolation from Potato Tubers

Scabby potato tubers were collected from major potato-producing areas in Egypt from 2021 and 2022. Each scabby tuber was rinsed with water to get rid of any soil particles and dried with a paper towel. Then a small piece of infected potato tissue was excised with a sterile scalpel, ensuring it included tissue beneath the necrotic zone. The tissues were disinfected on the surface using 1.5% NaOCl for 1.5 minutes, followed by four rinses with sterile distilled water (SDW). Thereafter, the tissue was ground in 1 ml of SDW and incubated for 60 min. at room temperature. 100 µL of the ground solution was plated onto oatmeal agar (OMA, oatmeal 20g, agar 18g, sterile water to 1000 mL, pH 7.2). After 10 -15 days of incubation at 28°C, *Streptomyces*-like colonies were selected and purified on OMA medium. These colonies were subsequently maintained in 15% glycerol at -80°C.

### Pathogenicity Tests

Tuber slice assay, radish seedling bioassay, and potato pot trial were performed to assess the pathogenicity of *Streptomyces* isolates according (Bignell et al., 2010; Loria et al., 1995) with some modifications.

For tuber slices pathogenicity assay, healthy tubers were surface sterilized with NaOCl, 1.5% for 1 min. and washed several times with SDW. Subsequently, the cores of the tubers were aseptically extracted, sliced into disks with a thickness of 0.5cm and placed on moistened filter paper (Whatman No.1, 9cm) in petri dishes. The isolates of *Streptomyces* were cultured in OMA medium for 10 days at 28°C. Agar mycelial plugs from the actively growing colonies were then inoculated upside-down onto potato disks. An agar plug of OMA was placed onto potato disks as a control. There were five replicates for each isolate. All petri dishes were incubated for seven days at 28°C. Following the incubation period, potato disks were observed for necrosis and photographed.

The pathogenicity assessment of the isolates was further investigated using a radish seedling bioassay. Radish seeds were surface disinfected with NaOCl for 2 min, afterwards, the seeds were washed 5 times with SDW. After that, they were allowed to germinate for a day at room temperature (23–25°C) in a petri dish containing moist filter paper. Germinated seedlings were placed into tubes (100ml) with water agar (1.5% w/v) and inoculated

with 200 µl cultures of *Streptomyces* cell suspension from 7-days-old Oatmeal Broth (OMB). Control seedlings were treated with non-inoculated OMB. There were three replicates of one isolate (for a total of 15 seedlings per isolate). Seedlings were grown at 25°C for 10 days and examined for the presence of necrosis as well as abnormal growth in the root or shoot system.

In addition, the pathogenicity of the *Streptomyces* isolates was tested using a potato pot trial. To prepare spore suspension for soil inoculation, 50 ml of Yeast-malt extract (YME) broth (0.4% Yeast Extract; 1% Malt Extract; 0.4% Glucose; PH 7.2) was inoculated with 1 ml of spore suspension collected from 15-day-old plate of *Streptomyces* isolates grown in YME agar. Cultures were shaken at 200 rpm at 28°C. After incubating for 7 days, the cultures were centrifuged to obtain spores which were then resuspended in sterile H<sub>2</sub>O and adjusted to a concentration of 10<sup>7</sup> CFU/ml. Potato tubers, cv. Spunta were procured from the Potato Brown Rot Project, Agricultural Research Center, Egypt, and were maintained at room temperature for several days. The tubers with sprouting eyes were planted into pots (35 cm in diameter) filled with a sterile mixture of sand-clay-soil (1:1; v/v). Inoculations (200ml, 10<sup>7</sup> CFU/ml) were added to the soil of the pots at the time of sowing. Non-inoculated pots were used as a control. Each treatment was repeated in three pots. After 90 days of planting, the disease incidence and disease index of potato scab were computed on harvested tubers (Hao et al., 2009; Liang et al., 2019). The disease severity was grouped as the following scale; 0 = no symptoms, 1 = 1 to 10% surface area with superficial or raised lesions, 2 = 11 to 25% surface area with superficial or raised lesions, 3 = 26 to 50% surface area with superficial or raised lesions, 4 = more than 50% surface area with superficial or raised lesions or 6 to 25% pitted lesion area, and 5 = >50% surface area with superficial or raised lesions or >25% pitted area. The disease index (percentage) was calculated as follows;  $[\sum (\text{number of diseased potatoes at each scale} \times \text{representative value at each scale})] / (\text{number of total potatoes investigated} \times \text{highest representative scale}) \times 100$ . The percentage of tubers in each treatment exhibiting CS symptoms was used to express the disease incidence. Data was subjected to a one-way analysis of variance using SPSS software, version 24 (SPSS Inc. USA).

### Morphological, Physiological, and Biochemical Traits of Pathogens

Morphological, physiological, and biochemical characterization were performed on the *Streptomyces* isolates identified as pathogens according to the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). For the morphological observations, the isolates were cultured on YME agar and incubated at 28°C for two weeks. The substrate mycelia, aerial mycelia, spore chains, and soluble pigments from the medium were examined. Melanin production assay was detected on peptone yeast iron (PYI) and tyrosine agar (TYR) plates. Cultures used for light microscope and transmission electron microscope (TEM) examination were obtained after incubation of tested isolates at 28°C for 15 days in OMA media. For TEM analysis, the carbon coated copper grids were gently pressed to the aerial surface of a culture with mature spores. Spore chains that adhere to the coated

surface of the grids were observed using a JEOL GEM-1010 transmission electron microscope at 80 kV at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt.

Physiological and biochemical characteristics were assessed following the methods described in (Williams et al., 1983). Briefly, the utilization of the sole carbon (1% w/v) and nitrogen sources (1g/l) was conducted, and growth results were recorded after 7 days of incubation (Goyer et al., 1996). The growth of strains was assessed at pH values (4-10), and NaCl concentrations (0-10) as described by Pridham et al. (1957). The potential effectiveness of penicillin G (10 IU/ml), oleandomycin (100 µg/ml), and streptomycin sulfate (20 µml) on bacterial cultures was evaluated (Lambert & Loria, 1989). The strains were tested for their capacity to hydrolyze xylan, Polygalacturonic acid, and arbutin (Williams et al., 1983). Additionally, The inhibitory effects of crystal violet (0.5 µg/ml), and phenol (0.1%) were assessed (Lambert & Loria, 1989).

### Molecular Identification

Isolates were cultured for two weeks on YME agar medium at 28°C (Shirling & Gottlieb, 1966). Then, mycelium and spores were collected from the plate and ground in liquid nitrogen. Genomic DNA was extracted using a commercial kit (Thermo Fisher Scientific, U.S.A) following the preparation instructions given by the manufacturers. The extracted DNA was quantified using gel electrophoresis and UV spectrophotometry (A260/A280) (Thermo/Scientific NanoDrop 200 Spectrometer). The 16S rRNA gene sequence of the isolates was obtained from fragments generated by PCR with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTGTTCAGACTT-3') (Lane, 1991). The PCR reaction mixture contained 2 µl of template DNA (25 ng/µL), 1µl of each of forward and reverse primer (10µM), 12.5 µl of 1x Master Mix (Takara Bio Inc.), and 8.5 µL of PCR grade water. The cycling conditions were as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The amplified DNA was electrophoresed on 1.5 % agarose gel stained with ethidium bromide. Sanger sequencing of the resultant products was carried out at Macrogen (Seoul, South Korea). The resulting sequences

were examined for sequence homology using BLASTn at the NCBI nucleotide sequence database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The 16S rRNA gene sequences from the highest homology with isolates Ag2 and Man along with sequences of *S. caviscabies* and *S. enissocaesilis* strains induced potato CS, were included in subsequent phylogenetic analyses. All sequences were aligned using the Clustal W algorithm in MEGA X software (Kumar et al., 2018). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987). The topology stability of the resultant tree was evaluated using the bootstrap method with 1000 repetitions (Felsenstein, 1985).

### Result

Samples of scabby tubers were collected from various regions of Egypt between 2021 and 2022. The disease symptoms of potatoes exhibited netted, superficial, raised, or pitted lesions (Table 1). Nine isolates of *Streptomyces* spp. were recovered from diverse symptoms of CS lesions.

To validate the pathogenicity of these isolates, tuber slice and radish seedling assays were performed. Among nine isolated *Streptomyces* spp. Ag2 and Man isolates showed pathogenicity traits (Table 1, and Figure 1). Ag2 and Man isolates produced brown, necrotize tissues associated with hyphal growth on potato slices (Figure 1g, h) and caused dwarfing abnormal growth of the radish seedlings (Figure 1d, e).

Positive isolates from the potato slices and radish seedlings were tested for pathogenicity using a potato pot trail. Additionally, the types of scab lesions and disease severity on harvested tubers were scored. Both Ag2 and Man isolates induced CS symptoms on potato tubers. The Ag2 isolate produced deep- pitted lesions with an incidence 98.33% and a disease index 73.30% (Figure 1j, and Table 2). Furthermore, tubers that were inoculated with the Man isolate showed apparent brown-raise lesions with an incidence 95.00% and a disease index 63.97% (Figure 1k, and Table 1). The scab symptoms did not observed on the control tubers (Figure 1, i). Bacteria that inoculated onto potato pot trail (tubers) were reisolated from scabbed lesions to confirm Koch's postulates by using morphological characteristics identity. So, Ag2 and Man isolates become known as potato scab pathogens and will be investigated further.

Table 1. Preliminary pathogenicity screening of *Streptomyces* spp. isolated from different potato growing areas and scab symptoms.

Isolate	Geographic origin	Lesion appearance	Pathogenicity	
			Potato	Radish
Beh-1	El-Beheira	Netted	-	-
Beh-9	El-Beheira	Raised	-	-
Nub-1	Nubaria	Netted	-	-
Nub-2	Nubaria	superficial	-	-
Nub-3	Nubaria	Netted	-	-
Qal-1	Qalyubia	Netted	-	-
IS-1	Ismailia	Netted	-	-
Ag2	Cairo	Pitted	+	+
Man	Mansoura	Raised	+	+

Note: (+) = indicates that the isolate induces necrosis on potato tuber slices and abnormal growth of radish seedlings. (-) indicates that the isolate did not induce necrosis on potato tuber slices and normal growth of radish seedlings.



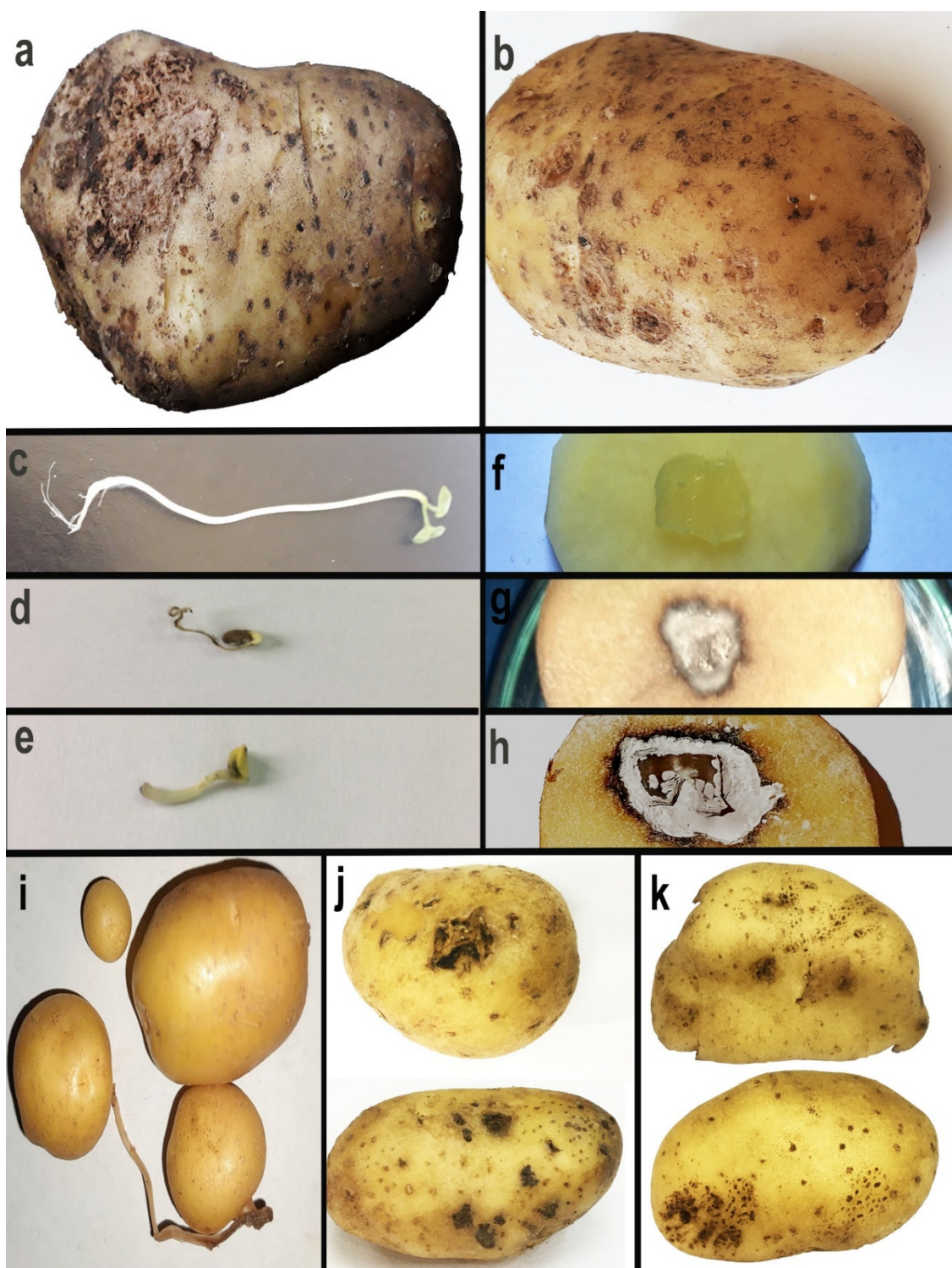


Figure 1. The field symptoms (Naturally infected potato showing symptoms of scab) of potato scab and Pathogenicity verification of *Streptomyces* isolates Ag2 and Man. Pitted and raised natural symptoms that are source of *Streptomyces* sp. Ag2 (a) and Man (b). Symptoms developed on radish seedlings treated with oatmeal culture of *Streptomyces* sp. Ag2 (d) and Man (e). Necrosis on potato tuber slices produced by agar plug from sporulating colonies of *Streptomyces* sp. Ag-2 (g) and Mans (h). Deep-pitted and raised lesions on potato cv. Spunta induced by *Streptomyces* sp. Ag2 (j) and Man (k). Blank control without pathogen inoculation (c,f, and i).

Table 2. Pathogenic properties of potato scab-causing *Streptomyces* isolates.

Isolate	Disease incidence	Disease index
Ag2	98.33 <sup>a*</sup>	73.30 <sup>a</sup>
Man	95.00 <sup>a</sup>	63.97 <sup>a</sup>
control	0.0 <sup>b</sup>	0.00 <sup>b</sup>
LSD <sub>0.05</sub>	6.66	13.18

\* Means with the same letters are not significantly different at 0.05 probability level.

Table 3. Comparison of Phenotypic, physiological and biochemical characteristics of strains Ag2 and Man with references strains.

Characteristics	Ag2	<i>S. caviscabies</i> *	Man	<i>S. enissocaesilis</i> **
Colony color on YME medium <sup>@</sup>	Gold	Gold to light brown	gray	N
Spore color	White	White	gray	gray
Spore ornamentation	Smooth	Smooth	Smooth	Smooth
Spore chain morphology	Flexuous	Flexuous	Spiral	Spiral
Melanin production on PYI <sup>#</sup>	-	-	-	-
Production of diffusible pigment	-	v	+	N
Utilization of carbon sources				
L-Arabinose	-	-	+	+
D-Fructose	-	-	+	+
D-Mannitol	-	-	+	+
Raffinose	+	+	+	+
Rhamnose	-	-	-	-
Sucrose	-	-	-	-
D-Xylose	-	-	+	+
D-Glucose	+	N	+	+
Meso-insitol	+	N	+	+
Utilization of nitrogen sources				
L - P r o l i n e	+	+	+	+
L-Methionine	+	+	-	+
L - Tyrosine	+	N	+	N
L - Histidine	+	N	+	+
Asparagine	+	N	+	N
L - Alanine	+	N		N
Hydrolysis of				
Arbutin	-	-	+	N
Polygalacturonic acid	-	-	-	N
Xylan	+	+	-	N
Minimum growth at pH 4.5	-	-	+	+
Growth in the presence of				
NaCl (4%)	+	+	+	+
NaCl (7%)	-	v	-	-
NaCl (10%)	-	-	-	-
Crystal violet (0.5 µg/ml)	+	v	+	+
Phenol (0.1%)	+	+	+	+
Penicillin (10 IU/ml)	+	+	+	+
Oleandomycin (100 µg/ml)	+	v	+	N
Streptomycin (20 µg/ml)	+	+	-	-

The phenotypic, physiological and biochemical characteristics data of *S. caviscabies* and *S. enissocaesilis* from references Goyer et al., 1996; Faucher et al 1995; + : Positive reaction ; - : Negative reaction ; V: variable; N : Not available; @: YME medium, yeast malt extract medium; #: PYI, Peptone-yeast extract-iron medium.

The morphological, biochemical, and physiological properties of the pathogenic strains are given in Table 3 and Figure. 2. Strain Ag2 exhibited a golden-colored mycelium on YME medium (Figure 2a) and lacked melanin pigment production on PYI. No soluble pigments were detected in the media used. Additionally, it produced white, cylindrical, smooth spores in flexuous chains (Figure 2c). The Ag-2 utilized raffinose, D-glucose, and meso-insitol as carbon sources as well as grew on all tested nitrogen sources. Notably, it could degrade xylan but not hydrolyze polygalacturonic acid or arbutin. Furthermore, this strain cannot grow at a minimum pH of 4.5 but can grow with 4% NaCl or less. It was tolerant to penicillin (10 IU/ml), oleandomycin (100 µg/ml), and streptomycin (20 µg/ml). Also, this strain exhibited growth in the presence of crystal violet (0.5 µg/ml) and phenol (0.1%).

In contrast, strain Man developed gray aerial mycelium, which developed smooth and gray spores arranged in spiral chains (Figure. 2b, d). It produced a brown diffusible pigment but no melanin pigment was

detected. The strain used almost all sugars as a carbon source except rhamnose and sucrose. Also, proline, tyrosine, histidine, asparagine, and alanine were utilized as nitrogen sources, but no growth was observed on methionine. The strain had a minimum growth pH of 4.5 and growth in the presence of NaCl 4%. This strain was resistant to oleandomycin (100 µg/ml), penicillin (10 IU/ml), crystal violet (0.5 µg/ml), phenol (0.1%), and arbutin, but its growth was inhibited by polygalacturonic acid, xylan, and streptomycin (20 µg/ml).

A comparison of the phenotypic, biochemical, and physiological traits of pathogenic strains Ag2 and Man with reference *Streptomyces* strains is given in Table 3 and Figure. 2. Therefore, Ag2 and Man strain are nearly identical to *S. caviscabies* and *S. enissocaesilis* respectively.

PCR and sequencing of the 16S rDNA gene were performed to confirm the identity of strains Ag2 and Man (Figure 3).

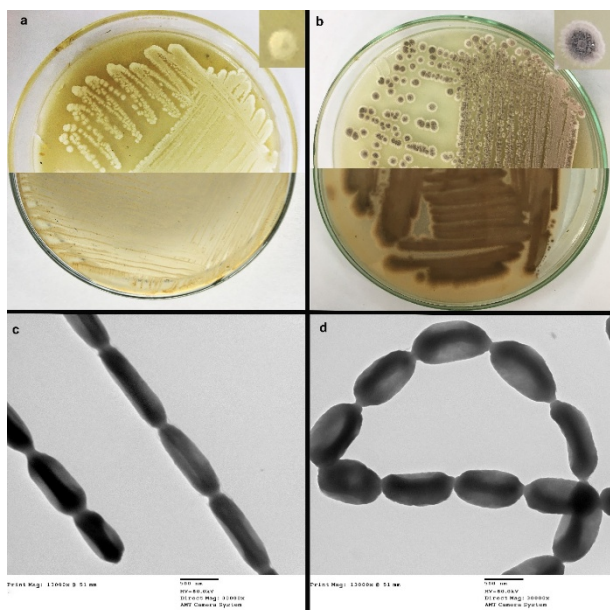


Figure 2. Morphological observation of potato scab-inducing *Streptomyces* isolates Ag2 and Man. spore color and substrate mycelium of Ag2 (a) and Man (b) culture on yeast malt extract medium incubated at 28°C for 14 days. Spores chain morphology and spores ornamentation under transmission electron microscope of Ag2 (c) and Man (d) grown on oatmeal agar at 28°C for 14 days.

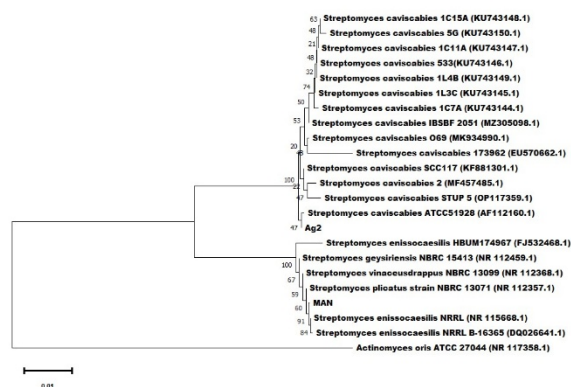


Figure 3. Phylogenetic tree derived from 16S rDNA gene sequences, showing the relationships of strains Ag2 and Man with related *Streptomyces* spp. The tree was rooted with *Actinomyces oris* and constructed using the neighbor-joining method with bootstrap values calculated from 1,000 repetitions. The bar indicates 1% estimated sequence divergence

The sequencing data of the Ag2 and Man strains have been deposited in the GenBank (NCBI) under accession numbers OR447472 and OR447473, respectively. A nucleotide BLAST search revealed that the Ag2 and Man strains share 100% similarity with *S. caviscabies* ATCC 51928 and *S. enissocaesilis* NRRL B-16365, respectively. The 16S rDNA phylogenetic tree (Figure 3) demonstrated that the pathogenic strains (Ag2 and Man) were separated into two distinct groups supported by high bootstrap values (100%). All *S. caviscabies* strains and the Ag2 strain formed a cluster in the first group, with Ag2 closely related to the *S. caviscabies* (ATCC 51928) type strain. The second group comprised *Streptomyces* species with nearly identical 16S DNA sequences, and the Man strain clustered

closely with type strain of *S. enissocaesilis* NRRL B-16365 with a bootstrap value of 91%.

## Discussion

Potato CS is a widespread disease incited by different *Streptomyces* species that significantly decreases the marketable yield for potato growers (Braun et al., 2017). While *S. scabies* is widely recognized as the main cause of scab disease, there are actually over 27 scab-causing *Streptomyces* species that have been reported worldwide (Vincent & Bignell, 2024).

Despite the frequent occurrence of scab disease in Egypt, which poses a significant threat to potato crops and has been reported as early as 1966 (EL.Kashier, 1966; Mehiair & El-Samra, 1978), there is a noticeable absence of comprehensive studies that specifically aim to characterize the pathogenic *Streptomyces* species in the country.

In this investigation, nine isolates of *Streptomyces* spp. were recovered from potato tubers naturally infected and collected from various locations in Egypt. Out of the nine isolates, only the Ag2 and Man strains showed positive results in both the tuber slice and radish seedling tests. In the potato pot trail, these strains induced symptoms of CS on potato tubers that were identical to those observed on the original infected potatoes. A significant number of *Streptomyces* spp. isolated from scabby potatoes have been previously reported to be non-pathogenic (Gouws, 2013; Henao et al., 2022; Jordaan & Van der Waals, 2016; Wanner, 2007). Also, it has been found that non-pathogenic *Streptomyces* can colonize tubers that display symptoms of CS. This colonization may potentially contribute to the development of the disease (Chalupowicz et al., 2022). The study of Cui et al. (2021) reported that isolate 5A-1 exhibited pathogenicity among 20 isolated *Streptomyces* spp.

The pathogenic isolates in our study were identified as *S. caviscabies* and *S. enissocaesilis*. This identification was based on an evaluation of their morphological and biochemical characteristics, along with the analysis of their 16S rDNA gene sequences. Both pathogens are identified for the first time as causative agents of potato common scab in Egypt.

*Streptomyces caviscabies* was initially described as a pathogen responsible for the development of deep-pitted potato scab in Canada (Faucher et al., 1995; Goyer et al., 1996). Additionally, Corrêa et al. (2015) detected this pathogen in several potato fields in Brazil where it was associated with potato scab. In Iran, a group of *S. caviscabies* strains was isolated from scabby potatoes and these strains caused significant disease severity under both natural and greenhouse conditions (Khodakaramian & Khodakaramian, 2013). Recently, five isolates from typical CS lesions in China were identified as *S. caviscabies*. These isolates exhibited typical symptoms of CS on potatoes under greenhouse conditions (Gong et al., 2017).

The species *S. enissocaesilis* was first reported as a novel potato scab pathogen in China (Zhang et al., 2010; Zhang et al., 2009). Yang et al. (2018) investigated the species composition of pathogenic *Streptomyces* responsible for causing potato scab in Yunnan Province, China. Out of the 67 pathogenic *Streptomyces* spp., 29 were identified as *S. enissocaesilis* and caused tuber

disease with CS symptoms in greenhouse pots. Recently, *S. enissocaesilis* was used as a pathogen of potato CS to screen for highly efficient bactericidal agents (Pu et al., 2022). On the other hand, *S. enissocaesilis* has previously been documented as an effective biological control agent against a wide range of diseases and pests (Aallam et al., 2021; Abbasi et al., 2019; Boukelloul et al., 2024; Ganesan et al., 2018). In the rhizosphere, *S. galilaeus* and *S. griseoplanus* have a dual role. In addition to being a pathogen that can cause potato CS, they can also act as a potential bioagent for controlling plant diseases (Cui et al., 2021; Cui et al., 2018; Nimnoi et al., 2017). Biocontrol agent assays, particularly those involving *Streptomyces* spp., require careful attention. It is crucial to conduct radish seedling and potato slice assays to verify the pathogenicity of the tested agents used for controlling plant diseases. The emergence of new pathogen causing CS may be attributed to the horizontal gene transfer of pathogenicity genes among *Streptomyces* spp. (Armijos-Jaramillo et al., 2017; Bukhalid et al., 2002).

In Egypt, the precise date of the initial recording of potato CS disease is uncertain. However Egyptian quarantine acts and regulations indicate that it was recognized as early as 1932 (EL.Kashier, 1966). Many studies have indicated that *S. scabiei* is the main causal agent of potato CS disease in Egypt (Abd El-Hafez & El Shishtawy, 2021; Eid et al., 2022; Gabr, 1988; Galal et al., 1999; Mehari & El-Samra, 1978). Additionally, a few other causal agents have been identified, including *S. acidiscabies* (El-Sayed, 2000), and *S. alkaliscabies* (Abdel-Rahman et al., 2012). The low diversity of scab-causing strains isolated from Egypt may be related to previous studies that performed only phenotypic (morphology and biochemical) identification tests, except for *S. alkaliscabies*. Therefore, phenotypic and molecular characteristics should be performed for an accurate and clear diagnosis of pathogenic strains.

## Conclusion

The current study reports, for the first time, the presence of scab-causing pathogens *S. caviscabies* and *S. enissocaesilis* in Egypt. These pathogens were identified based on their phenotypic and molecular traits. Their pathogenicity was validated through potato slices and radish seedling assays, as well as a greenhouse evaluation. Further research is necessary to define the range of pathogens and develop effective control strategies.

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## Phenotypic and Molecular Characterization of *Streptomyces enissocaesilis* and *Streptomyces caviscabies* Induced Potato Common Scab in Egypt

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### ABSTRACT

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Potato common scab incited by pathogenic *Streptomyces* spp. is a significant soil-borne disease leading to serious economic losses in potato tubers worldwide. However, there is limited information available in Egypt regarding the pathogenicity, prevalence, and variety of *Streptomyces* spp. inciting common scab. Therefore, this study aims to clarify the aspects of identifying and characterizing *Streptomyces* spp. obtained from scabby tubers as well as to evaluate their pathogenicity. In the present investigation, nine isolates of *Streptomyces* spp. were obtained from various scab lesion symptoms. Of these, the Ag2 and Man strains exhibited pathogenic traits. The pathogenicity assays demonstrated that the strains induced necrotic lesions on tuber slices and abnormal growth of radish seedlings. In potato pot trail, The Ag2 isolates caused deep-pitted lesions with a disease index of 73.30%. Additionally, tubers inoculated with the Man isolate exhibited visible brown raised lesions, resulting in a disease index of 63.97%. Subsequently, the strains were characterized based on morphological, physiological, biochemical and phylogenetic levels. Phylogenetic tree derived from 16S rRNA gene sequences revealed that Ag2 and Man strains share 100% sequence similarity with *Streptomyces caviscabies* ATCC 51928 and *Streptomyces enissocaesilis* NRRL B-16365, respectively. The results of this study demonstrate that *S. caviscabies* and *S. enissocaesilis* are capable of causing CS disease in potatoes and may pose a potential threat to potato cultivation in Egypt.

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## Introduction

The potato (*Solanum tuberosum*) is the world's third-largest crop and a critical component of global food security. Potato tubers provide essential carbohydrates, protein, antioxidants, minerals, and vitamins. they also have various industrial processes and serve as animal feed (Dongyu, 2022). In Egypt, potatoes are a major export commodity, with production reaching approximately 6.9 million metric tons (FAO, 2020).

Among the numerous diseases affecting potato crops, potato common scab (CS) is a significant disease affecting most potato-growing regions worldwide (Braun et al., 2017). Symptoms of CS disease appear as scab-like surface, shallow-pitted, netted, raised, or russet lesions on the tuber (Gong et al., 2017; Li et al., 2019; Loria et al., 1997; Natsume et al., 2005). Recently, deep longitudinal cracks with scabby lesions (fissure scab) have also been produced (Cruywagen et al., 2021). These symptoms cause economic losses for farmers, decrease the quality of the potatoes, and impact potato marketability (Lankau et al., 2020; Loria et al., 1995). The CS disease of potatoes is

induced by gram-positive bacteria in the genus *Streptomyces* which comprises over 900 species (Parte, 2018). Although *Streptomyces* exhibits a wide range of morphological, ecological, and molecular genetic variations, only a specific closely related group is responsible for the widespread occurrence of this disease worldwide (Braun et al., 2017). To date, many studies have reported that potato scab is caused by more than 27 species of pathogenic *Streptomyces*, including *S. scabies*, *S. acidiscabies*, *S. europaeiscabiei*, *S. luridiscabiei*, *S. reticuliscabiei*, *S. stelliscabiei*, *S. turgidiscabies*, *S. puniscabiei*, and *S. niveiscabiei* (Aallam et al., 2021; Braun et al., 2017; Cruywagen et al., 2021; Vincent & Bignell, 2024). Other species that are also associated with CS include *S. caviscabies*, (found in Canada, China, Iran, and Brazil) and *S. enissocaesilis*, (found in China) (Corrêa et al., 2015; Gong et al., 2017; Goyer et al., 1996; Khodakaramian & Khodakaramian, 2013; Yang et al., 2018).

In Egypt, the prevalence of potato scab disease has recently become a significant concern for potato growers, and the incidence of CS across three governorates ranges from 22.5% to 60% (El-Sheikh, 2010; El-Sheikh et al., 2012). Several reports indicate that *S. scabies* is the most frequent pathogen associated with CS in potato cultivation (Abd El-Hafez & El Shishtawy, 2021; Eid et al., 2022; El-Sayed, 2000; Hosny et al., 2016; Hussein et al., 2019). Potato CS is also associated with *S. acidiscabies* (El-Sayed, 2000) and *S. alkaliscabies*, (Abdel-Rahman et al., 2012).

The current investigation aims to identify and characterize the *Streptomyces* species that have the ability to cause scab disease in potatoes under Egyptian conditions.

## Materials and Methods

### *Streptomyces* Isolation from Potato Tubers

Scabby potato tubers were collected from major potato-producing areas in Egypt from 2021 and 2022. Each scabby tuber was rinsed with water to get rid of any soil particles and dried with a paper towel. Then a small piece of infected potato tissue was excised with a sterile scalpel, ensuring it included tissue beneath the necrotic zone. The tissues were disinfected on the surface using 1.5% NaOCl for 1.5 minutes, followed by four rinses with sterile distilled water (SDW). Thereafter, the tissue was ground in 1 ml of SDW and incubated for 60 min. at room temperature. 100 µL of the ground solution was plated onto oatmeal agar (OMA, oatmeal 20g, agar 18g, sterile water to 1000 mL, pH 7.2). After 10 -15 days of incubation at 28°C, *Streptomyces*-like colonies were selected and purified on OMA medium. These colonies were subsequently maintained in 15% glycerol at -80°C.

### Pathogenicity Tests

Tuber slice assay, radish seedling bioassay, and potato pot trial were performed to assess the pathogenicity of *Streptomyces* isolates according (Bignell et al., 2010; Loria et al., 1995) with some modifications.

For tuber slices pathogenicity assay, healthy tubers were surface sterilized with NaOCl, 1.5% for 1 min. and washed several times with SDW. Subsequently, the cores of the tubers were aseptically extracted, sliced into disks with a thickness of 0.5cm and placed on moistened filter paper (Whatman No.1, 9cm) in petri dishes. The isolates of *Streptomyces* were cultured in OMA medium for 10 days at 28°C. Agar mycelial plugs from the actively growing colonies were then inoculated upside-down onto potato disks. An agar plug of OMA was placed onto potato disks as a control. There were five replicates for each isolate. All petri dishes were incubated for seven days at 28°C. Following the incubation period, potato disks were observed for necrosis and photographed.

The pathogenicity assessment of the isolates was further investigated using a radish seedling bioassay. Radish seeds were surface disinfected with NaOCl for 2 min, afterwards, the seeds were washed 5 times with SDW. After that, they were allowed to germinate for a day at room temperature (23–25°C) in a petri dish containing moist filter paper. Germinated seedlings were placed into tubes (100ml) with water agar (1.5% w/v) and inoculated

with 200 µl cultures of *Streptomyces* cell suspension from 7-days-old Oatmeal Broth (OMB). Control seedlings were treated with non-inoculated OMB. There were three replicates of one isolate (for a total of 15 seedlings per isolate). Seedlings were grown at 25°C for 10 days and examined for the presence of necrosis as well as abnormal growth in the root or shoot system.

In addition, the pathogenicity of the *Streptomyces* isolates was tested using a potato pot trial. To prepare spore suspension for soil inoculation, 50 ml of Yeast-malt extract (YME) broth (0.4% Yeast Extract; 1% Malt Extract; 0.4% Glucose; PH 7.2) was inoculated with 1 ml of spore suspension collected from 15-day-old plate of *Streptomyces* isolates grown in YME agar. Cultures were shaken at 200 rpm at 28°C. After incubating for 7 days, the cultures were centrifuged to obtain spores which were then resuspended in sterile H<sub>2</sub>O and adjusted to a concentration of 10<sup>7</sup> CFU/ml. Potato tubers, cv. Spunta were procured from the Potato Brown Rot Project, Agricultural Research Center, Egypt, and were maintained at room temperature for several days. The tubers with sprouting eyes were planted into pots (35 cm in diameter) filled with a sterile mixture of sand-clay-soil (1:1; v/v). Inoculations (200ml, 10<sup>7</sup> CFU/ml) were added to the soil of the pots at the time of sowing. Non-inoculated pots were used as a control. Each treatment was repeated in three pots. After 90 days of planting, the disease incidence and disease index of potato scab were computed on harvested tubers (Hao et al., 2009; Liang et al., 2019). The disease severity was grouped as the following scale; 0 = no symptoms, 1 = 1 to 10% surface area with superficial or raised lesions, 2 = 11 to 25% surface area with superficial or raised lesions, 3 = 26 to 50% surface area with superficial or raised lesions, 4 = more than 50% surface area with superficial or raised lesions or 6 to 25% pitted lesion area, and 5 = >50% surface area with superficial or raised lesions or >25% pitted area. The disease index (percentage) was calculated as follows;  $[\sum (\text{number of diseased potatoes at each scale} \times \text{representative value at each scale})] / (\text{number of total potatoes investigated} \times \text{highest representative scale}) \times 100$ . The percentage of tubers in each treatment exhibiting CS symptoms was used to express the disease incidence. Data was subjected to a one-way analysis of variance using SPSS software, version 24 (SPSS Inc. USA).

### Morphological, Physiological, and Biochemical Traits of Pathogens

Morphological, physiological, and biochemical characterization were performed on the *Streptomyces* isolates identified as pathogens according to the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966). For the morphological observations, the isolates were cultured on YME agar and incubated at 28°C for two weeks. The substrate mycelia, aerial mycelia, spore chains, and soluble pigments from the medium were examined. Melanin production assay was detected on peptone yeast iron (PYI) and tyrosine agar (TYR) plates. Cultures used for light microscope and transmission electron microscope (TEM) examination were obtained after incubation of tested isolates at 28°C for 15 days in OMA media. For TEM analysis, the carbon coated copper grids were gently pressed to the aerial surface of a culture with mature spores. Spore chains that adhere to the coated



surface of the grids were observed using a JEOL GEM-1010 transmission electron microscope at 80 kV at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt.

Physiological and biochemical characteristics were assessed following the methods described in (Williams et al., 1983). Briefly, the utilization of the sole carbon (1% w/v) and nitrogen sources (1g/l) was conducted, and growth results were recorded after 7 days of incubation (Goyer et al., 1996). The growth of strains was assessed at pH values (4-10), and NaCl concentrations (0-10) as described by Pridham et al. (1957). The potential effectiveness of penicillin G (10 IU/ml), oleandomycin (100 µg/ml), and streptomycin sulfate (20 µml) on bacterial cultures was evaluated (Lambert & Loria, 1989). The strains were tested for their capacity to hydrolyze xylan, Polygalacturonic acid, and arbutin (Williams et al., 1983). Additionally, The inhibitory effects of crystal violet (0.5 µg/ml), and phenol (0.1%) were assessed (Lambert & Loria, 1989).

### Molecular Identification

Isolates were cultured for two weeks on YME agar medium at 28°C (Shirling & Gottlieb, 1966). Then, mycelium and spores were collected from the plate and ground in liquid nitrogen. Genomic DNA was extracted using a commercial kit (Thermo Fisher Scientific, U.S.A) following the preparation instructions given by the manufacturers. The extracted DNA was quantified using gel electrophoresis and UV spectrophotometry (A260/A280) (Thermo/Scientific NanoDrop 200 Spectrometer). The 16S rRNA gene sequence of the isolates was obtained from fragments generated by PCR with the universal primers 27F (5'-AGAGTTTGGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991). The PCR reaction mixture contained 2 µl of template DNA (25 ng/µL), 1µl of each of forward and reverse primer (10µM), 12.5 µl of 1x Master Mix (Takara Bio Inc.), and 8.5 µL of PCR grade water. The cycling conditions were as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The amplified DNA was electrophoresed on 1.5 % agarose gel stained with ethidium bromide. Sanger sequencing of the resultant products was carried out at Macrogen (Seoul, South Korea). The resulting sequences

were examined for sequence homology using BLASTn at the NCBI nucleotide sequence database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The 16S rRNA gene sequences from the highest homology with isolates Ag2 and Man along with sequences of *S. caviscabies* and *S. enissocaesilis* strains induced potato CS, were included in subsequent phylogenetic analyses. All sequences were aligned using the Clustal W algorithm in MEGA X software (Kumar et al., 2018). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987). The topology stability of the resultant tree was evaluated using the bootstrap method with 1000 repetitions (Felsenstein, 1985).

### Result

Samples of scabby tubers were collected from various regions of Egypt between 2021 and 2022. The disease symptoms of potatoes exhibited netted, superficial, raised, or pitted lesions (Table 1). Nine isolates of *Streptomyces* spp. were recovered from diverse symptoms of CS lesions.

To validate the pathogenicity of these isolates, tuber slice and radish seedling assays were performed. Among nine isolated *Streptomyces* spp. Ag2 and Man isolates showed pathogenicity traits (Table 1, and Figure 1). Ag2 and Man isolates produced brown, necrotize tissues associated with hyphal growth on potato slices (Figure 1g, h) and caused dwarfing abnormal growth of the radish seedlings (Figure 1d, e).

Positive isolates from the potato slices and radish seedlings were tested for pathogenicity using a potato pot trail. Additionally, the types of scab lesions and disease severity on harvested tubers were scored. Both Ag2 and Man isolates induced CS symptoms on potato tubers. The Ag2 isolate produced deep- pitted lesions with an incidence 98.33% and a disease index 73.30% (Figure 1j, and Table 2). Furthermore, tubers that were inoculated with the Man isolate showed apparent brown-raise lesions with an incidence 95.00% and a disease index 63.97% (Figure 1k, and Table 1). The scab symptoms did not observed on the control tubers (Figure 1, i). Bacteria that inoculated onto potato pot trail (tubers) were reisolated from scabbed lesions to confirm Koch's postulates by using morphological characteristics identity. So, Ag2 and Man isolates become known as potato scab pathogens and will be investigated further.

Table 1. Preliminary pathogenicity screening of *Streptomyces* spp. isolated from different potato growing areas and scab symptoms.

Isolate	Geographic origin	Lesion appearance	Pathogenicity	
			Potato	Radish
Beh-1	El-Beheira	Netted	-	-
Beh-9	El-Beheira	Raised	-	-
Nub-1	Nubaria	Netted	-	-
Nub-2	Nubaria	superficial	-	-
Nub-3	Nubaria	Netted	-	-
Qal-1	Qalyubia	Netted	-	-
IS-1	Ismailia	Netted	-	-
Ag2	Cairo	Pitted	+	+
Man	Mansoura	Raised	+	+

Note: (+) = indicates that the isolate induces necrosis on potato tuber slices and abnormal growth of radish seedlings. (-) indicates that the isolate did not induce necrosis on potato tuber slices and normal growth of radish seedlings.

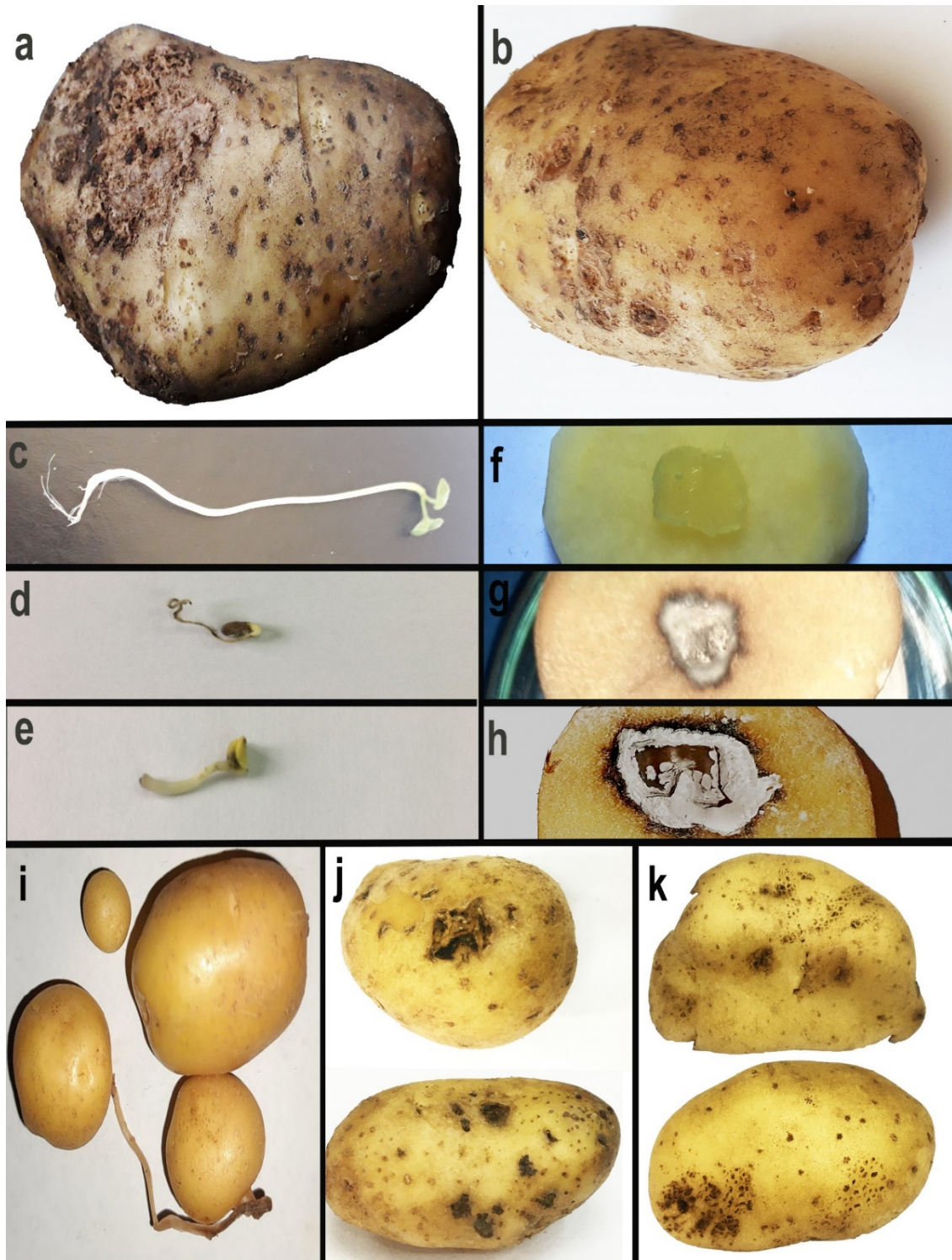


Figure 1. The field symptoms (Naturally infected potato showing symptoms of scab) of potato scab and Pathogenicity verification of *Streptomyces* isolates Ag2 and Man. Pitted and raised natural symptoms that are source of *Streptomyces* sp. Ag2 (a) and Man (b). Symptoms developed on radish seedlings treated with oatmeal culture of *Streptomyces* sp. Ag2 (d) and Man (e). Necrosis on potato tuber slices produced by agar plug from sporulating colonies of *Streptomyces* sp. Ag-2 (g) and Mans (h). Deep-pitted and raised lesions on potato cv. Spunta induced by *Streptomyces* sp. Ag2 (j) and Man (k). Blank control without pathogen inoculation (c,f, and i).

Table 2. Pathogenic properties of potato scab-causing *Streptomyces* isolates.

Isolate	Disease incidence	Disease index
Ag2	98.33 <sup>a*</sup>	73.30 <sup>a</sup>
Man	95.00 <sup>a</sup>	63.97 <sup>a</sup>
control	0.0 <sup>b</sup>	0.00 <sup>b</sup>
LSD <sub>0.05</sub>	6.66	13.18

\* Means with the same letters are not significantly different at 0.05 probability level.

Table 3. Comparison of Phenotypic, physiological and biochemical characteristics of strains Ag2 and Man with references strains.

Characteristics	Ag2	<i>S. caviscabies</i> *	Man	<i>S. enissocaesilis</i> **
Colony color on YME medium@	Gold	Gold to light brown	gray	N
Spore color	White	White	gray	gray
Spore ornamentation	Smooth	Smooth	Smooth	Smooth
Spore chain morphology	Flexuous	Flexuous	Spiral	Spiral
Melanin production on PYI#	-	-	-	-
Production of diffusible pigment	-	v	+	N
Utilization of carbon sources				
L-Arabinose	-	-	+	+
D-Fructose	-	-	+	+
D-Mannitol	-	-	+	+
Raffinose	+	+	+	+
Rhamnose	-	-	-	-
Sucrose	-	-	-	-
D-Xylose	-	-	+	+
D-Glucose	+	N	+	+
Meso-inositol	+	N	+	+
Utilization of nitrogen sources				
L - P r o l i n e	+	+	+	+
L-Methionine	+	+	-	+
L - Tyrosine	+	N	+	N
L - Histidine	+	N	+	+
Asparagine	+	N	+	N
L - Alanine	+	N		N
Hydrolysis of				
Arbutin	-	-	+	N
Polygalacturonic acid	-	-	-	N
Xylan	+	+	-	N
Minimum growth at pH 4.5	-	-	+	+
Growth in the presence of				
NaCl (4%)	+	+	+	+
NaCl (7%)	-	v	-	-
NaCl (10%)	-	-	-	-
Crystal violet (0.5 µg/ml)	+	v	+	+
Phenol (0.1%)	+	+	+	+
Penicillin (10 IU/ml)	+	+	+	+
Oleandomycin (100 µg/ml)	+	v	+	N
Streptomycin (20 µg/ml)	+	+	-	-

The phenotypic, physiological and biochemical characteristics data of *S. caviscabies* and *S. enissocaesilis* from references Goyer et al., 1996; Faucher et al 1995; + : Positive reaction ; - : Negative reaction ; V: variable; N : Not available; @: YME medium, yeast malt extract medium; #: PYI, Peptone-yeast extract-iron medium.

The morphological, biochemical, and physiological properties of the pathogenic strains are given in Table 3 and Figure. 2. Strain Ag2 exhibited a golden-colored mycelium on YME medium (Figure 2a) and lacked melanin pigment production on PYI. No soluble pigments were detected in the media used. Additionally, it produced white, cylindrical, smooth spores in flexuous chains (Figure 2c). The Ag-2 utilized raffinose, D-glucose, and meso-inositol as carbon sources as well as grew on all tested nitrogen sources. Notably, it could degrade xylan but not hydrolyze polygalacturonic acid or arbutin. Furthermore, this strain cannot grow at a minimum pH of 4.5 but can grow with 4% NaCl or less. It was tolerant to penicillin (10 IU/ml), oleandomycin (100 µg/ml), and streptomycin (20 µg/ml). Also, this strain exhibited growth in the presence of crystal violet (0.5 µg/ml) and phenol (0.1%).

In contrast, strain Man developed gray aerial mycelium, which developed smooth and gray spores arranged in spiral chains (Figure. 2b, d). It produced a brown diffusible pigment but no melanin pigment was

detected. The strain used almost all sugars as a carbon source except rhamnose and sucrose. Also, proline, tyrosine, histidine, asparagine, and alanine were utilized as nitrogen sources, but no growth was observed on methionine. The strain had a minimum growth pH of 4.5 and growth in the presence of NaCl 4%. This strain was resistant to oleandomycin (100 µg/ml), penicillin (10 IU/ml), crystal violet (0.5 µg/ml), phenol (0.1%), and arbutin, but its growth was inhibited by polygalacturonic acid, xylan, and streptomycin (20 µg/ml).

A comparison of the phenotypic, biochemical, and physiological traits of pathogenic strains Ag2 and Man with reference *Streptomyces* strains is given in Table 3 and Figure. 2. Therefore, Ag2 and Man strain are nearly identical to *S. caviscabies* and *S. enissocaesilis* respectively.

PCR and sequencing of the 16S rDNA gene were performed to confirm the identity of strains Ag2 and Man (Figure 3).

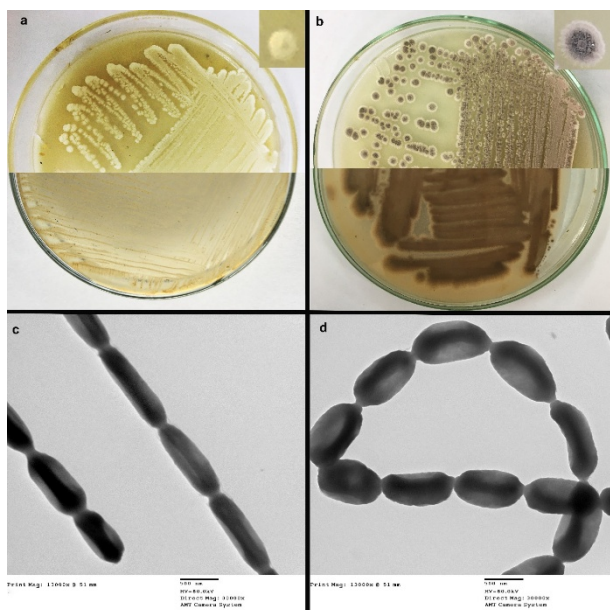


Figure 2. Morphological observation of potato scab-inducing *Streptomyces* isolates Ag2 and Man. spore color and substrate mycelium of Ag2 (a) and Man (b) culture on yeast malt extract medium incubated at 28°C for 14 days. Spores chain morphology and spores ornamentation under transmission electron microscope of Ag2 (c) and Man (d) grown on oatmeal agar at 28°C for 14 days.

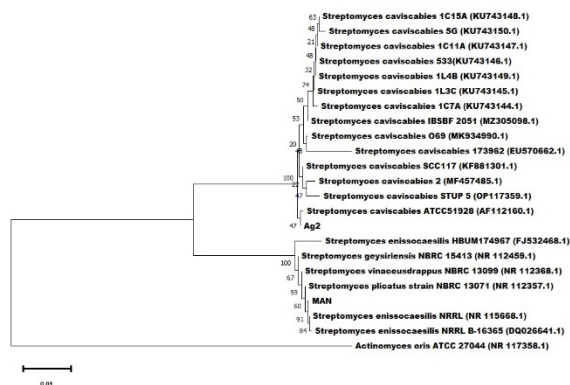


Figure 3. Phylogenetic tree derived from 16S rDNA gene sequences, showing the relationships of strains Ag2 and Man with related *Streptomyces* spp. The tree was rooted with *Actinomyces oris* and constructed using the neighbor-joining method with bootstrap values calculated from 1,000 repetitions. The bar indicates 1% estimated sequence divergence

The sequencing data of the Ag2 and Man strains have been deposited in the GenBank (NCBI) under accession numbers OR447472 and OR447473, respectively. A nucleotide BLAST search revealed that the Ag2 and Man strains share 100% similarity with *S. caviscabies* ATCC 51928 and *S. enissocaesilis* NRRL B-16365, respectively. The 16S rDNA phylogenetic tree (Figure 3) demonstrated that the pathogenic strains (Ag2 and Man) were separated into two distinct groups supported by high bootstrap values (100%). All *S. caviscabies* strains and the Ag2 strain formed a cluster in the first group, with Ag2 closely related to the *S. caviscabies* (ATCC 51928) type strain. The second group comprised *Streptomyces* species with nearly identical 16S DNA sequences, and the Man strain clustered

closely with type strain of *S. enissocaesilis* NRRL B-16365 with a bootstrap value of 91%.

## Discussion

Potato CS is a widespread disease incited by different *Streptomyces* species that significantly decreases the marketable yield for potato growers (Braun et al., 2017). While *S. scabies* is widely recognized as the main cause of scab disease, there are actually over 27 scab-causing *Streptomyces* species that have been reported worldwide (Vincent & Bignell, 2024).

Despite the frequent occurrence of scab disease in Egypt, which poses a significant threat to potato crops and has been reported as early as 1966 (EL.Kashier, 1966; Mehiair & El-Samra, 1978), there is a noticeable absence of comprehensive studies that specifically aim to characterize the pathogenic *Streptomyces* species in the country.

In this investigation, nine isolates of *Streptomyces* spp. were recovered from potato tubers naturally infected and collected from various locations in Egypt. Out of the nine isolates, only the Ag2 and Man strains showed positive results in both the tuber slice and radish seedling tests. In the potato pot trail, these strains induced symptoms of CS on potato tubers that were identical to those observed on the original infected potatoes. A significant number of *Streptomyces* spp. isolated from scabby potatoes have been previously reported to be non-pathogenic (Gouws, 2013; Henao et al., 2022; Jordaan & Van der Waals, 2016; Wanner, 2007). Also, it has been found that non-pathogenic *Streptomyces* can colonize tubers that display symptoms of CS. This colonization may potentially contribute to the development of the disease (Chalupowicz et al., 2022). The study of Cui et al. (2021) reported that isolate 5A-1 exhibited pathogenicity among 20 isolated *Streptomyces* spp.

The pathogenic isolates in our study were identified as *S. caviscabies* and *S. enissocaesilis*. This identification was based on an evaluation of their morphological and biochemical characteristics, along with the analysis of their 16S rDNA gene sequences. Both pathogens are identified for the first time as causative agents of potato common scab in Egypt.

*Streptomyces caviscabies* was initially described as a pathogen responsible for the development of deep-pitted potato scab in Canada (Faucher et al., 1995; Goyer et al., 1996). Additionally, Corrêa et al. (2015) detected this pathogen in several potato fields in Brazil where it was associated with potato scab. In Iran, a group of *S. caviscabies* strains was isolated from scabby potatoes and these strains caused significant disease severity under both natural and greenhouse conditions (Khodakaramian & Khodakaramian, 2013). Recently, five isolates from typical CS lesions in China were identified as *S. caviscabies*. These isolates exhibited typical symptoms of CS on potatoes under greenhouse conditions (Gong et al., 2017).

The species *S. enissocaesilis* was first reported as a novel potato scab pathogen in China (Zhang et al., 2010; Zhang et al., 2009). Yang et al. (2018) investigated the species composition of pathogenic *Streptomyces* responsible for causing potato scab in Yunnan Province, China. Out of the 67 pathogenic *Streptomyces* spp., 29 were identified as *S. enissocaesilis* and caused tuber

disease with CS symptoms in greenhouse pots. Recently, *S. enissocaesilis* was used as a pathogen of potato CS to screen for highly efficient bactericidal agents (Pu et al., 2022). On the other hand, *S. enissocaesilis* has previously been documented as an effective biological control agent against a wide range of diseases and pests (Aallam et al., 2021; Abbasi et al., 2019; Boukelloul et al., 2024; Ganesan et al., 2018). In the rhizosphere, *S. galilaeus* and *S. griseoplanus* have a dual role. In addition to being a pathogen that can cause potato CS, they can also act as a potential bioagent for controlling plant diseases (Cui et al., 2021; Cui et al., 2018; Nimnoi et al., 2017). Biocontrol agent assays, particularly those involving *Streptomyces* spp., require careful attention. It is crucial to conduct radish seedling and potato slice assays to verify the pathogenicity of the tested agents used for controlling plant diseases. The emergence of new pathogen causing CS may be attributed to the horizontal gene transfer of pathogenicity genes among *Streptomyces* spp. (Armijos-Jaramillo et al., 2017; Bukhalid et al., 2002).

In Egypt, the precise date of the initial recording of potato CS disease is uncertain. However Egyptian quarantine acts and regulations indicate that it was recognized as early as 1932 (EL.Kashier, 1966). Many studies have indicated that *S. scabiei* is the main causal agent of potato CS disease in Egypt (Abd El-Hafez & El Shishtawy, 2021; Eid et al., 2022; Gabr, 1988; Galal et al., 1999; Mehiar & El-Samra, 1978). Additionally, a few other causal agents have been identified, including *S. acidiscabies* (El-Sayed, 2000), and *S. alkaliscabies* (Abdel-Rahman et al., 2012). The low diversity of scab-causing strains isolated from Egypt may be related to previous studies that performed only phenotypic (morphology and biochemical) identification tests, except for *S. alkaliscabies*. Therefore, phenotypic and molecular characteristics should be performed for an accurate and clear diagnosis of pathogenic strains.

## Conclusion

The current study reports, for the first time, the presence of scab-causing pathogens *S. caviscabies* and *S. enissocaesilis* in Egypt. These pathogens were identified based on their phenotypic and molecular traits. Their pathogenicity was validated through potato slices and radish seedling assays, as well as a greenhouse evaluation. Further research is necessary to define the range of pathogens and develop effective control strategies.

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## Analysis of Drying Kinetics and Mathematical Modelling of Peanut Pods using Sunlight, Hot Air, and Microwaves Drying Processes

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### ABSTRACT

This study analyzed the drying kinetics of peanut pods employing sun, hot air, and microwave drying techniques, and evaluated their mathematical modeling. The findings demonstrated that sun-drying decreased the moisture content from 26.47% to 8-10% over a duration surpassing 72 hours. Hot air drying at temperatures of 60°C, 80°C, and 100°C, commencing with an initial moisture content of 29.92%, necessitated 810 minutes, 360 minutes, and 660 minutes, respectively. Microwave drying, commencing with an initial moisture content of 23.01%, required 40 minutes, 45 minutes, and 60 minutes at belt velocities of 3 mm/s, 4.9 mm/s, and 6.2 mm/s, respectively, at 300 W. At 400 W, the durations were 24 minutes, 30 minutes, and 40 minutes, respectively. All drying kinetics curves exhibited decreasing rates characteristic of agro-food products. Mathematical modeling analysis identified the Midilli model as the most appropriate, succeeded by the Page, Henderson, and Pabis models, for characterizing moisture loss during the sun, hot air, and belt microwave drying of peanut pods.

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## Introduction

Peanut (*Arachis hypogea* L.), one of the most widely cultivated and largest oilseed crops globally, has an annual production of approximately 54 million tons. According to FAOSTAT (2022), the leading producing countries are China (17.8 million tons), India (10,134,199 tons), Nigeria (3.4 million tons), the United States of America (2.575 million tons), and Argentina (1.346 million tons). Peanut kernels are a substantial source of oils (44–56%) and proteins (25–35%). Moreover, peanuts are abundant in various health-promoting nutrients, including vitamin A, vitamin B6, and minerals, which may enhance metabolism, bolster memory, improve learning capacity, and retard aging (Xie et al, 2023, Krzyzanowski et al, 2006).

During harvest, peanut pods with a moisture content of 30 to 40 % need drying to prevent spoilage. Handling overly fresh pods, with kernels still attached to the shell, can cause irreversible biological deterioration. Immediate drying reduces moisture to 20-25 %, gradually to 8-10 %. Drying duration varies from days to weeks, affected by weather and drying methods, such as open-air or artificial drying (John and Otten, 1989). Open-air drying exposes products to adverse weather, potentially causing losses

from precipitation-induced mold and aflatoxin, compromising quality. Producers mitigate risks using tarps, but improper use leads to poor drying, quality depreciation, and economic losses. Exploring alternative drying methods to ensure safe, rapid drying in any weather is crucial.

Researchers extensively study factors affecting peanut drying, e.g., temperature profiles and moisture reduction under different microwave powers (Boldor et al., 2005; John and Otten, 1989). Comparing traditional practices and solar dryers allowed to evaluate drying kinetics (El-Sayed et al., 2006; Goneli et al., 2017; Hürdoğan et al., 2021). Studies using various methods investigated performance and relate mathematical models to experimental data (Hürdoğan et al., 2021) (Yang et al., 2007).

Conventional drying methods (forced air convection, oven, microwave) need suitable units with specific advantages and disadvantages (Chiewchan et al., 2015). Regardless of method, all dried products lose moisture. Ideal drying faces challenges altering peanuts' qualities and by-products (Babalıs et al., 2017), affecting nutritional security, and organoleptic properties.



Although research on hot air and microwave drying emphasizes kinetics and energy consumption, there is a deficiency in the modeling of optimal parameters. The specific objectives are to analyze drying kinetics under various conditions and to model peanut drying using established mathematical frameworks to identify the most appropriate models.

**Materials and Methods**

**Drying Experiments**

In this study, Batem-5025 peanut varieties from the Oilseed Research Institute of Osmaniye Province served as the biological materials. The experiments were performed at the Agricultural Faculty of Çukurova University in Adana, Türkiye. The peanut pods were subjected to multiple drying techniques to attain a moisture content appropriate for extended storage or subsequent processing. Initially, the drying process aimed to attain a safe moisture content of 8-10%, selected for its low water activity (*a<sub>w</sub>* 0.67), which is favorable for storage. Three desiccation methods were utilized: solar drying, hot air drying, and microwave drying.

**Sun Drying:** Sun drying involved exposing samples directly to sunlight on mats or racks placed on the roof terrace at Çukurova University, under Adana/Türkiye’s weather conditions. Samples were sheltered at night to prevent moisture absorption. Drying took place over four days, with daytime temperatures around 28°C and nighttime temperatures around 19°C. Humidity remained below 32 %, with constant wind speed of 10 km/h. Moisture loss was monitored by weighing every 12 h.

Peanut samples were dried in the oven at 60°C, 80°C and 100°C in 3 cycles. Two different powers (300 W and 400 W) and three belt speeds (3.7 mm/s, 4.9 mm/s and 6.2 mm/s) were used in the belt microwave drying process. Triplicate samples of approximately 20 g were dried until the required moisture content was reached. The samples were subjected to multiple drying cycles with the exposure time proportional to the number of cycles. The linear velocity was determined by the number of passes and tunnel length.

**Determination of Moisture Content Profile and Drying Kinetics**

Before initiating any drying process, laboratory procedures were essential to determine the initial moisture content of peanut pods. The FAO method, outlined by Karmas (1980), was employed for this purpose. Approximately 20 g of peanut samples were subjected to 6

hours at 130°C, as outlined by Young et al. (1982), until a stable weight was attained. Mass measurements were conducted utilizing a digital balance (Sartorius GP3202, Göttingen, Germany) with a precision of 0.01 g. Thereafter, the moisture content, represented as a percentage on a wet basis, was computed utilizing Equation 1.

$$MC(\%) = \frac{M_w}{M_w + M_d} \times 100 \tag{1}$$

Where *M<sub>C</sub>* is the moisture content (%), *M<sub>w</sub>* is the mass of water (g), and *M<sub>d</sub>* is the material’s dry mass (g)

Drying kinetics involve continuously weighing samples using an electronic balance to assess mass loss and drying rate of the product. As moisture evaporates during drying, the product’s mass diminishes. The reduction in mass attributed to moisture loss was computed using Equation 2. Essentially, establishing the necessary moisture content at a specified time, referred to as the required time, signifies the duration needed to achieve the desired moisture content during drying operations.

$$\Delta W = W_1 \frac{N_1 - N_2}{100 - N_2} \text{ with } \Delta W = W_1 - W_2 \tag{2}$$

Where  $\Delta W$  is the mass loss in the product (g); *W<sub>1</sub>* is the initial mass of the sample (g), *W<sub>2</sub>* is the final mass of the sample (g), *N<sub>1</sub>* is the initial moisture content of the sample (% w.b) and *N<sub>2</sub>* is the final moisture content of the sample (% w.b).

The drying rate refers to the change in moisture content of the product as measured internally. It is determined by applying the formula outlined in Equation 3:

$$V = \frac{dM}{M_S * dt} \tag{3}$$

Where *V* is drying rate or kinetics (g/g.dm), *M* is the total mass of sample (g), *M<sub>S</sub>* is the mass of dry matter (g), and *t* is the drying time (s).

**Mathematical Modelling**

In this study, nine empirical models, each possessing distinct characteristics, are utilized across three different drying methods to establish a characteristic drying law for each process. Several empirical and semi-empirical models have been developed to describe agri-food products’ drying kinetics (Midilli and Kucuk, 2003). These models, presented in Table 1, serve to mathematically depict experimental drying curves.

Table 1. Mathematical models provided by various authors for the description of the drying curves

N°	Models	Equations	References
1	Midilli-Kucuk	$Xr = a \exp(-kt^n) + bt$	Midilli et al. (2002)
2	Henderson And Pabis	$Xr = a \exp(-kt)$	Zhang and Litchfield (1991)
3	Two-Term Exponential	$Xr = a \exp(-kt) + (1-a) \exp(-kat)$	Sharaf-Eldeen et al. (1980)
4	Newton	$Xr = \exp(-kt)$	Ayensu and Asiedu-Bondzie (1986)
5	Wang And Singh	$Xr = 1 + at + bt^2$	Akpınar et al. (2003)
6	Page (PG.)	$Xr = \exp(-kt^n)$	Morey and Li (1984)
7	Verma (VM.)	$Xr = a \exp(-kt) + (1-a) \exp(-gt)$	Verma et al. (1985)
8	Diffusion Approach	$Xr = a \exp(-kt) + (1-a) \exp(-kbt)$	Yaliz and Ertekin (2001)
9	Logarithmic (LG.)	$Xr = a \exp(-kt) + c$	Yağcıoğlu et al. (1999)

**Statistical Analysis**

The data collected underwent statistical analysis via Sigma Plot software for mathematical modelling. Regression analysis was carried out using the General Nonlinear Modelling procedure to simulate and compare various mathematical drying models with the experimental data on drying kinetics. Procedures such as R<sup>2</sup> and standard error estimate were utilized to identify variables with the highest correlation and lowest standard deviation.

**Results**

**Moisture Loss Profiles and Drying Kinetics**

*Sun Drying*

Figure 1 show the profiles of moisture loss. It was noted that the fluctuations in moisture content of peanut pods during natural drying closely correlate with environmental weather conditions, despite the slight decrease in moisture content loss curve with an initial moisture content of 26.47 %. When sun-drying peanut pods at varying layer thicknesses of 5 cm, 10 cm, and 15 cm, the average moisture content (w.b) decreased from an initial level of 54.39 % to around 13 % in 52 h, 56 h, and 66 h, respectively (El-Sayed et al., 2006). The findings of the present study underscore that the drying time of peanuts is influenced not only by prevailing weather conditions but also by the thickness of the layers of peanuts destined for drying. The duration of 72 h required to achieve the desired moisture content of 9.04 % confirms this slow dehydration process. Over the course of 72 h, the reduction in humidity was approximately 65.84 %.

Figure 2 shows a gradual decline in the development of the experimental drying kinetics curve. Initially, significant changes in moisture content occur in the early stages of drying and stabilize as drying progresses towards the desired moisture level. According to Can (2000), drying kinetics, including rate variations, are affected by the drying air temperature as evidenced in experiments with pumpkin seeds. In particular, Xie et al, Moukhtar Lati and Bechki (2015) observed that increasing and constant ratesre almost non-existent. Analysis of the decreasing period indicates a slight decrease in the drying rate over time, a phenomenon recorded by Xie et al. (2023) and Akoto et al (2018) on hot air and sun drying of peanuts. These trends can be attributed to the increasing internal

resistance to mass transfer, as suggested by Nguyen (2015). According to Nguyen (2015), the drying behavior is related to a tight comparison of the external resistance due to the velocity, temperature and humidity of the air flow and the internal resistance related to the effective diffusivity and size of the product. Moreover, drying curves and speed variations are highly dependent on the moisture content of the product.

*Hot Air Drying*

Different temperatures, namely 100°C, 80°C, and 60°C were applied to hot air dry pre-treated peanut samples. Figure 3 illustrates drying curves showing moisture loss trends in the peanut pod samples. Initially, all samples had a moisture content of 29.92 %. The drying process at each temperature showed a gradual reduction in moisture content over time, followed by stabilization to a constant level. Studies on agro material drying have consistently highlighted the positive impact of air temperature, especially for organic products with high internal water resistance (Belghit et al., 1999a; Mariem and Mabrouk, 2017). However, the time needed to reach the desired moisture content varied significantly with temperature. For example, drying times to achieve 8-10 % moisture content were 6 h at 100°C, 7 h at 80°C, and 13.5 h at 60°C, all shorter than the 14 h reported by Krzyzanowski et al. (2006) for peanuts dried at 34.6°C using pipe technology. These differences can be attributed to initial moisture levels and higher drying temperatures in this study. Munir Ahmad and Mirani (2012) dried peanuts from 23.3 % to 14 % moisture content in 2.4 h using a mobile flat-bed dryer under specific conditions. Increasing temperature decreased drying time for peanut pods, significantly reducing moisture content. Specifically, when temperature rose from 60°C to 100°C, drying time halved. Apart from moisture loss, drying affects other factors, such as the volatility of certain compounds in the dried product. External weather parameters, like ambient air conditions during repeated weighing, may also indirectly influence samples (Belghit et al., 1999a). Generally, higher drying temperatures correlate with lower moisture content, creating a greater deficit in water vapor pressure, which drives outward moisture diffusion (Belkacem, 2016). Higher drying temperatures enhance heat transfer, increasing water molecule energy, facilitating quicker migration within the product, and accelerating their escape.

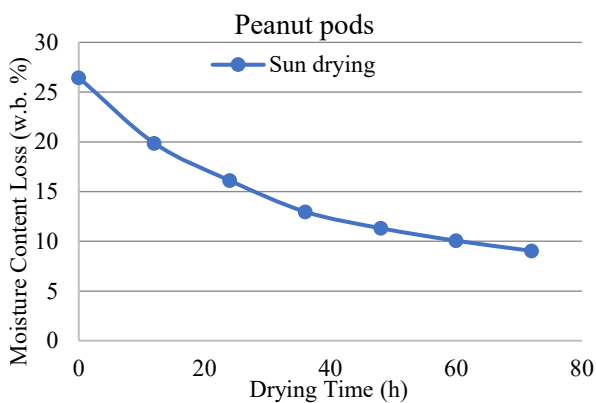


Figure 1. Effect of natural conditions on average moisture loss of sun dried peanuts pods

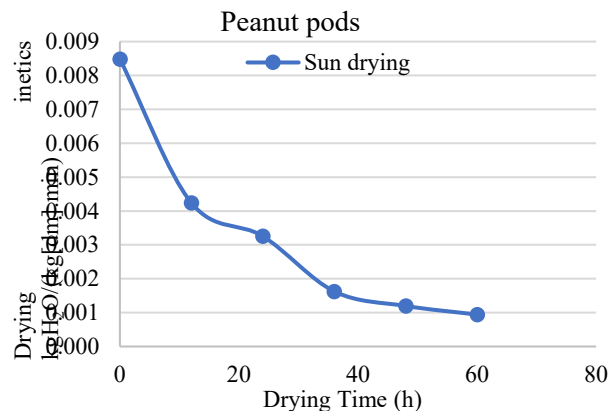


Figure 2. Drying kinetics variations of natural conditions dried peanut pods

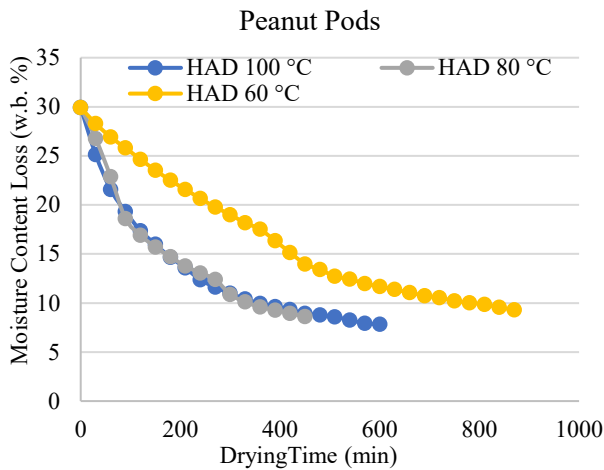


Figure 3. Effect of drying temperatures on the average moisture loss of peanut pods

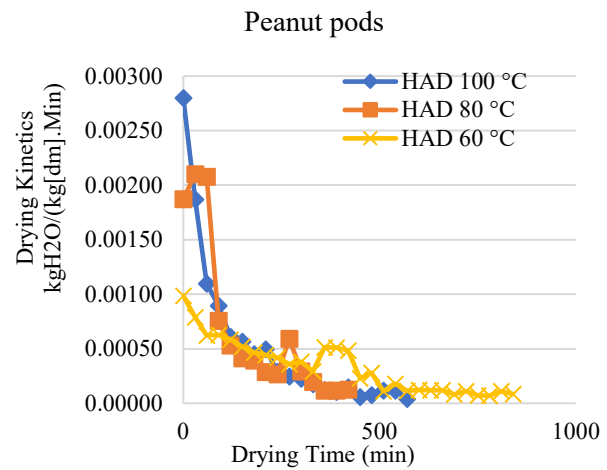


Figure 4. Drying kinetics variations of hot air dried peanut pods

Figure 4 illustrates the progression of drying kinetics over time, highlighting drying temperature as a critical factor. Traditionally, materials such as cellulose, wood, and clay, which are non-hygroscopic and minimally deformable, exhibit three distinct phases: initial product warming, constant speed drying, and decreasing pace period drying (Bonazzi and Bimbenet, 2003). However, analysis of peanut drying curves reveals a deviation from this pattern, characterized by the absence of an initial heating phase and a sustained decline in drying rate. These observations, reflecting agro-food product drying kinetics, are consistent with findings in various studies (Belghit et al., 1999b; Ndukwu, 2009). The phase of decreasing drying rate is likely influenced by structural changes within the dried material and the absence of free water on the product's surface.

The analysis of decreasing periods uncovers two distinct phases in drying kinetics:

Initially, there is a phase characterized by a decreasing drying rate, where the material's physical properties progressively impact the kinetics. This decrease in drying rate is often attributed to the increasing internal resistance to mass transfer, as indicated by Nguyen (2015). Increasing the drying temperature generally boosts the drying rate, particularly at the start of drying, prominently seen in the data at 100°C and 80°C. However, towards the end of the operation, the influence of temperature diminishes as nearly all free and bound water molecules have evaporated, leaving only those bound to the product's structure unaffected by the drying process (Mariem and Mabrouk, 2017). Temperature is identified as the most influential factor affecting drying kinetics.

The second phase occurs as the material enters the hygroscopic domain entirely. Here, the drying kinetics gradually diminishes until it aligns with the material's equilibrium with the external conditions.

### **Belt Microwave Drying**

In the section of Belt Microwave Drying, the effects of two power intensities (300 W and 400 W) and three belt velocities of microwave dryers were examined. Figure 5 illustrates the moisture content loss profiles. Initially, all samples had a consistent moisture content of 23.01 %.

Results indicated a gradual decrease in moisture content with increasing power intensities. Towards the end of drying, where the rate stabilizes, the water potential at the surface remains insignificant compared to that of the microwaves, resulting in a slight variation in the water potential differential.

Delwiche et al. (1986) similarly observed this progressive decrease in moisture content in peanut pods and kernels with varying power levels. With a power intensity of 300 W, exposure times to achieve the desired moisture content were 40 min, 45 min, and 60 min, corresponding to velocities of 6.2 mm/s (250 rpm), 4.9 mm/s (200 rpm), and 3.7 mm/s (150 rpm). Using 400 W, exposure times were 24 min, 30 min, and 40 min, with corresponding velocities. These findings suggest that increasing power intensity or belt velocity gradually reduces the required drying time.

In essence, higher power leads to increased microwave energy (Boldor et al., 2005), while lower belt velocity extends exposure time in the dryer, consequently affecting the temperature and moisture of peanuts. These observations align with the findings of Schirack et al. (2007), who studied a continuous belt microwave system for blanching peanuts and noted that higher power and temperature levels could rapidly reduce moisture content.

Figure 6 illustrates the variation in drying kinetics. Analysis of the drying kinetics curves indicates a single phase characterized by a decreasing drying rate, without an initial phase of increasing and constant heating rate. These findings are consistent with experiments conducted by (Lamyae L. et al., 2015). Examining the warming phase emphasizes the significant influence of microwave power intensity and belt velocity on drying kinetics. Higher power levels and lower belt velocities initially accelerate the drying rate. However, as drying progresses within the tunnel, the rate gradually diminishes until it reaches equilibrium with external conditions. These trends are influenced by the inherent properties of peanuts and the conduction properties of the boundary layer, which maintain relative constancy during this phase. As the product's surface continues to dry, changes in these properties impede mass transfer, leading to a reduction in moisture content.

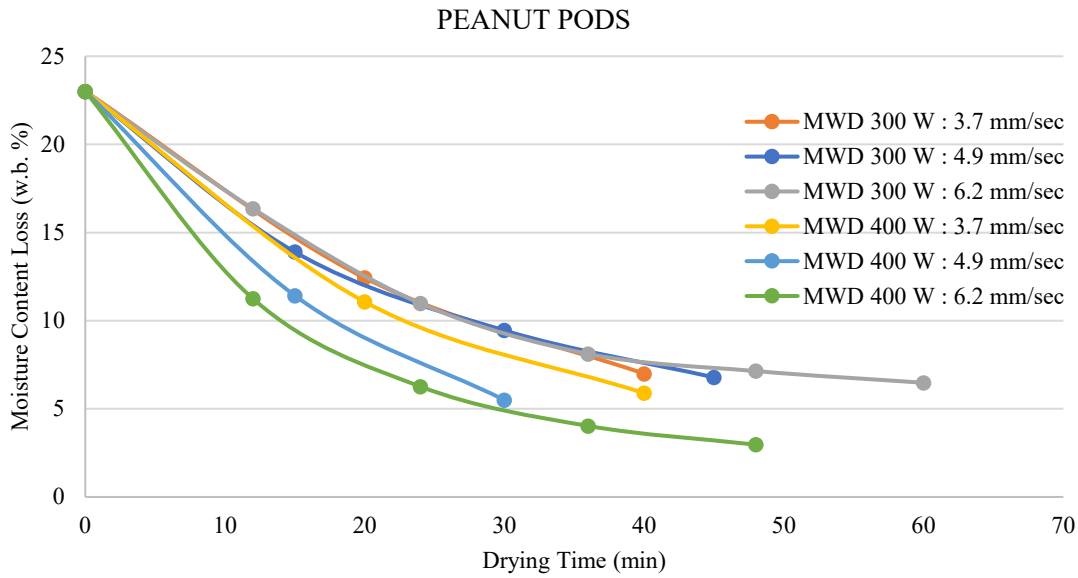


Figure 5. Effect of power intensities and belt velocities on average moisture loss in belt microwave dried peanuts pods

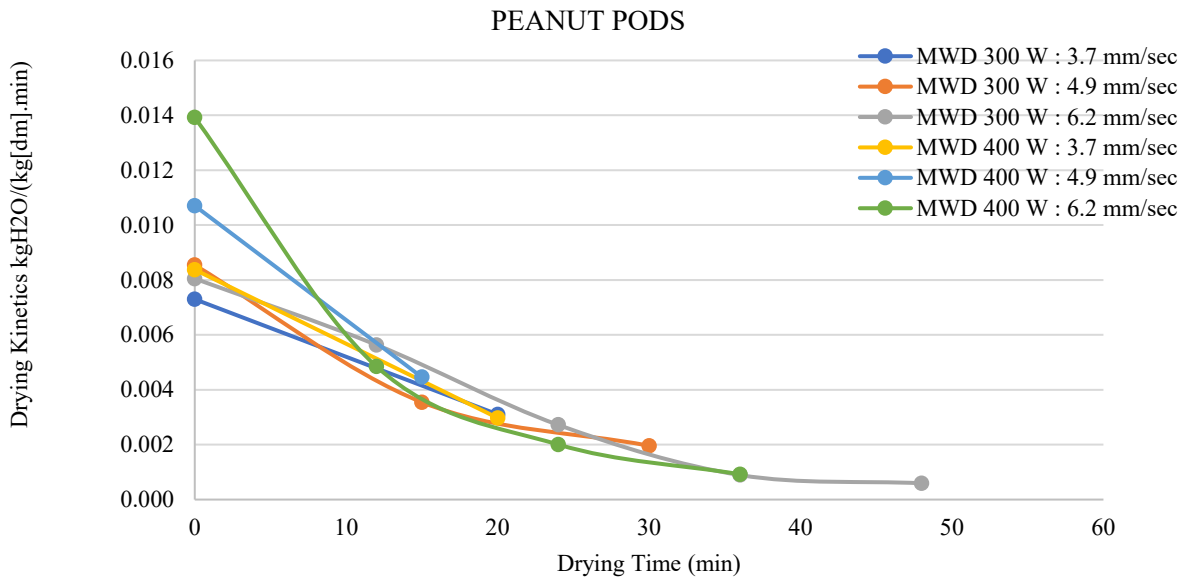


Figure 6. Drying kinetics variations of belt microwave dried peanut pods

**Mathematical Modelling of Drying Peanuts**

The drying kinetics data from drying methods were analysed using mathematical models, with semi-empirical equations representing the moisture content variation over time during drying. To ensure agreement with experimental data, these equations include adjusted constants determined through nonlinear regression. The efficiency of each model was assessed through statistical parameters such as a high correlation coefficient ( $R^2$ ) approaching 1 and a minimum standard deviation ( $\chi$ ) approaching 0.

**Modelling of Sun Drying**

Table 2 illustrates that in terms of sun drying, the Midilli-Kucuk and Page models demonstrate superior coefficients for describing peanut pod drying under natural meteorological conditions. Correlation coefficients ( $R^2$ )

range from 0.1864 to 0.8600, with standard deviations between 0.0015 and 0.0028. Hürdoğan et al. (2021) identified the random tree and Quintic models as best suited for estimating moisture content and drying rate of solar-dried peanuts. Conversely, Midilli and Kucuk (2003) found the Two-Term Exponential model effective for thin-layer sun drying of pistachios. The Page model accurately described moisture content evolution during thin-layer solar drying of raw rice seeds (Basunia and Abe, 2001). For vegetables, Yaliz and Ertekin (2001) reported the diffusion approximation model as optimal for pumpkin and green pepper, the Two-Term Exponential model for stuffed pepper and onion, and the Page model for green beans. Furthermore, the Exponential model was found to better represent the characteristics of thin-layer sun drying of mulberry fruits compared to Page's model.

Table 3. Modelling of moisture content according to drying time for peanut pods in the sun drying

N°	Models	R <sup>2</sup>	χ
1	Midilli-Kucuk	0.8600	0.0015
2	Henderson And Pabis	0.1070	0.0030
3	Two-Term Exponential	NAN	0.4472
4	Newton	NAN	0.4083
5	Wang And Singh	NAN	0.5109
6	Page	0.1864	0.0028
7	Verma	NAN	0.5000
8	Diffusion Approach	NAN	0.5000
9	Logarithmic	0.2050	0.0031

Bests mathematical models; 1<sup>st</sup>. Midilli-Kucuk model's; 2<sup>nd</sup>. Page model's

Table 4. Modelling of moisture content according to drying time for peanut pods in the hot air drying

N°	Models	60 °C		80 °C		100 °C	
		R <sup>2</sup>	χ	R <sup>2</sup>	χ	R <sup>2</sup>	χ
1	Midilli-Kucuk	0.9065	0.0001	0.9023	0.0003	0.9930	0.0001
2	Henderson And Pabis	0.8799	0.0001	0.8371	0.0003	0.9694	0.0001
3	Two-Term Exponential	NAN	0.0004	NAN	0.0009	0.2532	0.0006
4	Newton	NAN	0.0004	NAN	0.0009	0.2532	0.0006
5	Wang And Singh	NAN	0.3216	NAN	0.3106	NAN	0.3161
6	Page	0.8030	0.0001	0.7217	0.0004	0.9812	0.0001
7	Verma	0.8948	0.0000	0.8371	0.0003	0.9818	0.0001
8	Diffusion Approach	0.8948	0.0001	0.8371	0.0003	0.9818	0.0001
9	Logarithmic	0.8811	0.0001	0.8380	0.0003	0.9860	0.0001

Bests mathematical models; 1<sup>st</sup>. Midilli-Kucuk's model; 2<sup>nd</sup>. Logarithmic's mod

Table 5. Modelling of moisture content according to drying time for peanut pods in belt microwave drying at 300 W

N°	Models	300 W					
		3.7 mm/s (150 rpm)		4.9 mm/s (200 rpm)		6.2 mm/s (250 rpm)	
		R <sup>2</sup>	χ	R <sup>2</sup>	χ	R <sup>2</sup>	χ
1	Midilli-Kucuk	1.000	(+inf)	1.000	(+inf)	0.9999	0.0005
2	Henderson And Pabis	0.1150	0.0975	0.0006	0.0671	0.0283	0.0425
3	Two-Term Exponential	NAN	1.0004	NAN	0.7076	NAN	0.5002
4	Newton	NAN	0.7076	NAN	0.5779	NAN	0.4476
5	Wang And Singh	NAN	1.000	NAN	0.7174	NAN	0.5100
6	Page	0.6720	0.0593	0.4106	0.0515	0.2062	0.0385
7	Verma	NAN	(+inf)	NAN	1.0000	NAN	0.5770
8	Diffusion Approach	NAN	(+inf)	NAN	1.0000	NAN	0.5770
9	Logarithmic	0.6720	(+inf)	0.4110	0.0729	0.2350	0.0436

Bests mathematical models: 1<sup>st</sup>. Page's model; 2<sup>nd</sup>. Midilli-Kucuk's model

Table 6. Modelling of moisture content according to drying time for peanut pods in belt :microwave drying at 400 W

N°	Models	400 W					
		3.7 mm/s (150 rpm)		4.9 mm/s (200 rpm)		6.2 mm/s (250 rpm)	
		R <sup>2</sup>	χ	R <sup>2</sup>	χ	R <sup>2</sup>	χ
1	Midilli-Kucuk	1.0000	(+inf)	1.000	(+inf)	1.0000	0.0000
2	Henderson And Pabis	0.0173	0.1560	0.1100	0.1080	0.0000	0.0400
3	Two-Term Exponential	NAN	1.0000	-75.907	1.0004	-207.18	0.5776
4	Newton	NAN	0.7071	-75.967	0.7076	-207.28	0.5003
5	Wang And Singh	NAN	1.0000	-75.850	1.0000	-211.42	0.5834
6	Page	0.4143	0.1200	0.6626	0.0663	0.3252	0.0329
7	Verma	NAN	(+inf)	-75.850	(+inf)	-207.05	0.7070
8	Diffusion Approach	NAN	(+inf)	-75.850	(+inf)	-207.05	0.7070
9	Logarithmic	0.4140	(+inf)	0.6630	(+inf)	0.3250	0.0403

Bests mathematical models: 1<sup>st</sup>. Page's model; 2<sup>nd</sup>. Midilli-Kucuk's model

**Modelling of Hot Air Drying**

Table 3 presents the main findings from the statistical analysis of the mathematical modelling of peanut pod drying. The Midilli-Kucuk model accurately predicts the drying process of dried peanut pods at 60°C, 80°C, and 100°C, with a correlation coefficient (R<sup>2</sup>) ranging from 0.9023 to 0.9930 and a standard deviation ranging from

0.0001 to 0.0003. Additionally, the Logarithm model effectively characterizes the drying process of peanut pods at 80°C and 100°C, while the Verma model yields favourable coefficients particularly for drying at 60°C. nut pods in hot air drying.

In this study, observations confirmed by Yang et al. (2007) indicated that the Henderson-Pabis model is the most suitable for describing the drying of thin peanut layers. Conversely, Tayel et al. (2015) found that the Lewis model effectively describes the drying behaviour of peanut pods under high humidity conditions. Xie et al. (2022) demonstrated that the logarithmic model is appropriate for peanut pod drying under hot air at 40°C with an air speed of 0.5 m/s. Goneli et al. (2017) concluded that the Two-Term, Midilli-Kucuk, Page, and Thompson Diffusion Approximation models are suitable for representing peanut kernel drying kinetics during forced air drying. Fokone et al. (2013) found that the exponential model is a perfect fit for describing carrot drying behaviour across different temperatures, air velocities, and relative humidity. Akpinar et al. (2003) indicated that an approximation of the diffusion model satisfactorily describes the drying curve of red peppers under specific drying conditions.

### **Belt Microwave Drying**

The findings in Tables 4 and 5 regarding microwave drying demonstrate that, the Page and Midilli-Kucuk model adequately describe the drying of peanut pods. For power intensities of 300 W and 400 W applied to three belt velocities, correlation coefficients ( $R^2$ ) range from 0.2062 to 1.0000, with standard deviations ranging from 0.0000 to 0.1202. These results are consistent with Beyza (2018) research on microwave drying of pomegranate grains. However, John and Otten (1989) found that the Two-Term Exponential model accurately predicts the microwave drying of peanut pods and kernels.

### **Conclusion**

This study examined the independent effects of sun drying, hot air drying, and belt microwave drying on the moisture content of peanuts, using numerical models to assess their consistency. Peanut pods were initially sun dried for 72 h to reach optimal moisture levels. Subsequently, hot air drying at 80°C reduced the drying time to 7 h, while microwave belt drying at 400 W and 6.2 mm/s (250 rpm) further reduced it to 24 min. Microwave drying is economically feasible with sun drying whereas hot air drying of peanuts pods needs more energy to achieve the safe moisture content.

Results indicate that drying kinetics followed typical patterns observed in agricultural products across all methods and variables. Theoretical models such as "Midilli-Kucuk" and "Henderson and Pabis" accurately represented moisture content variations in hot air drying. Conversely, Page's and Midilli's models effectively described moisture content variations during continuous belt microwave and sun drying. Among the nine models examined, the "Wang Sangh" model was found to be the least suitable for all drying conditions.

### **Declarations**

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**Conflict of interest:** This study does not have any conflict of interest.

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**Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## Determination of Optimum Insulation Thickness in Building Insulation in Tokat Province and Its Effect on CO<sub>2</sub> Emission

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### ABSTRACT

Agricultural structures are generally classified as rural structures, animal-plant production structures and protection-storage structures. As in all types of buildings, it is necessary to reduce energy costs by reducing thermal losses/gains in agricultural structures. Thus, the damage caused by the waste gases released to people and the environment is also reduced. For these reasons, it has become important to determine the type and thickness of the insulation material used to reduce thermal losses/gains in a way that will provide the most economic benefit. In this study, optimum insulation thicknesses, annual savings, payback period and reduction in CO<sub>2</sub> emissions on the outer walls of the building for heating and heating-cooling applications in Tokat province were calculated. Although the desired temperature values differ according to the building types, this study for residences is also an adaptive prediction for agricultural building types. XPS (extruded polystyrene) and RW (rock wool) insulation materials were used for two different wall types. Natural gas for heating and electricity for cooling were selected as energy sources. DD (degree day) and LCA (life cycle analysis) methods were used for thermal and economic analysis. According to the wall type, the optimum insulation thickness of XPS insulation material is 0.032m-0.029m for heating, 0.071m-0.068m for heating-cooling, payback period is 5.13years-6.92years for heating, 2.32years-2.94years for heating-cooling, and the reduction in CO<sub>2</sub> emission is calculated as 64.57%-57.77% for heating, 80.46%-76.28% for heating-cooling. According to the wall type, the optimum insulation thickness of RW insulation material was 0.023m-0.019m for heating, 0.055m-0.051m for heating-cooling, payback period 8.20years-11.93 years for heating, 3.33years-4.3years for heating-cooling, and the reduction in CO<sub>2</sub> emission was determined as 53.98%-44.65% for heating, 73.69%-68.49% for heating-cooling. In the insulation application of buildings, it has been determined that it is more appropriate to prefer XPS insulation material because it is more advantageous than RW insulation material in terms of both payback period and economy and environmental impact in reducing CO<sub>2</sub> emissions, at the calculated optimum insulation thicknesses.

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### Introduction

Economic, political, and social factors are the most important factors influencing energy use (Coyle 2014). Total energy consumption in the world is 37.8% in industry, 30.1% in buildings, 26% in transportation and 6.1% in other sectors, respectively. Energy consumption in the building sector has increased by an average of 1% over the past decade. Natural gas meets 23% of the energy demand in buildings. It is estimated that the global total area in residential buildings will increase by 55% by 2050 and energy consumption in buildings will increase between 4.63%-5.26% by 2030 (International Energy Agency [IEA], 2023).

In 2020, 34.4% of Türkiye's total energy consumption was in industry, 25.5% in transportation, 24.5% in residential and 15.6% in services. It is estimated that in

2030, total energy consumption will increase by 2.9% for industry and decrease by 3.3% for residences (Turkey's National Energy Plan, 2022).

Energy consumption in buildings in the world and in Türkiye constitutes approximately 25% of total energy consumption. Monitoring and controlling CO<sub>2</sub> emissions caused by energy consumption in buildings, as in all sectors, is important in terms of its impact on climate change.

Total greenhouse gas emissions per capita in Türkiye were calculated as 4 tons of CO<sub>2</sub> equivalent in 1990 and 6.7 tons of CO<sub>2</sub> equivalent in 2021 (TUIK, 2023). As of 2021, the emissions of the EU-27 countries per capita in CO<sub>2</sub> equivalent are 7.9 tonnes/capita (EUROSTAT, 2020).



When compared in terms of construction costs, insulated buildings cost 15-40% more than non-insulated buildings, but when examined in terms of energy consumption costs, insulated buildings provide approximately 60% energy savings compared to non-insulated buildings (Paraschiv et al., 2020). For this reason, the importance of insulation applications is increasing to reduce energy use and negative environmental impacts of buildings. However, applications must be compatible with technology and public policies (Coyle&Simmons, 2014).

Tokat province is in the 3rd climate zone of the four climate zones listed from hot climate to cold climate according to Degree Day regions. Many studies based on economic analysis have been carried out using the DD method to determine the optimum insulation thickness in the heating and cooling of buildings in all climatic zones of Turkey. Some of the studies in the literature are given below.

Kaynaklı (2013) determined the optimum insulation thicknesses, energy savings and payback periods of all cities in Türkiye according to their energy needs for heating, cooling and both. Effects of thermal and economic parameters on energy saving and optimum insulation thickness were determined.

Kürekci (2016) determined the optimum insulation thicknesses according to four different insulation materials and five different fuel types for heating, cooling and both heating and cooling applications for all provinces of Türkiye using DD and LCA methods. According to the 10-year lifetime and 9.83 PWF (present worth factor) the optimum insulation thickness in natural gas heating for Tokat province is calculated as 0.050 m for XPS insulation material, 0.071m for EPS (expanded polystyrene) insulation material, 0.096m for GW (glass wool) insulation material, 0.092m for RW insulation material and 0.036 m for Polystyrene insulation material, respectively.

Özel et al. (2015) claimed that the LCA method, which is widely used in determining the optimum insulation thickness due to the increasing effects of global warming, will not be sufficient to determine the optimum insulation thickness, and calculated the optimum insulation thickness for natural gas heating in Bilecik province for a lifetime of 10 years with a method called the Entasy method, which calculates the environmental impact using the life cycle analysis of materials, processes and systems. In the study, GW and RW were selected as insulation materials. According to environmental impact analysis, optimum insulation thicknesses were determined as 0.15 m and 0.064 m, and according to the LCA method, 0.012 m and 0.007 m.

Güven (2019) used DD method and exergetic environmental impact factor to examine the optimum insulation thicknesses and the change in CO<sub>2</sub> emissions for the provinces of Antalya, Bursa, Isparta and Erzurum in different climatic zones of Türkiye in heating with natural gas fuel. The study was carried out according to GW and RW insulation materials. The optimum insulation thickness was determined as the lowest 0.43 m and the highest 0.11 m, the reduction in CO<sub>2</sub> emissions between the minimum 64% and the highest 92%, and the net ecological environmental savings between the lowest 345 mPts/m<sup>2</sup>.yr and 2249 mPts/m<sup>2</sup>.yr according to the insulation material type.

Aşıkoğlu (2023) determined the heat losses and gains from the roofs and walls for four different insulation materials in the provinces of Türkiye located in the 1st degree day region with the Builder Simulation program according to the insulation thicknesses. When energy savings and investment costs were evaluated according to the net present value method, it was seen that the use of GW insulation materials for the roof and RW insulation materials for the wall were the most efficient options.

Ozkan&Onan (2011) calculated the optimum insulation thicknesses, energy savings, payback periods, reduction in CO<sub>2</sub> and SO<sub>2</sub> emissions of buildings according to two different insulation materials and two different fuel types, using the P<sub>1</sub>-P<sub>2</sub> method for four different climate zones of Türkiye. In addition, it has shown that the increase in the percentage of glass area in buildings will cause a large increase in the optimum insulation thickness, revealing its disadvantage in terms of energy saving and emission of harmful gases.

Dombayci et al. (2017) calculated the energy requirement due to heat loss from the outer wall for four provinces located in different climate zones of Türkiye with DD method. Exergy analysis was used to calculate natural gas fuel consumption to cover heat losses. EPS and PU (polyurethane) made an economic analysis using LCA method for insulation materials and found optimum insulation thicknesses and energy savings. The optimum insulation thicknesses were calculated as between 2.3 cm and 10.7 cm depending on the type of insulation material, and energy savings were calculated as between 22% and 56% depending on the type of insulation material. The results showed that both the optimum insulation thickness and energy saving received the highest value in the province located in the colder climate zone.

Akan (2021) calculated the optimum insulation thicknesses, energy savings and payback periods for four different insulation materials for all provinces of Türkiye with DD and LCA methods for a life span of 10 years. Since the calculations contain many parameters and take time, it has developed 12 different models using regression analysis to make it easier to determine the optimum insulation thicknesses. Although the regression values of the models were found to be slightly lower than both heating and cooling only, they were over 96.8%, so they were successful in finding values close to the theoretical calculations. It has been suggested that the use of RW insulation material in all cities will be more appropriate.

Akan&Akan (2022) developed a model that predicts CO<sub>2</sub> emissions according to DD values and optimum insulation thicknesses according to four different insulation materials in all cities of Türkiye. It has been calculated that CO<sub>2</sub> emissions will be reduced by 66-76% and 46-69%, respectively, in heating-only and cooling-only application. It has been determined that the application of insulation will provide energy savings of 54.6-80.5% and 10.1-61.1%, respectively, in heating-only and cooling-only application.

Aktemur et al. (2021) calculated energy savings, payback periods and CO<sub>2</sub> emissions according to the optimum insulation thicknesses in natural gas heating application in all cities of Türkiye using XPS, EPS, GW and RW insulation materials. Researchers determined RW as the most advantageous insulation material and GW

insulation material as the most disadvantageous insulation material. The optimum insulation thicknesses were found to be between 0.07 m and 0.23 m, the total annual energy savings were 4.4-53.5 (\$/m<sup>2</sup>.year), the payback period was 0.11-0.38 years, and the CO<sub>2</sub> emissions were between 53.2%-94%.

Küçüktopçu&Cemek (2019) determined the optimum insulation thickness, energy savings, payback periods and CO<sub>2</sub> emissions for heating and heating-cooling application in laying hen houses. The study was conducted for ten provinces by selecting two different insulation materials and five different energy types.

In this study, optimum insulation thicknesses, payback periods, annual fuel savings and reduction in CO<sub>2</sub> emissions for XPS and RW insulation materials according to two different wall types in the heating and heating-cooling applications of Tokat province were calculated using DD and LCA methods. Although there are similar studies in the literature, most studies do not have CO<sub>2</sub> emission values. There is only one study on CO<sub>2</sub> emissions for Tokat province. The fact that the heating and cooling day values that change with the effect of global warming and the inflation, interest, unit price of insulation material and fuel prices that change according to the years using up-to-date data makes the study different from previous studies.

**Materials and Methods**

**The Properties of the Buildings Wall**

The wall design for the insulation application to be made on the outer wall of the building is shown in Figure 1. Two different thickness wall types were selected to study the optimum change of insulation thicknesses in the exterior wall with different thermal resistances. The thicknesses and thermal properties of the construction materials in the exterior wall design are given in Table 1.

Where, k is thermal conductivity, R is thermal resistance, R<sub>o</sub> is outside thermal resistance, R<sub>i</sub> is inside thermal resistance and R<sub>w</sub> is thermal resistance of wall layers in Table 1. In the literature, thermal resistances of the exterior wall were selected between 0.503-0.627 for Tokat province. (Kaynaklı, 2012; Kürekçi, 2016; Akan, 2021; Aktemur et al., 2021). In this study, the thermal resistance of two different wall structures was selected at 0.503 and 0.604 for Tokat province.

**The Properties of the Insulation Materials**

The thermal and economic values of XPS and RW insulation materials used in wall insulation are shown in Table 2.

**Degree day's method**

The Degree Day (DD) method, which is used to determine energy losses and gains in heating and cooling applications in building, is the sum of the difference between the preferred comfort temperatures and the outside temperatures over a certain period, usually between 14-18 °C for heating and 22-26 °C for cooling (Bulut at al., 2007; Bayram&Yeşilata, 2009).

The following equations were used to calculate Heating Degree Days (HDD) and Cooling Degree Days (CDD).

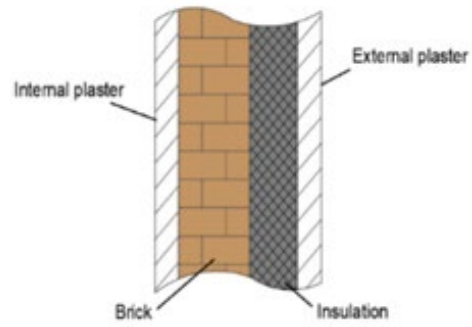


Figure 1. External wall structure (anonymous)

Table 1. Physical parameters of wall structure

WS	Thickness(m)		k (W m <sup>-1</sup> K <sup>-1</sup> )		R (m <sup>2</sup> K W <sup>-1</sup> )	
	Type I	Type II	Type I	Type II	Type I	Type II
IP	0.02	0.02	0.87	0.87	0.023	0.029
B	0.13	0.19	0.45	0.50	0.289	0.380
EP	0.03	0.03	1.40	1.40	0.021	0.025
R <sub>i</sub>	-	-	-	-	0.130	0.130
R <sub>o</sub>	-	-	-	-	0.040	0.040
R <sub>w</sub> *	-	-	-	-	0.503	0.604

WS Wall structure; IP: Internal plaster; B Bricks; EP: External plaster; \*(uninsulated)

Table 2. Features of insulation material

Insulation Material	k (W m <sup>-1</sup> K <sup>-1</sup> )	Price (\$/m <sup>3</sup> )
XPS	0.035	198
RW	0.039	309

\* İzocam Company

$$T_o < T_b$$

$$HDD = \sum_{i=1}^n (T_b - T_o) \tag{1}$$

$$T_o > T_b$$

$$CDD = \sum_{i=1}^n (T_o - T_b) \tag{2}$$

Where, T<sub>b</sub> is inside temperature, T<sub>o</sub> is outside temperature.

The average HDD and CDD values of the city of Tokat used in the study were accepted as 2068 and 171, respectively. These values were determined based on the base temperature of T<sub>b</sub> ≤ 15°C and T<sub>o</sub> > 22 °C recommended by Turkish State Meteorological Services (MGM,2024) between 2014 and 2023.

**Economic Analysis**

The heat loss per unit from the external building wall is given by,

$$q = U \cdot (T_b - T_o) \tag{3}$$

Where, U is the overall heat transfer coefficient. The annual heat loss per unit area can be calculated from,

$$q_a = 86400 \cdot DD \cdot U \tag{4}$$

Annual heating requirement per unit area of building wall,

$$E_{Ah} = \frac{86400 \cdot DD \cdot U}{\eta} \tag{5}$$

Annual cooling requirement per unit area of building wall,

$$E_{Ac} = \frac{86400 \cdot DD \cdot U}{COP} \quad (6)$$

Overall heat transfer coefficient for a typical building wall is given by,

$$U = \frac{1}{R_i + R_w + R_{ins} + R_o} \quad (7)$$

Where,  $R_i$  and  $R_o$  are convection resistances for inside and outside of the wall.  $R_w$  is the total thermal resistance of wall substances and  $R_{ins}$  is the thermal resistance of insulation material,

$$R_{ins} = x/k \quad (8)$$

Where,  $x$  and  $k$  are the thickness and thermal resistance of insulation material.

The cost of energy used in heating and cooling applications per unit area is calculated by the following equations.

Annual heating cost per unit area,

$$C_h = \frac{86400 \cdot HDD \cdot C_f}{\left[ (R_{wt} + \frac{x}{k}) \cdot H \cdot \eta \right]} \quad (9)$$

Annual cooling cost per unit area,

$$C_c = \frac{86400 \cdot CDD \cdot C_e}{\left[ (R_{wt} + \frac{x}{k}) \cdot COP \cdot \eta \right]} \quad (10)$$

In the study, the fuel price ( $C_f$ ), the lowest calorific value ( $H_u$ ), the fuel efficiency ( $\eta$ ) and the price ( $C_e$ ) of the electricity used in the cooling application and the cooling efficiency coefficient ( $COP$ ) of the natural gas used as fuel in building heating are given in Table 3.

Table 3. Characteristics of fuels

Fuel	$C_f$ (\$/m <sup>3</sup> )	$H_u$ (J.m <sup>-3</sup> )	COP	$\eta$ (%)
N. Gas	0.26*	345.18 105	-	90
Electricity	1.98**	-	2.5	-

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In calculating the annual energy cost, it is necessary to determine the present worth factor;

If  $i > g$  then

$$r = \frac{i-g}{1+g} \quad (11)$$

If  $i < g$  then

$$r = \frac{g-i}{1+i} \quad (12)$$

$$PWF = \frac{(1+r)^N - 1}{r \cdot (1+r)^N} \quad (13)$$

Where,  $i$  is interest rate,  $g$  is inflation and  $N$  is lifetime.

The inflation rate, interest rate and present value factor ( $PWF$ ) calculated for the 10-year lifetime of the insulation material are given in Table 4.

Table 4. Financial Parameters

% Inflation (g)	% Interest (i)	PWF
61.78*	60*	9.4

\*TCMB

The cost of insulation per unit area

$$C_i = C_l \cdot x \quad (14)$$

Where,  $C_l$  is the cost of the insulation material and  $x$  is the insulation thickness

The total heating cost of an insulated wall as per the life cycle analysis

$$C_{t,h} = C_h \cdot PWF + C_l \cdot x \quad (15)$$

Both heating and cooling cost of an insulated wall as per the life cycle analysis

$$C_{t,h,c} = C_h \cdot PWF + C_c \cdot PWF + C_l \cdot x \quad (16)$$

Total energy savings and payback period for buildings both heated and cooled are calculated with the following equations.

Optimum insulation thickness minimizing the total both heating and cooling;

$$x_{opt-h} = 293,94 \cdot \sqrt{\frac{HDD \cdot PWF \cdot k \cdot C_f}{C_l \cdot H \cdot \eta} - k \cdot R_{wt}} \quad (17)$$

$$x_{opt-hc} = 293,94 \cdot \sqrt{\frac{HDD \cdot PWF \cdot k \cdot C_f}{C_l \cdot H \cdot \eta} + \frac{CDD \cdot PWF \cdot k \cdot C_e}{C_l \cdot COP} - k \cdot R_{wt}} \quad (18)$$

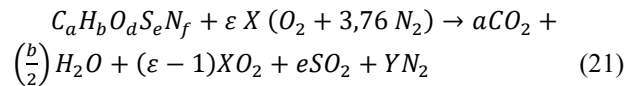
$$A_{years} = C_{t,pre-ins} - C_t \quad (19)$$

$$PP = \frac{C_i}{C_{t,pre-ins} - C_t} \quad (20)$$

### Emission Analysis

CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O and fluorinated gases are directly called greenhouse gases. Million-ton CO<sub>2</sub> equivalent of direct greenhouse gases in Türkiye increased by 157.1% in 2021 compared to 1990. 85.2 % of total CO<sub>2</sub> emissions originated from the energy sector in 2021. CO<sub>2</sub> emissions due to electricity and heat generation in the energy sector are 32.7% (TUIK, 2021).

The general combustion equation of fuels used to obtain heat energy is given in the equation below.



The X and Y coefficients given in the general combustion equation are found by equations (22) and (23) below.

$$X = \left( a + \frac{b}{4} + e - \frac{d}{2} \right) \quad (22)$$

$$Y = \left( 3,76 \varepsilon X + \frac{f}{2} \right) \quad (23)$$

The molecular weight of the fuel,

$$M = 12a + b + 16d + 32e + 14f \quad (24)$$

Annual fuel consumption,

$$M_f = \frac{86400 \cdot HDD}{(R_{wt} + R_{ins}) \cdot \eta \cdot H} \quad (25)$$

Total annual CO<sub>2</sub> emissions due to fuel consumption,

$$M_{CO_2} = \frac{44a}{M} M_f \quad (26)$$

**Result and Discussion**

Optimum insulation thicknesses, energy savings, payback periods and reduction in CO<sub>2</sub> emissions calculated only in heating and heating and cooling application according to the type of insulation material in Tokat province are given in Table 5.

Table 5. Optimum insulation thickness, payback period, energy saving and reduction in CO<sub>2</sub> emissions compared to the insulation material of Tokat province.

Insulation material	Wall Type	X <sub>opt-h</sub> (m)	X <sub>opt-hc</sub> (m)	A <sub>year-h</sub> (%)	A <sub>year-hc</sub> (%)	PP <sub>h</sub> (years)	PP <sub>hc</sub> (years)	CO <sub>2(h)</sub> (%)	CO <sub>2(hc)</sub> (%)
XPS	Type I	0.032	0.071	41.838	64.28	5.133	2.32	64.57	80.46
	Type II	0.029	0.068	33.166	58.06	6.922	2.94	57.77	76.28
RW	Type I	0.023	0.055	28.544	54.55	8.194	3.33	53.98	73.69
	Type II	0.019	0.051	19.426	47.07	11.928	4.30	44.65	68.49

Table 6. Comparison with other studies in the literature for Tokat province

Parameters	Present study Type I/ Type II	Kaynaklı (2012)	Kürekci (2016)	Akan (2021)	Aktemur et al. (2021)
R <sub>wt</sub>	0.503/0.604	0.617	0.503	0.604	0.627
HDD	2068	2399	2399	2060	2150
CDD	171	5	97	106	-
η (N.Gas)	0.90	0.93	0.90	0.90	0.90
C <sub>f</sub> (\$ m <sup>-3</sup> )	0.26	0.386	0.385	0.232	0.367
C <sub>e</sub> (\$ kWh <sup>-1</sup> )	1.98	0.132	-	0.0912	-
C <sub>1</sub> XPS (\$ m <sup>-3</sup> )	198	-	180	150	93.921
C <sub>1</sub> RW (\$ m <sup>-3</sup> )	309	-	80	70	131.193
C <sub>1</sub> PS (\$ m <sup>-3</sup> )	-	80	-	-	-
COP	2.5	2.5	2.5	2.5	-
N	10	20	10	10	10
PWF	9.400	15.100	9.830	9.640	8.759
k					
XPS	0.035	-	0.031	0.030	0.031
RW	0.039	-	0.040	0.040	0.040
PS	-	0.035	-	-	-
X <sub>opt-h</sub> (m)					
XPS	0.032/0.029	-	0.050	0.032	0.06
RW	0.023/0.019	-	0.092	0.061	0.05
PS	-	0.107	-	-	-
X <sub>opt-hc</sub> (m)					
XPS	0.071/0.068	-	0.052	0.034	-
RW	0.055/0.051	-	0.094	0.064	-
PS	-	0.107	-	-	-
A <sub>year-h</sub>					
XPS	1.244/0.821	-	-	1.399	18
RW	0.849/0.481	-	-	1.570	14
A <sub>year-hc</sub>					
XPS	6.064/4.561	-	-	1.530	-
RW	5.146/3.698	-	-	1.828	-
A <sub>year-h</sub> (%)					
XPS	41.837/33.166	-	-	-	-
RW	28.543/19.425	-	-	-	-
A <sub>year-hc</sub> (%)					
XPS	64.28/58.06	-	-	-	-
RW	54.55/47.07	-	-	-	-
PS	-	83.2	-	-	-
PP <sub>h</sub> (year)					
XPS	5.133/6.922	-	-	3.431	0.30
RW	8.184/11.928	-	-	2.720	0.45
PP <sub>hc</sub> (year)					
XPS	2.32/2.94	-	-	3.333	-
RW	3.33/4.30	-	-	2.451	-
PS	-	6.89	-	-	-
Heating CO <sub>2</sub> (%)					
XPS	64.57/57.77	-	-	-	-
RW	53.98/44.65	-	-	-	-
Heating/Cooling CO <sub>2</sub> (%)					
XPS	80.46/76.28	-	-	-	74
RW	73.69-68.49	-	-	-	66

Optimum insulation thicknesses, energy savings, and reduction in CO<sub>2</sub> emissions were found to be higher in Type I wall type of both insulation materials compared to Type II wall type, while payback periods were calculated to be lower. In the heating-cooling application of the same insulation material and wall type, optimum insulation thicknesses, energy savings, reduction in CO<sub>2</sub> emissions are quite high and the payback period is very low compared to the heating application alone. For this reason, it will be advantageous to prefer the Type I wall type with XPS insulation material, which has a low payback period and a high reduction in CO<sub>2</sub> emissions, in buildings where cooling is expected to be used effectively in terms of comfort as well as in heating applications.

The comparison between this study and four similar studies in the literature for Tokat province is shown in Table 6.

Optimum insulation thicknesses vary due to the differences in thermal and economic parameters. In addition, since the equations used to calculate the payback period and energy savings are similar in some studies and differ in others, a wide variety of results have been obtained. In terms of some of the thermal parameters ( $HDD$  and  $R_{w,t}$ ), the most similar study to the Type II wall type of this study is the study of Akan (2021). In the heating application alone, the optimum insulation thickness of the XPS insulation material was calculated as 0.029 m in this study and the Akan (2021) was calculated as 0.32 m. The optimum insulation thickness of the RW insulation material was found to be 0.019 m in this study and 0.061 m in Akan (2021)'s study. The fact that there is a large difference in the unit price of RW insulation material between the two studies shows that economic parameters cause large differences in the optimum insulation thickness.

As the price of insulation material increases, the optimum insulation thickness decreases, and according to Kaynaklı (2013)'s study, our study has shown similar results.

## Conclusion

According to the two different wall thermal properties of XPS insulation material, the optimum insulation thicknesses are calculated as 0.032 -0.029 m, annual savings are 41.83%-33.17% and payback periods are 5.13-6.92 years, respectively, in heating application.

The optimum insulation thickness for heating-cooling application was found to be 0.071-0.068 m, annual savings were 64.28%-58.06% and payback periods were 2.32-2.94 years.

According to the two different wall thermal properties of the RW insulation material, the optimum insulation thicknesses for heating application were determined as 0.023-0.019 m, annual savings as 28.54%-19.42%, payback periods as 8.19-11.93 years.

The optimum insulation thicknesses for the heating-cooling application of RW insulation material were 0.055-0.051m, annual savings were 54.55%-47.07%, payback periods were 3.33-4.30 years.

The reduction in CO<sub>2</sub> emission in the heating-only application of XPS insulation material was 64.57%-57.77%, and the reduction in CO<sub>2</sub> emission in heating-only application of RW insulation material was 53.98%-44.65%.

The reduction in CO<sub>2</sub> emission for both heating and cooling application was 80.46%-76.28% for XPS and 73.69%-68.49% for RW.

Since the payback period and CO<sub>2</sub> emission were calculated with a lower value in the use of XPS insulation material than in RW insulation material, it has been determined that it is more appropriate to prefer XPS insulation material in terms of economy and environment only in heating and heating-cooling application in the province of Tokat.

## Declarations

### Author Contribution Statement

Yunus Kültürel: Conducted the study, data collection, review, formal analysis, and writing the original draft.

Lütfullah Dağkurs: Management, supervision, review, editing, and calculations of the study.

### Conflict of Interest

"We declare that the authors have no conflict of interest."

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## Effects of EC, Mycorrhiza and Vermicompost Applications on Tomato (*Solanum Lycopersicum* L.) Seedling Development

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 12.10.2024 Accepted : 28.11.2024</p> <p><b>Keywords:</b> Ec Mycorrhiza Seedling Tomato Vermicompost</p>	<p>The tomato is one of the most significant vegetable species cultivated globally. In both field and greenhouse tomato cultivation, seedlings are typically employed as the initial planting material, rather than seeds. This study aimed to examine the impact of varying doses of mycorrhiza, vermicompost, and fertiliser on the growth of tomato seedlings. The Cuma F1 tomato variety was utilised in the investigation. The study was conducted in accordance with the coincidence plots experimental design, with three replications. In the study, different doses of vermicompost (0, 10 and 20 %) with EC 0.5-1.00 with and without mycorrhizae were applied to 70% peat and 30% perlite media for tomato seedling cultivation. The seedlings were uprooted in one and a half month. In this study, the following morphological (seedling height, hypocotyl length, stem diameter, number of leaves, leaf wet weight, leaf dry weight, root wet weight and root dry weight) characteristics of tomato plants were investigated. As a result of the study, the presence of vermicompost and mycorrhiza treatments in the medium caused significant differences in many parameters. It was observed that seedling quality improved at full fertiliser doses (EC1) and that the addition of vermicompost to the medium had a positive effect on seedling development at low fertiliser doses (EC 0.5).</p>

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## EC, Mikoriza ve Vermikompost Uygulamalarının Domates (*Solanum Lycopersicum* L.) Fidesi Gelişimine Etkileri

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 12.10.2024 Kabul : 28.11.2024</p> <p><b>Anahtar Kelimeler:</b> Domates Ec Fide Mikoriza Vermikompost</p>	<p>Domates, dünyada üretilen en önemli sebze türlerinden birisidir. Hem tarla hemde serada domates yetiştiriciliğinde başlangıç materyali olarak genellikle tohumdan ziyade fide kullanılmaktadır. Bu çalışmanın amacı, farklı dozlarda mikoriza, vermicompost ve gübre uygulamalarının domates fidelerinin gelişimi üzerindeki etkisini araştırmaktır. Çalışmada Cuma F1 domates çeşidi kullanılmıştır. Araştırma, tesadüf parselleri deneme desenine uygun olarak 3 tekerrürlü olarak gerçekleştirilmiştir. Çalışmada, domates fidesi yetiştiriciliği için %70 torf %30 perlit karışımı olan ortamlara EC 0.5-1.00 ile hem mikorizalı hemde mikorizasız şekilde farklı oranlarda vermicompost (% 0, 10 ve 20) dozları uygulanmıştır. Fideler bir buçuk ayda sökülümü yapılmıştır. Bu çalışmada, domates bitkilerinin şu morfolojik (fide boyu, hipokotil uzunluğu, gövde çapı, yaprak sayısı, yaprak yaş ağırlığı, yaprak kuru ağırlığı, kök yaş ağırlığı ve kök kuru ağırlığı) özellikleri incelenmiştir. Çalışma sonucunda, vermicompost ve mikoriza uygulamalarının ortamda bulunması birçok parametre bakımından önemli farklar oluşturmaktadır. Tam gübre (EC1) dozlarında fide kalitesinin arttığı ve düşük gübreleme (EC 0.5) dozlarında ise ortama vermicompost ilave edilmesinin fide gelişimini olumlu yönde etkilediği görülmüştür.</p>

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## Giriş

Domates (*Solanum lycopersicum*), *Solanaceae* familyasının bir türüdür ve ülkemizde yaygın olarak yetiştirilen ve tüketilen sebzedir (Şa ve ark., 2023). Türkiye, yıllık 31.7 milyon tonluk sebze üretimiyle dünyanın dördüncü sebze üreticisidir. Toplam sebze üretiminin yaklaşık % 40'ını domates oluşturmaktadır (Tüik, 2022). Yaklaşık olarak 100 işletme ve tesiste çeşitli yollarla işlenen ve ürün haline getirilen domates, insan beslenmesinde vazgeçilmez ürünler arasında yerini almaktadır (Ertürk ve Çirka, 2015). Domates bitkisinde ya doğrudan tohum ekimi yada fide dikimi yoluyla yetiştiricilik yapılmaktadır (Şa ve ark., 2023). Doğrudan tohum ekimine göre fide yetiştiriciliği, daha hızlı büyüme, daha fazla verim, kök hastalıklarına karşı daha fazla dayanıklılık ve daha az işgücü gereksinimi gibi bir dizi avantaj sunmaktadır (Özer ve Kandemir, 2016; Yılmaz ve ark., 2018; Demir ve ark., 2020; Tüzel ve ark., 2021). Bitki köklerinin büyüme ve gelişme süresinin kısalığı ve bu büyümenin gerçekleştiği ortamın sınırlı hacmi göz önünde bulundurulduğunda, gübre uygulamalarına fide üretiminde çok dikkat edilmesi gerekmektedir (Şa ve ark., 2023). Azotlu gübreleme, fide büyümesini etkileyen en önemli faktördür. Önceki araştırmalar, bitkiler tarafından azotun hızlı bir şekilde emilmesinin domates fidelerinin yaş ve kuru ağırlıklarını belirgin bir şekilde artırdığını göstermiştir (Tüzel ve ark., 2021). Fide yetiştiriciliğinde ilave gübre kullanımı, yetiştiriciler için önemli bir maliyet unsurudur. Ancak organik gübre kullanımı, hem girdi maliyetlerini azaltma hem de fide kalitesini artırma potansiyeli sunmaktadır (Şa ve ark., 2023). Organik gübrelerin bitki besin elementleri sağlaması, toprağın yapısını iyileştirmesi ve çevresel sürdürülebilirliği desteklemesi nedeniyle fide yetiştiriciliğinde önem kazanmaktadır. Farklı sebze türleri üzerine yapılan çalışmalarda, fide büyümesi ve kalitesi üzerine organik gübrelerin kullanılmasının önemli bir etkiye sahip olduğu bildirilmiştir (Yedidia ve ark., 2001; Bal ve Altıntaş, 2008; Azarmi ve ark., 2011; Kumar, 2017; İkiz, 2019). Bitkisel-hayvansal atıklardan oluşan organik gübreler, doğada çözünebilir, sürdürülebilir ve bünyesinde değişen miktarlarda azot, fosfor, potasyum ve ayrıca diğer besin maddeleri bulunduran gübrelerdir. Organik gübreler, makro ve mikro besin maddeleri sağlamanın yanı sıra mikrobiyolojik aktiviteyi hızlandırarak toprağın yapısını, havalanmasını ve su tutma kapasitesini artırması gibi toprak üzerinde çok yönlü olumlu etkilere sahiptir (Ulus ve Yavuzaslanoğlu, 2017). Çok çeşitli organik gübreler bulunmaktadır. Bunlar içerisinde vermikompost (Solucan gübresi), toprağın biyolojik yapısını destekleyen ve hareketlilik sağlayan farklı bakteri (*Azotobacter*) (Blair ve ark., 1997; Cortez ve ark., 2000; Amador ve Görres, 2005; Amador ve ark., 2006; McDaniel ve ark., 2013) ve mikoriza mantarları içermektedir (Kızılkaya, 2008). Vermikompostta bulunan mikroorganizmalar, toprakta bulunan ve bitki tarafından kolayca alınmayan besin maddelerinin parçalanmasını kolaylaştırır ve böylece bunları bitki tarafından kullanılabilir bir forma dönüştürür (Ulus ve Yavuzaslanoğlu, 2017). Vermikompost, solucanlar tarafından salgılanan ve bitkinin büyümesini destekleyen oksin, sitokinin ve gibberellin gibi bir dizi hormon içerir (Nagavallema ve ark., 2004). Ayrıca, solucan gübresinde enzimler, vitaminler, amino asitler ve büyüme hormonları

dahil olmak üzere biyomoleküllerin varlığının, olumsuz çevre koşullarına maruz kalan bitkilerde daha hızlı büyüme ve dayanıklılığı artırdığı gözlemlenmiştir (Demir ve ark., 2010). Çirka ve ark. (2022) yapılan bir çalışmada, buğday bitkisinde artan dozlarda vermikompos uygulamalarının vejetasyon sıcaklığı hariç diğer tüm incelenen parametreler üzerinde kayda değer bir artış gösterdiği tespit edilmiştir. Erşahin ve ark. (2017) yılında solucan gübresi uygulamalarının domates bitkilerinin büyümesi üzerindeki etkisini inceledikleri çalışmada, ortama % 20 oranında solucan gübre uygulamasının büyüme teşvik ettiği bildirilmiştir. Mikoriza, belirli bitki türlerinin kök sistemleriyle mutualistik bir ilişki kuran mantar türleri için kullanılan bir terimdir. Bu yapıların varlığı mikroskopik inceleme ile tespit edilebilir ve yüksek hif üretimleri ile diğer mantar üreme biçimlerinden ayrılırlar (Ortaş, 1997). Bitkilerin yaklaşık %95'inde görülen bitki köklerindeki mikorizal enfeksiyonun, besin eksikliği olan topraklarda bitki büyümesini desteklediği kanıtlanmıştır (Ceylan ve ark., 2016). Mikorizalar, bitkiler tarafından alınmayan fosforu, bitkiler tarafından rizosferden dokularına alınmasını kolaylaştırmaktadır (Ulus ve Yavuzaslanoğlu, 2017). Dahası, topraktaki diğer iyonların birleşmesiyle oluşan trikalsiyum fosfat gibi tuzlar da mikoriza yoluyla bitki tarafından kullanılabilir (Ulus ve Yavuzaslanoğlu, 2017). Mikorizanın hif yapısı, toprağa üstün fiziksel özellikler kazandırarak, özellikle kurak koşullarda bitkilerde su kullanım etkinliğinin artmasını kolaylaştırmaktadır (Soyergin, 2003). Mikoriza üzerine yapılan çalışmalarda, mikorizanın bitkiler tarafından besin ve su alımını kolaylaştırdığı, fide gelişimini artırdığı ve köklerin ömrünü uzatarak yeni çimlenen tohumların hayatta kalmasını sağladığı (Harley ve Smith, 1983; Malajczuk ve ark., 1992), ve ayrıca yapılan bir başka çalışmada, mikoriza kullanımının kaliteli fide üretimiyle sonuçlandığı ve bunun da verim üzerinde olumlu bir etkisi olduğu bildirilmiştir (Dinç ve ark., 1978). Bu çalışmada, farklı dozlarda vermikompost, mikoriza ve kimyasal gübre uygulamalarının domates fidesinin büyüme ve gelişim parametreleri üzerine etkileri araştırılmıştır.

## Materyal ve Yöntem

Çalışma, 2021 yılında Tokat Gaziosmanpaşa Üniversitesi'nde ısıtmalı serada kekkila torfu kullanılarak 216'lık fide yetiştirme viyollerinde yapılmış olup torf ve vermikompost özellikleri Çizelge 1'de gösterilmiştir. Her uygulama konusuna bir viyol domates tohumu ekilmiş ve viyollerin kenar sırası kenar tesiri olarak kabul edilmiş, viyoller 3 eşit şekilde bölünüp ve 3 tekerrür olmak üzere her tekerrürde 10 bitki üzerinde ölçüm yapılmış ortalama değerleri dikkate alınmıştır.

Fidelerin gübrenmesi için kullanılan besin çözeltisi, mikro elementlerle birlikte 100 ppm N<sup>-3</sup>, 50 ppm P<sup>-3</sup>, 100 ppm K<sup>+</sup>, 100 ppm Ca<sup>+2</sup>, 50 ppm Mg<sup>+2</sup> ve 50 ppm S<sup>-2</sup> içermektedir. Çalışma, tam gübre (EC 1) ve yarım gübre (EC 0,5) dozu ile farklı vermikompost dozlarının besleme koşulları üzerindeki etkisini araştırmayı ve gübre dozu ile vermikompost dozları arasındaki ilişkiyi incelemeyi amaçlamıştır. EC uygulamaları ve her hafta için karşılık gelen dozları Çizelge 2'de gösterilmiştir.



Çizelge 1. Sphagnum torfu ve vermikompost özellikleri  
Table 1. Properties of sphagnum peat and vermicompost

Sphagnum Torfu	
Özellikler	Değerler
Organik madde (%)	95
pH	5,5
EC (dS m <sup>-1</sup> )	2,5
Azot (mg l <sup>-1</sup> )	140
Fosfor (mg l <sup>-1</sup> )	160
Potasyum (mg l <sup>-1</sup> )	180
Magnezyum (mg l <sup>-1</sup> )	10
Silisyum (mg l <sup>-1</sup> )	187
Demir (mg l <sup>-1</sup> )	0,9
Mangan (mg l <sup>-1</sup> )	1,6
Bor (mg l <sup>-1</sup> )	0,3
Çinko (mg l <sup>-1</sup> )	0,4
Bakır (mg l <sup>-1</sup> )	1,5
Molibden (mg l <sup>-1</sup> )	0,5
Vermikompost	
Organik madde (%)	64
pH	7,5
EC (dS m <sup>-1</sup> )	2,15
C/N	18,71
Azot (mg l <sup>-1</sup> )	1,25
Fosfor (mg l <sup>-1</sup> )	0,55
Potasyum (mg l <sup>-1</sup> )	1,02

Çizelge 2. EC uygulamalarının haftalara göre dağılımı  
Table 2. Distribution of EC applications by week

Uygulamalar	Haftalar				
	1.	2.	3.	4.	5.
EC 1 (dS m <sup>-1</sup> )	-	1,2	1,4	1,6	1,9
EC 0.5 (dS m <sup>-1</sup> )	-	0,92	1,02	1,12	1,22

Domates bitkisinde gerçek yapraklar görülmeye başladıktan sonra EC 1,4 ve 1,8 mmhos/cm ile muamele edilmiştir. Mikoriza uygulaması, bitki kökleriyle simbiyotik etkileşime girebilen Endo Roots Soluble (ERS) ticari mikoriza kullanılarak gerçekleştirilmiştir. Mikoriza BioGlobal şirketinden satın alınmış ve 5000 mg/7kg konsantrasyonda hazırlanmıştır. Mikoriza daha sonra ekimden dört gün sonra her bir viyolaya 50 ml saf su ile uygulanmıştır. Uygulamaların fideler üzerindeki etkisini belirlemek için, biber fideleri ortalama 1,5 içerisinde toprak üstü kısımlarından kesilip gözlemler yapılmıştır. Çalışmada yapılan gözlemler; fide boyu: Yetiştirme ortamının yüzeyinden en uzun yaprağın ucuna kadar ölçülmüştür. Hipokotil boyu: Kök boğazından kotiledon yapraklarına kadar olan mesafe ölçülerek belirlenmiştir. Gövde çapı: Fidelerin kök boğazı üzerinden dijital bir kumpas aracılığıyla belirlenmiştir. Yaprak sayısı: Hasat işleminden önce yapılmıştır. Hasat işleminin ardından fideler distile su ile temizlenmiş ve kısa bir süre oda sıcaklığında bekletilmiş ve ağırlıklar hassas terazi (0,01 g hassasiyet) kullanılarak belirlenmiştir. Toprak üstü kısımlar ve kökler ayrı ayrı tartılmış ve ağırlık sabit kalana kadar 65 °C'de bekletilmiştir. Son olarak, yeşil aksam ve kökler kurumaya bırakılmıştır. Araştırma sonunda, veriler SPSS 20.0 yazılım paketi kullanılarak analiz edilmiş ve ortalamaları %1-5 düzeyinde karşılaştırmak için Duncan'ın çoklu karşılaştırma testi kullanılmıştır.

## Bulgular ve Tartışma

Araştırmada, vermikompost, mikoriza ve gübre uygulamalarının domates fidelerinin büyümesi üzerindeki etkisi Çizelge 3, 4, 5, 6, 7, 8, 9 ve 10'da sunulmuştur.

Domates bitkisinin fide boyu üzerine, Ec, Ec+vermikompost ve Ec+mikoriza+vermikompost interaksiyonları arasında %1 düzeyinde önemli bir fark olduğu, Ec+mikoriza ve mikoriza+vermikompost uygulamalarında ise fide boyu üzerine farkın istatistiksel olarak önemsiz olduğu görülmektedir (Çizelge 3). Fide boyu açısından farkın istatistiksel olarak önemsiz olduğu görülmektedir. Bu bulgular, EC ile vermikompostun sinerjik bir etki yaratarak fide boyunu artırabileceğini, ancak mikoriza uygulamalarının bu etkiyi değiştirmediğini göstermektedir. En yüksek fide boyu 11,25 cm ile %0 vermikompost uygulamasında, en düşük fide boyu 10,91 cm ile %20 vermikompost uygulamasında gözlenmiştir. Jeevitha ve ark. (2019) yılında domates fidesi üzerine organik (toprak, çiftlik gübresi, vermikompost) ve inorganik (kum) gübrelerle yaptıkları çalışmada, %75 vermikompost + %25 çiftlik gübresi uygulamasından en iyi fide boyu (19,80 cm) elde etmişlerdir. Şa ve ark. (2023) yılında domateste fide kalitesi üzerine yaptıkları çalışmanın sonucunda, en iyi fide boyu 16,5 cm ile kül solüsyon uygulamasından elde edilmiştir. Yılmaz ve ark. (2017) yılında domates fide kalitesi üzerine yaptıkları çalışmada, torf, zeolit, vermikompost ve bunların farklı karışımlarını kullanmışlardır. Çalışma sonunda, en düşük fide boyu 4,88 cm ile %100 zeolit uygulamasından en yüksek fide boyu 23,18 cm ile %65 torf + %15 zeolit + %20 vermikompost uygulamasından elde edilmiştir. Benzer şekilde Çelebi, (2019) domates, biber ve hıyar yetiştiriciliğinde sera koşullarında çeşitli substratlarla (torf, perlit, tınlı toprak+perlit) yaptığı çalışmada, 4,5 ile 9,8 cm arasında değişen fide boylarıyla en iyi sonucu torf ortamının verdiğini bildirmiştir. Bizim çalışmamızda elde edilen bulgular, Olympios (1992), Kreen ve ark. (2002) ve Şirin ve ark. (2010) tarafından yapılan çalışmalarla uyumludur.

Domates bitkisi hipokotil boyu üzerine yapılan analizlerde, Ec uygulamasında %0,05, Ec+vermikompost uygulamasında %0,01 düzeyinde anlamlı farklılık gösterirken, diğer uygulamalar arasında herhangi bir fark görülmemiştir (Çizelge 4). En yüksek hipokotil uzunluğu 6,40 cm ile %10 Ec+vermikompost dozunun EC 1 uygulamasında elde edilmiştir. EC 0.5 dozunda ise 6,00 cm hipokotil uzunluğu ile %0 Ec+vermikompost uygulamasından elde edilmiştir (Çizelge 4). Tüm uygulamalar arasında 5,97 cm ile Ec+mikoriza+vermikompost uygulamasının %10 vermikompost dozunda en iyi sonuç alınmıştır.

Domates fidelerinin gövde çapı üzerine, Ec+vermikompost ve Ec+mikoriza+vermikompost dozlarında P<0.01 düzeyinde bir fark oluşmuş ancak diğer uygulamalar arasında ise önemli bir fark gözlenmemiştir. Uygulamalar arasında en yüksek gövde çapı 4,62 mm ile %10 Ec+vermikompost dozunun Ec 1 uygulamasından, EC 0.5 dozunda ise en yüksek gövde çapı 4,42 mm ile %20 vermikompost+mikoriza uygulamasında tespit edilmiştir. Ec+mikoriza+vermikompost uygulamasında, en iyi sonuç 4,37 mm gövde çapı ile %20 vermikompost uygulamasında gözlenmiştir (Çizelge 5).

Çizelge 3. Domates fidelerinde uygulamaların fide boyu (cm) üzerindeki etkileri

Table 3. Effects of applications on seedling length (cm) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V**	M+Vöd	EC 1		EC+V**	M+Vöd	EC+M+V**
	Mikoriza (-)	Mikoriza (+)			Mikoriza (-)	Mikoriza (+)			
0	11,63	13,13	12,38a	11,17	10,70	9,53	10,12b	11,33	11,25a
10	11,87	10,30	11,08b	11,58	11,30	11,30	11,30a	10,80	11,19a
20	12,70	9,80	11,25b	11,32	9,93	11,20	10,57ab	10,50	10,91b
Ec+mikoriza öd	12,07	11,08			10,64	10,68			
Ec**	11,57a				10,67b				

(EC × MKRZ : öd EC × VRMKST : \*\* MKRZ × VRMKST : öd EC × MKRZ × VRMKST : \*\*); öd : önemsiz \* : % 1 düzeyinde önemli (P≤0,01).

Çizelge 4. Domates fidelerinde uygulamaların hipokotil boyu (cm) üzerindeki etkileri

Table 4. Effects of applications on hypocotyl length (cm) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V**	M+Vöd	EC 1		EC+V**	M+Vöd	EC+M+Vöd
	Mikoriza (-)	Mikoriza (+)			Mikoriza (-)	Mikoriza (+)			
0	5,57	6,43	6,00a	5,85	6,13	5,67	5,90b	6,05	5,95
10	5,47	5,60	5,53b	6,00	6,53	6,27	6,40a	5,93	5,97
20	5,80	5,77	5,78b	5,82	5,83	6,07	5,95b	5,92	5,87
Ec+mikoriza öd	5,61	5,93			6,17	6,00			
Ec*	5,77b				6,08a				

(EC × MKRZ : öd EC × VRMKST : \*\* MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd) öd : önemsiz \* : % 5 düzeyinde önemli (P≤0,05). \*\* : % 1 düzeyinde önemli (P≤0,01).

Çizelge 5. Domates fidelerinde uygulamaların gövde çapı (mm) üzerindeki etkileri

Table 5. Effects of applications on stem diameter (mm) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V**	M+Vöd	EC 1		EC+V**	M+Vöd	EC+M+V**
	Mikoriza (-)	Mikoriza (+)			Mikoriza (-)	Mikoriza (+)			
0	3,47	4,93	4,20b	3,95	4,43	3,90	4,17b	4,42	4,18b
10	3,80	3,93	3,87b	4,28	4,70	4,47	4,62a	4,20	4,24ab
20	4,80	4,03	4,42a	4,35	3,90	4,73	4,32b	4,38	4,37a
Ec+mikoriza öd	4,02	4,30			4,37	4,37			
Ec öd	4,16				4,37				

(EC × MKRZ : öd EC × VRMKST : \*\* MKRZ × VRMKST : öd EC × MKRZ × VRMKST : \*\*); öd : önemsiz \* : % 5 düzeyinde önemli olduğunu gösterir (P≤0,05). \*\* : % 1 düzeyinde önemli (P≤0,01).

Çizelge 6. Domates fidelerinde uygulamaların yaprak sayısı (adet) üzerindeki etkileri

Table 6. Effects of applications on the number of leaves in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+Vöd	M+Vöd	EC 1		EC+Vöd	M+Vöd	EC+M+Vöd
	Mikoriza (-)	Mikoriza (+)			Mikoriza (-)	Mikoriza (+)			
0	4,33	4,67	4,50	4,33	4,33	4,00	4,17	4,33	4,33
10	4,67	4,00	4,33	4,67	4,67	4,67	4,67	4,33	4,50
20	5,00	4,00	4,50	4,50	4,00	4,00	4,00	4,00	4,25
Ec+mikoriza öd	4,67	4,22			4,33	4,22			
Ec öd	4,45				4,28				

(EC × MKRZ : öd EC × VRMKST : öd MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd); öd : önemsiz

Yılmaz ve ark. (2017) domates fide kalitesi üzerine yaptıkları çalışmada, torf, zeolit, vermikompost ve bunların farklı karışımlarını kullandıkları çalışma sonucunda, en düşük gövde çapı 1,51 mm ile %100 zeolit uygulamasından en yüksek gövde çapı 2,93 mm ile %80 peat + %20 solucan gübresi uygulamasından elde edilmiştir. Alagöz ve Özer, (2017), domates fidelerinin kalitesi üzerine yaptıkları çalışmada, fideler toprak ve kompostlanmış çiftlik gübresi içeren bir harçta yetiştirilmiştir. Çalışmada, en yüksek gövde çapının (5,2 mm) organik olarak üretilen fidelerde gözlemlendiği, ticari fidelere göre organik olarak yetiştirilen fidelerin kalitesinin daha yüksek olduğunu belirtmişlerdir. Şa ve ark. (2023) yılında fide kalitesi üzerine domates ile yaptıkları araştırmada, en yüksek gövde çapı 3,2 mm kimyasal gübre uygulamasından, en düşük gövde çapı 2,7 mm ile kül uygulamasından tespit etmişlerdir. Buna ek olarak,

vermikompost uygulamalarının çilek, biber ve domates bitkilerinde sürgün uzunluğu, yaprak alanı ve pazar değerini artırdığı bildirilmiştir (Arancon ve ark., 2003).

Uygulamalar arasında domates bitkilerindeki yaprak sayısında istatistiksel olarak önemli bir fark gözlenmemiştir (Çizelge 6). Tüm uygulamalara bakıldığında ise yaprak sayısı 4,50-4,25 adet arasında olduğu görülmektedir. Şa ve ark. (2023) yılında domateste fide kalitesi üzerine yaptıkları çalışmada, yaprak sayısını 4,3-6,0 adet arasında sırasıyla kontrol ve kimyasal gübre uygulamasından elde edilmiştir. Fadilloğlu, (2022) yılında domates, biber ve patlıcan bitkileri üzerinde farklı ortamların denendiği bir çalışmada, en yüksek yaprak sayısı (4,38 adet) çiftlik gübresi+torf ortamından, en düşük yaprak sayısı (2,82 adet) çiftlik gübresi+zeolit ortamından elde edilmiştir. Elde edilen sonuçlar yapılan farklı çalışmalarla benzerlik göstermektedir.

Çizelge 7. Domates fidelerinde uygulamaların yaprak yaş ağırlığı (g) üzerindeki etkileri

Table 7. Effects of applications on leaf fresh weight (g) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V **	M+V öd	EC 1		EC+V **	M+V öd	EC+M+V **
	Mikoriza (-)	Mikoriz a (+)			Mikoriza (-)	Mikoriza (+)			
0	29,00	50,67	39,83 a	32,67	36,33	30,00	33,17 c	40,33	36,50 a
10	29,00	30,67	29,83 c	36,33	43,67	37,67	40,67 a	34,17	35,25 b
20	40,33	34,33	37,33 b	33,67	27,00	43,33	35,17 b	38,83	36,25 a
Ec+mikoriza öd	32,78	35,56			35,67	37,00			
Ec öd	34,17				36,33				

( EC × MKRZ : öd EC × VRMKST : \*\* MKRZ × VRMKST : öd EC × MKRZ × VRMKST : \*\* ) öd : önemsiz \*\* : % 1 düzeyinde önemli (P≤0,01).

Çizelge 8. Domates fidelerinde uygulamaların yaprak kuru ağırlığı (g) üzerindeki etkileri

Table 8. Effects of applications on leaf dry weight (g) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V öd	M+V öd	EC 1		EC+V öd	M+V öd	EC+M+V öd
	Mikoriza (-)	Mikoriza (+)			Mikoriza (-)	Mikoriza (+)			
0	2,67	3,00	2,83	2,83	3,00	2,67	2,83	2,83	2,83
10	3,03	3,02	3,04	3,03	3,04	3,03	3,04	3,04	3,04
20	3,00	3,01	3,00	3,00	3,03	3,00	3,01	3,00	3,00
Ec+mikoriza öd	2,89	3,01			3,02	2,91			
Ec öd	2,95				2,97				

( EC × MKRZ : öd EC × VRMKST : öd MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd ) öd : önemsiz

Çizelge 9. Domates fidelerinde uygulamaların kök yaş ağırlığı (g) üzerindeki etkileri

Table 9. Effects of applications on root fresh weight (g) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V öd	M*V *	EC 1		EC+V öd	M+V *	EC+M+V öd
	Mikoriza (-)	Mikoriza (+)			Mikoriza (-)	Mikoriza (+)			
0	6,00	11,67	8,83	6,17b	6,33	6,67	6,50	9,17a	7,67
10	5,67	8,33	7,00	6,83b	8,00	6,67	7,33	7,50b	7,17
20	9,33	7,67	8,50	7,83a	6,33	6,00	6,17	6,83b	7,33
Ec+mikoriza*	7,00b	9,22 a			6,89 a	6,44b			
Ec *	8,11 a				6,67 b				

( EC . MKRZ : \* EC . VRMKST : öd MKRZ . VRMKST : \* EC . MKRZ . VRMKST : öd ) öd : önemsiz \* : % 5 düzeyinde önemli (P≤0,05).

Domates bitkisinde Ec+vermikompost ve Ec+mikoriza+vermikompost uygulamaları arasında yaprak yaş ağırlığı bakımından %1 düzeyinde anlamlı farklılık gösterirken, diğer uygulamalar arasında herhangi bir fark görülmemiştir (Çizelge 7). En yüksek yaprak yaş ağırlığı %0 vermikompost uygulamasında (36,50 g), en düşük yaprak kuru ağırlığı ise %10 vermikompost uygulamasında (35,25 g) görülmüştür. Jeevitha ve ark. (2019) yılında yaptıkları çalışmada, farklı yetiştirme ortamları (kokopit, vermikompost, toprak, kum ve çiftlik gübresi) değişen oranlarda domates fidesi üzerindeki etkilerini inceledikleri çalışmada, fide çapı 1,0 cm, sürgün boyu 14,13 cm, ve kök uzunluğu 5,68 cm olarak (%25 çiftlik gübresi+%75 vermikompost uygulanan ortamda) bulmuşlardır. Yapılan bir başka çalışmada, vermikompost ve mikorizanın birlikte kullanıldığı ortamlarda, biberde büyümeyi artırdığı, daha fazla besin sağladığı ve yaş ve kuru ağırlıkları artırdığı bildirilmiştir (Küçükçumuk ve ark., 2014).

Ec, mikoriza ve vermikompost uygulamaları arasında domates fidelerinde yaprak kuru ağırlığı açısından önemli bir fark olmadığı görülmüştür (Çizelge 8). Özbudak ve ark. (2013) yaptıkları çalışmada torf, vermikompost, farklı oranlarda torf ve vermikompost karışımlarının (% 0, 25, 75 ve 100 vermikompost) domates, biber ve patlıcan fideleri üzerindeki etkilerini inceledikleri çalışmanın sonucunda, yetiştirme ortamına vermikompost katılmasının fide büyümesini ve beslenme durumunu olumlu yönde etkilediğini bildirmişlerdir. Yılmaz ve ark. (2017) yılında serada farklı yetiştirme ortamlarının domates fideleri üzerindeki etkisini

inceledikleri çalışmada, zeolit, turba, vermikompost ve bu substratların farklı kombinasyonlarını test etmişlerdir. Çalışma sonunda yetiştirme ortamı seçiminin domates fidelerinin kalitesi üzerinde önemli bir etkisi olduğunu, %65 torf, %15 zeolit ve %20 vermikompost karışımının fide büyümesi, kalitesi, verimi ve besin maddesi dağılımı açısından en uygun karışım olduğu tespit edilmiştir.

Domates fidesi yetiştiriciliğinde, Ec, Ec+mikoriza ve mikoriza+vermikompost uygulamaları arasında istatistiksel olarak % 5 düzeyinde farklılık oluşmuş, diğer uygulamalar arasında önemli bir fark görülmemiştir (Çizelge 9). Ortamlar birbirleri ile karşılaştırıldığında en yüksek kök yaş ağırlık % 0 vermikompost uygulamasından en düşük ise %10 vermikompost uygulamasından elde edilmiştir. Yapılan benzer çalışmalarda, Yılmaz ve ark. (2017) yılında domates fide kalitesi üzerine yaptıkları çalışmada, torf, zeolit, vermikompost ve bunların farklı karışımlarını kullandıkları çalışmanın sonucunda, en düşük kök yaş ağırlık 7,36 g ile % 100 zeolit uygulamasından en yüksek kök yaş ağırlık ise 47,24 g ile %80 zeolit + %20 vermikompost uygulamasından elde edilmiştir. Namal, (2019) yılında domates fide kalitesini belirlemek amacıyla vermikompost, zeolit, turba ve diatomit gibi farklı yetiştirme ortamlarını birbiri ile karşılaştırdığı çalışmada, % 70 torf+% 10 zeolit+% 10 diatomit+% 10 vermikomposttan oluşan bu karışımın diğer ortamlara kıyasla hem verim hem de kalite açısından üstün sonuçlar verdiği, özellikle fide yaş-kuru ağırlığı ve kök yaş-kuru ağırlığı açısından daha iyi sonuçlar verdiği bildirilmiştir.

Çizelge 10. Domates fidelerinde uygulamaların kök kuru ağırlığı (g) üzerindeki etkileri

Table 10. Effects of applications on root dry weight (g) in tomato seedlings

Vermikompost oranları (%)	EC 0.5		EC+V öd	M+V öd	EC 1		EC+V öd	M+V öd	EC+M+V öd
	Mikoriza (-)	Mikoriz a (+)			Mikoriza (-)	Mikoriza (+)			
0	0,98	1,00	0,99	1,00	1,00	0,98	0,99	1,00	0,99
10	0,96	0,96	0,96	0,98	0,95	1,00	0,98	1,00	0,97
20	1,00	1,00	1,00	1,00	1,00	0,97	0,99	0,96	0,99
Ec+mikoriza öd	0,98	0,99			0,98	0,98			
Ec öd	0,99				0,98				

( EC × MKRZ : öd EC × VRMKST : öd MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd ) öd : önemsiz

Bir başka çalışmada, Fadıllıoğlu, (2022) yılında farklı organik ortamlar dendiği fide çalışmasında, en yüksek ve en düşük kök yaş ağırlıkları 4,01 g ile 3,59 g arasında olduğunu bildirmiştir.

Ec, mikoriza ve vermicompost uygulamaları domates fidelerinin kök kuru ağırlığında istatistiksel olarak önemli bir fark gözlenmemiştir (Çizelge 10). Namal, (2019) yılında domates fide kalitesini belirlemek amacıyla vermicompost, zeolit, turba ve diatomit gibi farklı yetiştirme ortamlarını birbiri ile karşılaştırdığı bir çalışma sonucunda, en iyi fide yaş ağırlığı, kuru ağırlığı, kök yaş ağırlığı ve kök kuru ağırlığı %70 torf+%10 zeolit+%10 diatomit+%10 vermicompost ortamından elde edilmiştir. Fadıllıoğlu, (2022) yılında yaptığı çalışmada, en yüksek kök kuru ağırlığı 0,57 g ile çiftlik gübresi+torf+zeolit ortamından elde etmiştir. Yine benzer bir çalışmada, Ahmed, (2017), yılında domates fidelerinin gelişimi üzerine yaptığı bir çalışmada, en yüksek ve en düşük kök yaş ağırlığını torf ortamından (1,60 g ile 0,12 g) elde etmiştir. Abdel-Razzak ve ark. (2019) kompost ve torf ortamlarının domates fide yetiştiriciliğinde kullandıkları çalışmada, kök kuru ağırlığı 0,57-1,27 g arasında bulmuşlardır. Bir başka çalışmada, Tüzel (2015), ise organik domates fidesi üretimi için yerel(torf, vermicompost) kaynakların kullandığı çalışmada, yerel kaynaklardan elde edilen torf ve vermicompost ortamının, sürgün yaş ve kuru ağırlıkları açısından en azından sadece torf ile elde edilenlerle karşılaştırılabilir sonuçlar verdiği ortaya konmuştur. Yapılan çalışmalarda elde edilen bulgular ile çalışmadan elde ettiğimiz sonuçlar benzerlik göstermektedir.

## Sonuç

Organik gübrelerin fide yetiştirme ortamına dahil edilmesi ve fide gelişimi için ortamdaki besin maddelerinin kullanımını etkileyen mikoriza gibi uygulamaların kullanılması, girdi maliyetlerinin azalmasına neden olacaktır. Farklı sebze türlerinin fide üretiminde kullanılan torf yerine, faydalı mikroorganizmaların (örneğin mikoriza ve bitki büyümesini destekleyen bakteriler) kullanılmasının, yetiştirme ortamının kimyasal özelliklerinden bağımsız olarak fide büyümesini ve kalitesini artırabileceği ve aynı zamanda vermicompost uygulamaları ile uygulanacak gübre miktarının azalması sağlanacaktır. Bu çalışmadan elde edilen bulgular ve daha önce yapılmış olan birçok çalışma, solucan gübresinin topraktan besin emilimini artırdığını, bitki büyümesini teşvik ettiğini, topraktaki organik madde içeriğini yükselttiğini ve mikrobiyal aktivitenin artmasına katkıda bulunduğunu göstermektedir. Sonuç olarak, vermicompost

kimyasal gübrenin yerini tutmaz ama hem ekolojik dengeyi olumlu etkileyecek hem de toprak için önemli bir destek sağlayacak çevre dostu bir gübre olarak değerlendirilmez.

## Beyanlar

### Yazar Katkı Beyanı

Yazarların makaleye eşit şekilde katkıları bulunmaktadır.

### Çıkar Çatışması

Yazarlar, herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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## Improvement of Fertility and Growth Characteristics of Hair Goats Kids Raised by Public in Kahramanmaraş Province

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 12/10/2024 Accepted : 25/11/2024</p> <p><i>Keywords:</i> Hair goat Breeding Fertility criteria Growth performance Survival rate</p>	<p>The current study was started in 2018 with 4867 female goats and 230 male goats with the voluntary participation of 25 Hair goat breeders breeding in Onikişubat district of Kahramanmaraş province and the study was conducted over five years. The aim of this study is to improve the fertility and growth characteristics of hair goats raised in the hands of the public. For this purpose, the fertility criteria for goats were determined and growth traits such as birth weight, weaning weight (90 days), daily live weight gain and survival rate of kids were recorded annually. When selecting the breeding material to be included in the herd each year, the morphological characteristics of the hair goat breed and the birth and weaning weights of the kids were taken into account and approximately 10% of the male kids and 50% of the female kids with the best growth and breeding characteristics were selected for breeding. At the end of the study, the birth rate was found to be between 54,21% and 76,93%, the twinning rate was between 21,64% and 27,19%, prolificacy was between 65,94% and 94,66% and the litter size was between 1,22 and 1,27. The birth weight of the kids was found to be between 3,11 kg and 3,19 kg, the weaning weight were between 17,09 kg and 18,63 kg, the daily live weight gain to weaning age were between 154,63 and 171,55 g and the survival rate was between 92,27% and 95,34%. According to these results, increases in the growth performance (<math>P&lt;0,001</math>) and vitality values of kids have been achieved in other years compared to 2018. In addition, when growth performance and survival rate are taken into account, it has been determined that male and single-born kids provide higher growth performance and survival rate values compared to female and twin-born kids, respectively, depending on gender and type of birth.</p>

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## Kahramanmaraş İlinde Halk elinde Yetiştirilen Kıl Keçilerinde Döl Verimi ve Oğlaklarında Büyüme Özelliklerinin İyileştirilmesi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 12/10/2024 Kabul : 25.11.2024</p> <p><i>Anahtar Kelimeler:</i> Kıl keçisi İslah Döl verim kriterleri Büyüme performansı Yaşama gücü</p>	<p>Mevcut çalışma, Kahramanmaraş ilinin Onikişubat ilçesinde yetiştiricilik yapan 25 Kıl keçisi yetiştiricisinin gönüllü katılımıyla 2018 yılında 4867 baş teke altı keçi ve 230 baş damızlık teke ile başlatılmış ve çalışma beş yıl boyunca sürdürülmüştür. Bu çalışma ile halk elinde yetiştirilen Kıl keçilerinin döl verimi ve büyüme özellikleri bakımından ıslahı amaçlanmıştır. Bu amaçla, keçilerde belirlenen döl verim kriterleri ile oğlakların doğum ağırlıkları, sütten kesim canlı ağırlıkları (90 gün), günlük canlı ağırlık kazançları ve yaşama gücü gibi büyüme özellikleri yıl bazında kaydedilmiştir. Her yıl sürüye eklenecek damızlık materyal seçimlerinde Kıl keçisi ırkına ait morfolojik özellikler ve oğlakların doğum ve sütten kesim ağırlıkları dikkate alınarak en iyi büyüme ve damızlık özelliği gösteren erkek oğlakların yaklaşık %10'u dişi oğlakların ise %50'si damızlık olarak seçilmiştir. Çalışmanın sonunda, yıllara göre doğum oranının %54,21 ile %76,93, ikizlik oranının %21,64 ile %27,19, oğlak veriminin %65,94 ile %94,66 ve doğum başına düşen oğlak sayısının 1,22 ile 1,27 arasında olduğu tespit edilmiştir. Kıl keçisi oğlaklarının yıllara göre doğum ağırlıkları 3,11 kg ile 3,19 kg, sütten kesim ağırlıkları 17,09 kg ile 18,63 kg, sütten kesime yaşama kadar günlük canlı ağırlık kazançları 154,63 ile 171,55 g ve yaşama gücünün %92,27 ile %95,34 arasında olduğu saptanmıştır. Bu sonuçlara göre oğlakların büyüme performansı (<math>P&lt;0,001</math>) ve yaşama gücü değerlerinde çalışmanın başladığı yıla göre diğer yıllarda artışlar sağlanmıştır. Ayrıca, büyüme performansı ve yaşama gücü dikkate alındığında, cinsiyete ve doğum tipine bağlı olarak erkek ve tek doğan oğlakların sırasıyla dişi ve ikiz doğan oğlaklara kıyasla daha yüksek performans ve yaşama gücü değerleri sağladığı belirlenmiştir.</p>

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## Giriş

Türkiye'nin son 20 yıldaki keçi varlığına bakıldığında 2001 yılında yaklaşık 7 milyon baş olan keçi varlığı 2001-2010 yılları arasında azalma eğilimine girmiş 2010-2024 yılları arasında artış göstererek günümüzde yaklaşık 10,5 milyon başa ulaşmıştır (TÜİK, 2024). Türkiye'de hayvansal üretim, sürekli artan insan nüfusunun gıda ihtiyacını karşılama noktasında yetersiz kalmaktadır. Bu durum son dönemlerde artan et fiyatları ile de kendisini göstermektedir. İnsanların beslenmesine kaynak oluşturan her türlü çiftlik hayvanından en yüksek oranda yararlanılması gerekmektedir. Bu kaynaklar arasında Kıl keçisi yetiştiriciliği Türkiye'nin hemen her bölgesinde faaliyet gösteren ve Türkiye keçi popülasyonunun yaklaşık %96'sını oluşturan payı ile hayvansal üretimde önemli bir yere sahiptir (Kolman ve ark., 2024). Kıl keçilerinin Türkiye geneline yayılmasında etkili olan başlıca faktörler arasında Anadolu'nun her türlü iklim ve arazi koşullarına adapte olması, yetersiz bakım ve besleme koşullarında yetiştirilebilmesi, güçlü vücut yapısına sahip olması, hastalıklara karşı dirençli olması, sıcak ve soğuğa karşı dayanıklı olması sayılabilir (Kolman ve ark., 2024). Ayrıca, bitkisel ürünler ve/veya atıkların değerli hayvansal gıdalara çevrilmesi bakımından oldukça değerli bir ırktır. Genellikle, yüksek rakımlı orman içi ve orman kenarı köy ve mezralarında bulunan fundalıklar, çalı formundaki bitkiler, orman içi meralar, anızlar ve nadasa bırakılmış alanlardaki otlarla neredeyse masrafsız bir şekilde yetiştirilir. Ancak, Türkiye'de kıl keçisi yetiştiriciliği daha çok kırsal kesimlerde ve halk elinde ekstansif üretim modelinde yapılması nedeniyle onlardan elde edilen verim düşük seviyelerde kalmaktadır. Bu nedenle, Türkiye'de ekstansif koşullar altında yetiştirilen Kıl keçisi sürülerinde et ve süt verimini artırmaya yönelik çalışmaların sayısı son yıllarda artmıştır. Bu bağlamda, Kahramanmaraş ilinde yürütülen ve Türkiye için önemli bir gen kaynağı olan Kıl keçisinin halk elinde seleksiyonla ıslahı çalışmaları önem arz etmektedir. Çünkü, Kahramanmaraş ilinin de içinde bulunduğu Akdeniz bölgesi, dağlık arazisi ve iklim

özellikleri nedeniyle Türkiye'nin en önemli keçi yetiştirme bölgesi olup keçi popülasyonu içerisinde Kıl keçisinin payı yaklaşık %27-28 arasındadır (Ertuğrul ve ark., 2000; Kolman ve ark., 2024). Mevcut çalışma ile halk elinde yetiştirilen Kıl keçilerinin ıslah edilmesiyle damızlık değeri yüksek bir elit sürü oluşturularak elde edilen verim miktarlarının artırılması amaçlanmıştır.

## Materyal ve Yöntem

Kıl keçilerinde yürütülen mevcut çalışma, 2018 yılında Doğu Akdeniz Bölgesinin Kahramanmaraş ilinde 4867 baş teke altı keçi ve 230 baş damızlık teke ile başlatılmış ve beş yıl boyunca sürdürülmüştür. Bu amaçla, Kahramanmaraş ilinin Onikişubat ilçesini temsil edecek şekilde Koyun ve Keçi Yetiştiricileri Birliği'nin veri tabanına kayıtlı olan ve ekstansif üretim sisteminde yetiştiricilik yapan 25 Kıl keçisi yetiştiricisi gönüllü olarak seçilmiştir. Buna göre mevcut çalışma Kahramanmaraş ilinin Onikişubat (enlem: 37.6003; boylam: 36.8397) ilçesine bağlı toplam 11 mahallesinde yürütülmüştür (Şekil 1). Bu işletmelerde yetiştirilen minimum hayvan sayısı 105 baş, maksimum hayvan sayısı 1130 baş ve ortalama hayvan sayısı ise 464 baştır. Çalışmanın yürütüldüğü bölge yaz aylarında sıcak ve kurak, kış aylarında ise ılıman ve yağışlı geçen iklim koşullarına sahip olması ile Akdeniz bölgesi iklim koşullarını yansıtmaktadır.

Çalışmanın yürütüldüğü yerleşim yerlerine göre yetiştirici ve hayvan sayılarına ait bilgiler Çizelge 1'de verilmiştir. Çiftliklerdeki üretim sürülerinde çiftleştirme işlemi serbest teke katımı ile yapılmış olup dişi/erkek oranı her 25 dişi için bir teke olarak hesaplanmıştır. Dişiler damızlık niteliklerini kaybedene kadar sürüde kalırken, tekeler oğullarının ilk yavruları çiftleşebilecek yaşa geldiğinde her iki yılda bir değiştirilmiştir. Her yıl, yaklaşık 100 erkek oğlak, elit sürülerde doğan ve anneleri ile kız kardeşlerinin doğurganlığına göre seçilerek diğer sürülere transfer edilmiştir.



Şekil 1. Kahramanmaraş Onikişubat ilçesinin harita görüntüsü  
Figure 1. Map image of Kahramanmaraş Onikişubat district



Çizelge 1. Çalışmanın yürütüldüğü yerleşim yerlerine göre yetiştirici ve hayvan sayıları

Table 1. Number of breeders and animals according to the settlements in which the study was conducted

İlçe	Köy	Yetiştirici Sayısı	Tekealtı Keçi Sayısı	Teke Sayısı
Onikişubat	Şahinkayası	1	178	8
Onikişubat	Kurucaova	4	611	29
Onikişubat	Kumarlı	3	949	47
Onikişubat	Beşbağlar	2	273	12
Onikişubat	Tekir	4	1081	49
Onikişubat	Avcılar	1	100	5
Onikişubat	Hacıbrahimuşağı	2	270	13
Onikişubat	Çamlıca	1	110	5
Onikişubat	Çağlayan	1	337	16
Onikişubat	Çevrepinarı	1	260	13
Onikişubat	Suçatı	5	698	33
Toplam		25	4867	230

Keçilerde çiftleştirme 15 Ağustos ile 30 Eylül arasında koçların sürüye katılması ile yapılmış bu süre dışında koç etkisinden yararlanmak amacıyla koçlar ayrı bir padokta barındırılmıştır. Çiftliklerde doğum sonrası bazı üreme kriterleri kaydedildikten sonra oğlakların doğum ağırlığı, ölüm oranı ve süttten kesim (90. gün) ağırlığı gibi performans verileri çalışma boyunca yetiştiriciler tarafından kaydedilmiştir. Doğan oğlakların kolostrum tüketmesinden sonra doğum ağırlığı yetiştiriciler tarafından dijital terazi kullanılarak kaydedilmiştir. Doğum kayıtları tutulurken oğlakların cinsiyeti, doğum tipi ve doğum tarihleri de kayıt altına alınmıştır. Oğlakların süttten kesim ağırlıkları ortalama 135 günlük olduklarında yapılmıştır. Tüm oğlakların 135. gün canlı ağırlıkları, doğum ile ikinci tartım arasındaki günlük vücut ağırlığı artışları esas alınmış ve İnterpol asyön yöntemi kullanılarak ayrı ayrı hesaplanmıştır. Günlük vücut ağırlığı artışı, iki tartım arasındaki toplam ağırlık artışının tartım günündeki yaşa bölünmesiyle bulunmuştur. Oğlakların yaşama oranı ise süttten kesim yaşına kadar yaşayan oğlak sayısının canlı doğan oğlak sayısına bölünmesiyle hesaplanmıştır. Her yıl çiftliğe damızlık olarak seçilen hayvanlar, morfolojik özellikler ve oğlakların bazı performans verileri gibi tanımlayıcı özellikler göz önünde bulundurularak çiftliğin ihtiyaçlarına göre seçilmiştir. Bu kriterlere göre, en iyi büyüme ve üreme özelliklerine sahip erkeklerin yaklaşık %10'u ve dişilerin %50'si çekirdek sürüler olarak seçilmiştir. Yetiştiricilere çiftleştirme, hayvan seçimleri ve performans ölçümleri yapılırken dikkat edilmesi gereken hususlar hakkında bilgilendirmek amacıyla 5 süreyle eğitim verilmiştir. Ayrıca, döl verim kriterlerini incelemek amacıyla doğum oranı (%), ikiz doğum oranı (%), doğum başına düşen oğlak sayısı ve oğlak verimi (%) Koluman ve ark. (2024)'andan yararlanılarak hesaplanmıştır.

$$DO = \frac{DKS}{TAKS} \times 100 \quad (1)$$

DO : Doğum oranı  
DKS : Doğuran keçi sayısı  
TAKS : Teke altı keçi sayısı

$$IDO = \frac{IDKS}{DKS} \times 100 \quad (2)$$

IDO : İkiz doğum oranı  
IDKS : İkiz doğuran keçi sayısı  
DKS : Doğuran keçi sayısı

$$DBDOS = \frac{DOS}{DKS} \quad (3)$$

DBDOS : Doğum başına düşen oğlak sayısı  
DOS : Doğan oğlak sayısı  
DKS : Doğuran keçi sayısı

$$OV = \frac{DOS}{TAKS} \times 100 \quad (4)$$

OV : Oğlak verimi  
DOS : Doğan oğlak sayısı  
TAKS : Teke altı keçi sayısı

Çalışmanın yürütüldüğü tüm çiftliklerde hayvanlar ilkbahar, yaz ve sonbaharda merada otlayarak beslenmiştir. Kışın üç ay boyunca hayvan başına yaklaşık 500-600 g/gün arpa, kepek, buğday, mısır gibi dane yemler ve yaklaşık 1000-1100 g/gün buğday samanı ve yonca gibi kaba yemler ile beslenmişlerdir. Çalışmanın verileri SPSS paket programı (SPSS, 2021) kullanılarak değerlendirildi ve iki grubun ortalamaları t testi ile karşılaştırıldı. İkidenden fazla grubun ortalamalarının karşılaştırılması One-way ANOVA testi kullanılarak yapıldı. One-Way ANOVA'nın matematiksel modeli aşağıdaki gibidir.

$$Y_{ij} = \mu + \alpha_i + e_{ij} \quad i = 1, 2, \dots, t \quad j = 1, 2, \dots, r$$

Burada;  
 $\mu$ : populasyon ortalamasını,  
 $\alpha_i$ : i'inci yıla ait etki payını,  
 $e_{ij}$ : Deneme hatasını,  
t: muamele sayısı,  
r: tekrar sayısı ifade etmektedir.

Gruplar arasındaki farkların karşılaştırılması Duncan testi kullanılarak yapıldı. Veriler ortalama ve standart hata olarak özetlenmiştir. Tüm testlerde istatistiksel anlamlılık düzeyi 0,05 olarak alınmıştır.

## Bulgular ve Tartışma

### Kıl Keçilerinde Döl Verim Özellikleri

Kıl keçilerinde döl verim özelliklerine ait bulgular yıllara bağlı olarak Çizelge 2'de sunulmuştur. Döl verim özellikleri incelendiğinde doğum oranının %54,21 ile %76,93, ikizlik oranının %21,64 ile %27,19, oğlak veriminin %65,94 ile %94,66 ve doğum başına düşen oğlak sayısının 1,22 ile 1,27 olduğu görülmektedir. Döl verim kriterleri içinde ikizlik oranının ilk yıla göre tüm yıllarda arttığı görülmüştür. Ayrıca, doğum başına düşen oğlak sayısının 2020 yılında en yüksek olduğu ve çalışmanın başlangıç yılına göre diğer yıllarda artış olduğu gözlenmiştir.

Kıl keçilerinde yapılan bazı çalışmalarda doğum oranı Erten ve Yılmaz (2013) %85,89, Erişir ve Gürdoğan (2004) %80,00, Şengonca ve ark. (2003) %79,00, Tozlu (2006) %82,93 ve Şimşek ve ark. (2006) %90,00 olarak bildirilmiştir. Oğlak verimi ise Erten ve Yılmaz (2013) %101,28, Çam ve ark. (2003) %100,00, Oral ve Altınel (2006) %96,27, Şengonca ve ark. (2003) %79,00, Tozlu (2006) %103,00, Erişir ve Gürdoğan (2004) %116,00 ve Şimşek ve ark. (2006) %118,00 olarak bildirmiştir. Mevcut çalışmada doğum oranının ve oğlak veriminin literatür bilgisine göre düşük olduğu tespit edilmiştir. Bu sonuç işletmelerde bulunan teke altı keçi sayısının çiftleşme sonucunda önemli bir oranda gebe kalmadığını göstermektedir. Bu durum kırsal kesimlerde ekstansif sistemde yetiştiricilik yapan işletmelerin her yıl yaklaşık %20-22 oranında yapılması gereken seleksiyon ve ayıklama uygulamalarını doğru bir şekilde uygulamadığından kaynaklandığı düşünülmektedir. Ancak, çalışmanın ilerleyen yıllarında döl tutma yeteneğini kaybetmiş hayvanların sürüden ayıklanması ve damızlık değeri yüksek analardan elde edilen yavruların sürüye seçilmesi ile doğum oranında ve oğlak veriminde yıllara bağlı olarak sürekli bir iyileşme sağlanmıştır. İkiz doğum oranını Erten ve Yılmaz (2013) %17,91, Erişir ve Gürdoğan (2004) %32,56 ve Tozlu (2006) %17,65 olarak

bildirmiştir. İkiz doğum oranı bakımından mevcut çalışmanın sonuçları literatür bilgisi ile büyük oranda uyumlu olduğu görülmekle birlikte, çalışmanın başlangıç yılına oranla diğer tüm yıllarda artış sağlanmıştır. Doğum başına düşen oğlak sayısını Özcan (1977) 1,43, Erten ve Yılmaz (2013) 1,18, Tozlu (2006) 1,17 ve Şimşek ve ark. (2006) 1,41 olarak belirtmişlerdir. Mevcut çalışmada doğum başına düşen oğlak sayısının literatür sınırları arasında olduğu, Özcan (1977) ve Şimşek ve ark. (2006)'nın sonuçlarına göre düşük diğer çalışmanın sonuçlarından yüksek olduğu tespit edilmiştir. Çalışmanın yürütüldüğü çiftliklerdeki sürüler 2018 yılından beri yapılan seleksiyon ve ayıklama yöntemleri ile genetik olarak iyileştirme programı uygulanmıştır. Buna göre döl verimi ve büyüme performansının sürü ortalamasının üzerinde performans gösteren erkek bireyler sürülerde tutularak verim seviyesinin yükseltilmesi sağlanmıştır. Bu tekelerin yine döl verimi ve büyüme performansına göre seçilmiş keçilerle çiftleşmesinden doğan dişi yavrular da seçildiği için bu sürülerde de performans artışları meydana gelmiştir. Ekstansif sistemde yetiştirilen Kıl keçilerinde üreme performansı her ne kadar entansif çiftliktekilere göre düşük olduğu düşünülse de uygulanan ıslah programının etkisiyle halk elinde yetiştirilen Kıl keçilerinde üreme ve büyüme performansının artacağı ortaya konmuştur.

### Kıl Keçisi Oğlaklarının Yıl Bazında Büyüme Özellikleri ve Yaşama Gücü

Yetiştirici şartlarında bakılan Kıl keçilerinden elde edilen oğlakların büyüme özellikleri bakımından doğum ağırlıkları, sütten kesim ağırlıkları, sütten kesime kadar günlük canlı ağırlık kazançları ve yaşama gücü oranları beş yıl boyunca kaydedilmiştir. Çalışmadan elde edilen verilere göre yıl bazında oğlakların yukarıda belirtilen performans değerleri Çizelge 3'te verilmiştir. Buna göre oğlakların doğum ağırlıkları 3,11 kg ile 3,19 kg, sütten kesim ağırlıkları 17,09 kg ile 18,63 kg, sütten kesime kadar günlük canlı ağırlık kazancı ortalamaları 154,63 ile 171,55 g ve yaşama gücünün %92,27 ile %95,34 arasında değiştiği saptanmıştır. Kıl keçisi oğlaklarında büyümeye bağlı performans değerlerinde gözlenen farkların yıllara göre önemli olduğu tespit edilmiş ve 2022 yılında en yüksek performans değerleri sağlanmıştır (P<0,001).

Çizelge 2. Kıl keçilerinde yıllar itibarıyla döl verim özellikleri

Table 1. Fertility characteristics in hair goats by year

Döl verim kriterleri	Yıllar					Ortalama
	2018	2019	2020	2021	2022	
Tekealtı keçi sayısı	5217	4920	5098	5210	4867	5062,40
Doğuran keçi sayısı	2828	3418	3553	3761	3744	3460,80
İkiz doğum sayısı	612	820	966	914	863	835,00
Doğan oğlak sayısı	3440	4238	4519	4675	4607	4295,80
Doğum oranı (%)	54,21	69,47	69,69	72,19	76,93	68,50
İkiz doğum oranı (%)	21,64	23,99	27,19	24,30	23,05	24,03
Oğlak verimi (%)	65,94	86,14	88,64	89,73	94,66	85,02
Doğum başına düşen oğlak sayısı	1,22	1,24	1,27	1,24	1,23	1,24

Çizelge 3. Karışık cinsiyetteki kıl keçisi oğlaklarının yıllar itibarıyla büyüme özellikleri ve yaşama gücü oranları

Table 3. Growth characteristics and survival rate of hair goat kids in mixed-sex by year

Yıllar	Doğum Ağırlığı (kg)	Sütten kesim ağırlığı (kg)	Günlük canlı ağırlık kazancı (kg)	Yaşama Gücü (%)
2018	3,13±0,01 <sup>a</sup>	17,60±0,08 <sup>a</sup>	160,72±0,92 <sup>a</sup>	92,27
2019	3,11±0,01 <sup>a</sup>	17,61±0,02 <sup>a</sup>	161,12±2,02 <sup>a</sup>	94,07
2020	3,17±0,01 <sup>b</sup>	17,09±0,08 <sup>b</sup>	154,63±0,84 <sup>b</sup>	95,34
2021	3,17±0,01 <sup>b</sup>	17,15±0,11 <sup>b</sup>	155,31±1,29 <sup>b</sup>	94,46
2022	3,19±0,01 <sup>b</sup>	18,63±0,11 <sup>c</sup>	171,55±1,21 <sup>c</sup>	93,86
P	P<0,001	P<0,001	P<0,001	-

Çizelge 4. Kıl keçisi oğlaklarının anaç yaşı, cinsiyet ve doğu tipine göre büyüme özellikleri ve yaşama gücü oranları

Table 4. Growth characteristics and survival rate of hair goat kids according to breeder age, sex and birth type

Anaç yaşı	Doğum ağırlığı (kg)	Sütten kesim ağırlığı (kg)	Günlük canlı ağırlık kazancı (kg)	Yaşama gücü (%)
1	2,99±0,03 <sup>c</sup>	16,90±0,03 <sup>b</sup>	154,65±3,07	89,43
2	3,13±0,01 <sup>b</sup>	17,34±0,02 <sup>ab</sup>	157,91±1,97	92,84
3	3,13±0,01 <sup>b3</sup>	17,70±0,01 <sup>a</sup>	161,88±1,46	93,62
4	3,20±0,01 <sup>a</sup>	17,75±0,09 <sup>a</sup>	161,60±1,00	92,80
5	3,18±0,01 <sup>ab</sup>	17,61±0,10 <sup>a</sup>	160,38±1,14	94,85
6	3,16±0,02 <sup>b</sup>	17,65±0,12 <sup>a</sup>	161,20±1,32	95,44
7 +	3,15±0,01 <sup>ab</sup>	17,34±0,10 <sup>ab</sup>	157,68±1,15	96,27
P	P<0,001	0,042	0,120	-
Cinsiyet				
Erkek	3,32±0,01	18,88±0,07	172,89±0,78	94,27
Dişi	2,98±0,01	16,20±0,08	146,86±0,89	93,94
P	P<0,001	P<0,001	P<0,001	-
Doğum tipi				
Tek	3,23±0,01	17,79±0,07	161,72±0,73	94,21
İkiz	2,88±0,01	16,78±0,07	154,37±0,81	93,32
P	P<0,001	P<0,001	P<0,001	-

Yapılan bazı araştırmalarda Kıl keçisi oğlaklarının doğum ağırlığını Erten ve Yılmaz (2013) 3,01 kg, Oral ve Altınel (2006) 2,58 kg, Öztürk (2000) 2,60 kg, Şengonca ve ark. (2003) 2,63 kg, Şimşek ve ark. (2006) 2,18 kg, Şimşek ve Bayraktar (2007) 2,77 kg, Şimşek (2005) 2,99 kg, Darcan (2000) 3,89 kg, Daş ve Savaş (2002) 3,80 kg, Karadağ (2006) 3,31 kg, Alşahan ve Öztürk (2019) 3,11 kg, Çam ve ark. (2003) 2,70 kg, Çelik ve Oflaz (2018) 3,70 kg, Gökdal ve ark. (2013) 2,75 kg, Atay ve ark. (2010) 3,12 kg ve Tozlu (2006) 3,72 kg olarak belirtilmiştir. Bu çalışmanın sonuçları literatür sonuçlarının sınırları içinde olup Alşahan ve Öztürk (2019) ve Atay ve ark. (2010) tarafından yapılan çalışmaların sonuçları ile benzer olduğu görülmüştür. Ayrıca, Darcan (2000), Daş ve Savaş (2002), Karadağ (2006), Çelik ve Oflaz (2018) ve Tozlu (2006) tarafından yapılan çalışmaların sonuçlarından düşük diğer çalışmaların sonuçlarından ise yüksek olduğu tespit edilmiştir. Kıl keçisi oğlaklarında üç aylık yaşta oğlakların sütten kesim ağırlıklarını Erten ve Yılmaz (2013) 12,32 kg, Çam ve ark. (2003) 13,70 kg, Cengiz ve ark. (1995) 11,84 kg, Darcan (2000) 18,00 kg, Oral ve Altınel (2006) 13,58 kg, Şimşek (2005) 17,77, Erduran ve Yaman (2012) 17,02 kg, Şimşek ve Bayraktar (2007) 16,05 kg ve Tozlu (2006) 16,0 kg olarak bildirmiştir. Yapılan çalışmada oğlakların sütten kesim ağırlıkları Darcan (2000), Şimşek (2005) ve Erduran ve Yaman (2012) tarafından yapılan çalışmaların sonuçları ile benzer, diğer çalışmaların sonuçlarına göre daha yüksek değerler elde edilmiştir. Kıl keçisi oğlaklarının sütten kesime kadar yaşama gücü oranını Erten ve Yılmaz (2013) %89,87, Odabaşoğlu ve Altın (1992) %78,16, Şengonca ve ark. (2003) %78,61, Şimşek

(2005) %82,5, Tozlu (2006) %88,11, Erduran ve Yaman (2012) %80 Şimşek ve Bayraktar (2007) %90,62, Eser (1998) %93,34, Çam ve ark. (2003) %93,3 ve Oral ve Altınel (2006) %95,44 olarak bildirmiştir. Mevcut çalışmada yaşama gücü verileri Erten ve Yılmaz (2013), Odabaşoğlu ve Altın (1992), Şengonca ve ark. (2003), Şimşek (2005), Tozlu (2006), Erduran ve Yaman (2012) ve Şimşek ve Bayraktar (2007) tarafından bildirilenlere göre yüksek Eser (1998), Çam ve ark. (2003) ve Oral ve Altınel (2006)'in bildirdiği sonuçlar ile benzerlik göstermektedir. Mevcut çalışmada oğlakların büyüme performanslarında ve yaşama gücü oranlarında çalışmanın başladığı yıla göre genellikle sonraki yıllarda iyileşmeler yaşanmıştır. Bu durum tutulan kayıtlara göre yavruların doğum ve sütten kesim ağırlığı gibi performans değerleri yüksek yavruların sürüye damızlık olarak bırakılması ile açıklanabilir. Ancak, bu yöntemin ebeveynlerin büyüme ve performans özelliklerine genetik yakınlığı ile yavrulardaki görülme olasılığı arasındaki ilişkiyi doğrudan yansıtmayacağı da unutulmamalıdır.

#### Kıl Keçisi Oğlaklarının Anaç Yaşı, Cinsiyet ve Doğum Tipine Göre Büyüme Özellikleri ve Yaşama Gücü

Kıl keçisi oğlaklarında anaç yaşı, oğlakların cinsiyeti ve doğum tipine bağlı olarak büyüme özellikleri tüm yılların ortalaması şeklinde Çizelge 4'te sunulmuştur. Anaç yaşına bağlı olarak oğlakların doğum ağırlıkları 2,99 kg ile 3,20 kg, sütten kesim ağırlığı 16,90 kg ile 17,75 kg, sütten kesime kadar ki günlük canlı ağırlık kazancı 154,65 ile 161,88 g ve yaşama gücü %89,43 ile %96,27 arasında değiştiği belirlenmiştir. Oğlakların erkek ve dişi cinsiyete

göre doğum ağırlığı sırasıyla 3,32 ve 2,98 kg, sütten kesim ağırlığı 18,88 ve 16,20 kg, sütten kesime kadar ki günlük canlı ağırlık kazancı 172,89 ve 146,86 g ve yaşama gücü %94,27 ve %93,94 olarak saptanmıştır. Oğlakların tek ve ikiz doğum tipine göre doğum ağırlığı sırasıyla 3,23 ve 2,88 kg, sütten kesim ağırlığı 17,79 ve 16,78 kg, sütten kesime kadar ki günlük canlı ağırlık kazancı 161,72 ve 154,37 g ve yaşama gücü %94,21 ve %93,32 olarak tespit edilmiştir.

Anaç yaşının oğlakların doğum ağırlığı ( $P<0,001$ ) ve sütten kesim ağırlığı ( $P<0,05$ ) üzerine etkisinin önemli olduğu; ancak oğlakların sütten kesime kadar ki günlük canlı ağırlık kazancının anaç yaşından etkilenmediği saptanmıştır ( $P>0,05$ ). Ayrıca, oğlakların büyüme ile ilgili performans değerleri doğum tipi ve cinsiyet tarafından önemli ölçüde etkilendiği tespit edilmiştir ( $P<0,001$ ). Erten ve Yılmaz (2013) tarafından yapılan çalışmada anaç yaşının 2, 3 ve 4 yaş ve üzeri olan kıl keçisi oğlaklarında doğum ağırlığı sırasıyla 2,81, 3,12 ve 3,10 kg, sütten kesim ağırlığı (90 günlük canlı ağırlığı) 11,65, 12,68 ve 12,65 kg, sütten kesime kadar günlük canlı ağırlık kazancı 95,95, 105,71 ve 105,88 g ve yaşama gücü %82,75, %100,00 ve %88,00 olarak bildirilmiştir. Aynı çalışmada, oğlakların tek ve ikiz doğumuna göre doğum ağırlığı sırasıyla 2,97 ve 3,05 kg, sütten kesim ağırlığı 12,52 ve 12,13 kg, sütten kesime kadar günlük canlı ağırlık kazancı 105,76 ve 99,27 g ve yaşama gücü %89,09 ve %91,66 olarak açıklanmıştır. Cinsiyete bağlı olarak erkek ve dişi oğlakların doğum ağırlığı sırasıyla 2,99 ve 3,03 kg, sütten kesim ağırlığı 12,41 ve 12,23 kg, sütten kesime kadar günlük canlı ağırlık kazancı 104,57 ve 100,46 g ve yaşama gücü %90,90 ve %88,57 olarak belirtilmiştir. Benzer bir çalışmada Tozlu (2006) anaç yaşı 3, 4, 5, 6 ve 7 olan kıl keçisi oğlaklarının doğum ağırlığını sırasıyla 4,03, 3,68, 4,05, 3,68 ve 3,40 kg, sütten kesim ağırlığı (75 günlük canlı ağırlığını) 15,88, 16,57, 17,11, 16,23 ve 15,21 kg ve sütten kesime kadar günlük canlı ağırlık kazancını 158,29, 169,40, 176,00, 165,20 ve 157,88 g olarak bildirmiştir. Aynı çalışmada, oğlakların tek ve ikiz doğum tipine göre doğum ağırlığı sırasıyla 3,90 ve 3,31 kg, sütten kesim ağırlığı 16,99 ve 13,91 kg ve sütten kesime kadar günlük canlı ağırlık kazancı 173,58 ve 141,56 g olarak açıklanmıştır. Cinsiyete bağlı olarak erkek ve dişi oğlakların doğum ağırlığı sırasıyla 3,76 ve 3,67 kg, sütten kesim ağırlığı 16,44 ve 15,22 kg ve sütten kesime kadar günlük canlı ağırlık kazancı 168,72 ve 153,50 g olarak belirtilmiştir. Mevcut çalışmada, oğlakların anaç yaşına, doğum tipine ve cinsiyete bağlı olarak gözlenen performans değerlerinin Erten ve Yılmaz (2013)'in sonuçlarına göre daha yüksek, Tozlu (2006)'nun yaptığı çalışma sonuçları ile büyük oranda tutarlı olduğu bulunmuştur. Ayrıca, ilk doğumunu yapmış anaçlar ile kıyaslandığında birden fazla kez doğum yapmış anaçların oğlaklarında büyüme performansı ve yaşama gücünde artış saptanmıştır. Bu durum yaşı ilerleyen damızlık anaçların yavrularını kabullenme davranışlarını daha yüksek oranda sergilemesi ve yavru büyütmedeki sahip olduğu tecrübenin artması ile açıklanabilir. Aynı zamanda cinsiyet ve doğum tipine bağlı olarak sırasıyla erkek ve tek doğan yavruların doğum ağırlığının dişi ve ikiz doğanlara göre daha yüksek olması onların yaşama gücünü arttırmış ve daha yüksek performans değerleri sağlamasına yardımcı olmuştur.

## Sonuç

Çalışmanın sonunda çiftçi koşullarında ve ekstansif sistemde saf olarak yetiştirilen Kıl keçilerinin seleksiyon ile üreme etkinliği ve sütten kesime kadar ki günlük canlı ağırlık artışına ilişkin performanslarının iyileştirilebileceği belirlenmiştir. Bu sonuçlar yerli Kıl keçilerine uygulanan seleksiyon ile ekstansif koşullarda Kıl keçi ırkında birim hayvan başına üreme ve sütten kesim dönemine kadar günlük canlı ağırlık kazancının artırılmış olduğu saptanmıştır.

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## The Effect of Mycorrhiza and Organic Fertiliser Applications on the Development of Pepper (*Capsicum annuum* L.) Seedlings

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 13.10.2024 Accepted : 22.11.2024</p> <p><b>Keywords:</b> EC Mycorrhiza Pepper Organic fertilizer Vermicompost</p>	<p>This study was conducted in a fully automated heated greenhouse at Tokat Gaziosmanpaşa University in 2021. The aim of this study was to examine the impact of varying doses of mycorrhiza and organic fertiliser applications on the growth of pepper seedlings. Bulut F1 pepper variety was used in the study. The study was conducted according to the randomized experimental design with 3 replications. In the study, different doses of nutrient solution and vermicompost with and without mycorrhizae were applied to peat-perlite mixture for pepper seedling cultivation. Seedlings were removed in one and a half month. In this study, seedling height, hypocotyl length, stem diameter, number of leaves, leaf wet weight, leaf dry weight, root wet weight and root dry weight were analysed. The findings of the study indicate that the combination of vermicompost, mycorrhiza and EC treatments yielded superior outcomes in terms of stem diameter. The application of vermicompost at increasing doses resulted in an increase in seedling height and hypocotyl length. However, the vermicompost treatments themselves had no effect. In general, the EC and EC+mycorrhiza treatments were observed to be more effective than the vermicompost treatments across all treatments.</p>

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## Mikoriza ve Organik Gübre Uygulamalarının Biber (*Capsicum annuum* L.) Fidelerinin Gelişimi Üzerindeki Etkisi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 13.10.2024 Kabul : 22.11.2024</p> <p><b>Anahtar Kelimeler:</b> Biber EC Mikoriza Organik gübre Vermikompost</p>	<p>Bu çalışma 2021 yılında Tokat Gaziosmanpaşa Üniversitesi bünyesindeki tam otomasyonlu ısıtmalı bir serada yürütülmüştür. Bu çalışmanın amacı, farklı dozlarda mikoriza ve organik gübre uygulamalarının biber fidelerinin gelişimi üzerindeki etkisini incelemektir. Çalışmada Bulut F1 biber çeşidi kullanılmıştır. Araştırma, tesadüf parselleri deneme desenine uygun olarak 3 tekrarlı olarak gerçekleştirilmiştir. Çalışmada, biber fidesi yetiştiriciliği için torf-perlit karışımına mikorizalı ve mikorizatsız farklı dozlarda besin çözeltisi ve vermicompost uygulanmıştır. Fidler bir buçuk ayda sökülümü yapılmıştır. Bu çalışmada, fide boyu, hipokotil uzunluğu, gövde çapı, yaprak sayısı, yaprak yaş ağırlığı, yaprak kuru ağırlığı, kök yaş ağırlığı ve kök kuru ağırlığı özellikleri incelenmiştir. Çalışma bugularına göre, gövde çapı üzerinde vermicompost+mikoriza+EC uygulamalarının daha iyi sonuç vermiştir. Vermikompost dozları arttıkça fide boyu ve hipokotil uzunluğunda bir artış olduğu ancak vermicompost uygulamalarının etkisinin olmadığı görülmektedir. Genel olarak tüm uygulamalara bakıldığı zaman vermicompost uygulamalarına göre EC ve EC+mikoriza uygulamalarının etkisinin daha fazla olduğu görülmektedir.</p>

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## Giriş

Biber (*Capsicum annum* L.), *Solanaceae* familyasına ait olup, sıcak iklimlerde tek yıllık, tropikal iklimlerde ise birkaç yıllık kültür sebzesidir. Türkiye topraklarının iklimsel ve coğrafi özellikleri göz önüne alındığında biber gen kaynağı açısından önemli bir konuma sahiptir. Dünya genelinde 36 milyon ton biber üretilmekte olup, bunun %6,5'i ülkemizden karşılanmaktadır. Türkiye, Çin ve Meksika'dan sonra yaklaşık 3,1 milyon ton üretimi ile önemli biber üreticisidir (TÜİK, 2022).

Türkiye, sivri, dolmalık ve kapa biberlerin yanı sıra salamura ve süs biberleri de dahil olmak üzere çok çeşitli biber türlerinin önemli bir üreticisidir. Gıda maddesi olarak kullanılmasına ek olarak, acı bir tat veren kapsaisin antioksidan maddesi nedeniyle biberler boya ve farmasötik ürünlerin üretiminde de kullanılmaktadır. Biber, besin değeri açısından, önemli miktarda protein, karbonhidrat, mineral ve vitamin içeren oldukça önemli bir sebzedir. Ek olarak acı biber, acı ve yakıcı bir tat veren alkaloid kapsaisin içerir. Kapsaisin, antioksidan aktivitenin yanı sıra antimutajenik ve tümör inhibisyonu süreçlerinde de önemli bir rol oynar (Arın, 2018), kardiyovasküler hastalıklar, kas ağrısı ve romatizma, sinir sistemi bozukluklarına iyi geldiği ve ayrıca, kan akışını teşvik ettiği ve kan basıncını düzenlediği bildirilmiştir (Vural ve ark., 2000; Şalk ve ark., 2008; Özalp, 2010).

Topraksız tarım ve fide yetiştiriciliğinde genellikle ortam olarak yaygın şekilde torf kullanılmaktadır. Ülkemizde torfun yüksek maliyeti göz önüne alındığında, organik gübre ihtiyacının tamamının tek başına torf ile karşılanması mümkün değildir. Torf (*Sphagnum* spp.) günümüzde pahalı, yenilenemeyen, istenmeyen değişken özelliklere sahip ve kullanımı kademeli olarak azaltılması gereken bir kaynak olduğu bildirilmektedir (Ceglie ve ark., 2015; Chrysargyris ve ark., 2017). Türkiye'de tarımsal ürünlerin işlenmesi sonucunda önemli miktarda bitkisel atık/artık ortaya çıkmaktadır (Baran ve ark., 1995). Yüksek organik madde içeriğine, uygun tuz içeriğine ve pH değerlerine, yüksek su tutma ve havalandırma kapasitesine sahip materyaller yetiştirme ortamı olarak kullanılma potansiyeline sahiptir. Yapılan pek çok çalışmada, atık malzemelerin doğrudan toprağa veya ortama ilave edilmesinin organik madde ve bitki besin kaynağı olarak hizmet edebileceğini göstermiştir. Ayrıca, bu materyallerin belirli oranlarda kullanılmasının yetiştirme ortamlarının geliştirilmesi için etkili bir yaklaşım olduğu görülmektedir (Aydeniz & Brohi, 1991; Özenç, 2004; Benito ve ark., 2005).

Toprağın doğal dengesinin iyileştirilmesine katkı sağlayacak materyallerden biri de vermikomposttur (Demir ve ark., 2010). Vermikompost, solucanlarla yapılan biyoteknolojik bir komposttur ve ayrıca organik atıkları geri dönüştüren bir gübre olarak tanımlanır (Bellitürk & Görres, 2012). Lazcano ve Dominguez (2011) vermikompostu, bitki büyümesi ve sağlığı için faydalarını gerekçe göstererek tarımda ve sera yetiştirme ortamlarında kimyasal gübrele umut verici bir alternatif olarak tanımlamıştır. Organik bir gübre olan vermikompostun kullanımı, bitkileri beslemeye ve toprak kalitesini arttırmaya hizmet ettiği için organik yetiştiriciliğin tamamında uygulanabilmektedir. Ayrıca solucan gübresi bitki büyüme hormonları içermesine ilaveten, insan sağlığı için risk oluşturabilecek bakteriyel veya patojenik ajanlar

içermemesidir (Dominguez ve ark., 1997; Yıldız, 2005; Şimşek-Erşahin, 2007; Demir, 2010; Joshi & Vig, 2010).

Mikoriza, belirli bitkilerin kökleriyle simbiyotik bir ilişki geliştiren bir grup mantarı tanımlamak için kullanılır. Bu tür mantarlar parazit olarak değil, bitkilerin destekçileri olarak hareket ederler. Mikorizalar, hifler aracılığıyla, topraktan emilmesi zor olan ve bitki köklerinin ulaşamayacağı besin maddelerinin taşınmasını kolaylaştırır. Ayrıca, vermikompost ve mikorizanın birlikte kullanılması bitkilerin verimini ve besin maddesi alımını artırabileceğini ve bitkisel üretimde faydalı bir uygulama olduğunu göstermiştir (Küçükyumuk, 2014). Bazı araştırmalar Veziküler-arbüsküler mikoriza (VAM), mantarlarının topraktan fosfor alımını teşvik ettiğini rapor etmişlerdir (Demirkaya ve ark. 2016).

Hem mikoriza hem de vermikompostun birlikte kullanıldığı çalışmada, biber bitkilerinin büyümesinde ve besin elementi alımında bir artış gözlemlenmiştir (Küçükyumuk ve ark., 2014). Bir başka vermikompost çalışmasında, mısır bitkisinin veriminde %50'e yakın artış sağladığı (Durukan ve ark., 2020), biochar ve vermikompostun ortamda birlikte kullanılması bitkide rizosfer kök bölgesi ve mikrobiyal biyokütle açısından olumlu etkileri olduğu (Yılmaz ve Kurt, 2020), ayrıca, çilek, biber ve domates bitkilerinde sürgün uzunluğunu, yaprak alanını ve meyve kalitesinde artış olduğu bildirilmiştir (Arancon ve ark., 2003). Yapılan benzer araştırmalarda, vermikompost ve mikoriza uygulamalarının domates, hıyar, lahana ve marul bitkisinde çimlenme, fide gelişimi ve ürün verimi üzerindeki etkileri incelenmiştir (Öcalan ve Sağlam, 2022). Ancak bu çalışmada, vermikompost ve mikoriza uygulamalarının birlikte ve ayrı kullanımına ek olarak sentetik gübre uygulamaları ile karşılaştırılması çalışmanın özgün yönünü oluşturmaktadır. Bu çalışmanın amacı, biber fidesi yetiştirme ortamına farklı dozlarda vermikompost, mikoriza ve EC (Tam gübre ve yarım gübre) uygulamalarının fide gelişimi ve kalitesi üzerindeki etkilerini incelemektir.

## Materyal ve Yöntem

Çalışma 2021 yılında Tokat Gaziosmanpaşa Üniversitesi'nde ısıtılmı serada kekkila torfu kullanılarak 150'lik fide yetiştirme viyollerinde yapılmış olup torf ve vermikompost özellikleri Çizelge 1'de gösterilmiştir. Her uygulama konusuna bir viyol biber tohumu ekilmiş ve viyollerin kenar sırası kenar tesiri olarak kabul edilmiş, viyoller 3 eşit şekilde bölünüp ve 3 tekerrür olmak üzere her tekerrürde 10 bitki üzerinde ölçüm yapılmış ve bir bitki ortalaması olarak alınmıştır.

Fideler ekimden yaklaşık 40 gün sonra hasat edilmiştir. Fidelerin yaş ağırlıkları, hipokotil uzunlukları, gövde çapları, yaprak sayıları, fidelerin biyomasi ve kuru ağırlıkları belirlenmiştir. Fidelerin gübrelenmesi için hazırlanan besin elementi çözeltisinde 100 ppm N, 50 ppm P, 100 ppm K, 100 ppm Ca, 50 ppm Mg ve 50 ppm S içermektedir. Mikro element uygulamaları da yapılmıştır. Çalışmada farklı vermikompost dozların etkisini tam gübre dozu (EC 1) ile yarım gübre (EC2) dozu ile besleme şartlarında da görmek ve gübre dozu ile vermikompost dozları arasında ilişkiye bakılmak istenmiştir.

Çizelge 1. Sphagnum torfu ve vermikompost özellikleri  
Table 1. Properties of sphagnum peat and vermicompost

Sphagnum Torfu	
Özellikler	Değerler
Organik madde (%)	95
pH	5,5
EC (dS m <sup>-1</sup> )	2,5
Azot (%)	0,014
Fosfor (%)	0,016
Potasyum (%)	0,018
Magnezyum (%)	0,001
Silisyum (mg l <sup>-1</sup> )	187
Demir (mg l <sup>-1</sup> )	0,9
Mangar (mg l <sup>-1</sup> )	1,6
Bor (mg l <sup>-1</sup> )	0,3
Çinko (mg l <sup>-1</sup> )	0,4
Bakır (mg l <sup>-1</sup> )	1,5
Molibden (mg l <sup>-1</sup> )	0,5
Vermikompost	
Organik madde (%)	64
pH	7,5
EC (dS m <sup>-1</sup> )	2,15
C/N	18,71
Azot (%)	1,25
Fosfor (%)	0,55
Potasyum (%)	1,02

Çizelge 2. EC uygulamalarının haftalara göre dağılımı  
Table 2. Distribution of EC applications by week

Uygulamalar	Haftalar				
	1.	2.	3.	4.	5.
EC 1 (dS/m)	-	1,2	1,4	1,6	1,9
EC 0,5 (dS/m)	-	0,92	1,02	1,12	1,22

Fidelere uygulanacak gübre çözeltilisinin hazırlanmasında bitki besin elementlerinin birbirine oranı, Azot (N) 2,5, Fosfor (P) 1,0, Potasyum (K) 2,0, Kalsiyum (Ca) 1,0 ve Magnezyum (Mg) 0,5 alınarak hazırlanmıştır. Çözeltinin içerisine uygun dozlarda Fe, Cu, Zn, Mn ve B ilavesi yapılarak EC gübre dozları hazırlanmıştır. EC 1 ve EC 0,5 besin çözeltilisinin EC miktarı ve haftalara göre verilme dozu Çizelge 2’de gösterilmiştir.

Biber bitkisinde gerçek yapraklar görülmeye başladıktan sonra EC 1,4 ve 1,8 mmhos/cm ile muamele edilmiştir. Mikoriza uygulaması, bitki kökleriyle simbiyotik etkileşime girebilen Endo Roots Soluble (ERS) ticari mikoriza kullanılarak gerçekleştirilmiştir. Mikoriza BioGlobal firmasından satın alınmış ve 5000 mg/7kg hazırlanmış ve tohum ekiminden dört gün sonra her bir viyole 50 ml saf su ile mikoriza uygulaması yapılmıştır. Fideler üzerinde uygulamaların etkisini belirlemek için, biber fideleri ortalama 1,5 ay içerisinde toprak üstü aksamından kesilmiş ve gözlemler (fide, hipokotil uzunluğu, gövde çapı, yaprak sayısı, yaprak yaş-kuru ağırlığı ve kök yaş-kuru ağırlığı) yapılmıştır. Çalışmada, fide boyu, yetiştirme ortamının yüzeyinden en uzun yaprağın ucuna kadar ölçülmüştür. Hipokotil uzunluğu,

kök boğazından kotiledon yapraklarına kadar ölçülmüştür. Gövde çapı, fidelerin kök boğazının üstünden dijital bir kumpas ile ölçülmüştür. Yaprak sayısı, hasattan önce yapılmıştır. Fideler hasat edildikten sonra damıtılmış su ile temizlenmiş ve oda sıcaklığında kısa bir süre bekletilmiş ve ağırlıklar hassas terazi kullanılarak belirlenmiştir. Toprak üstü kısımlar ve kökler ayrı ayrı tartılmış ve ağırlık sabit kalana kadar 65°C’de bekletilmiştir. Son olarak, yeşil aksam ve kökler kurumaya bırakılmıştır. Veriler SPSS 20.0 yazılım paketi kullanılarak analiz edilmiş ve ortalamaları %1-5 düzeyinde karşılaştırmak için Duncan’ın çoklu karşılaştırma testi kullanılmıştır.

## Bulgular ve Tartışma

Araştırmada biber fide gelişimine yönelik yetiştirme ortamına yapılan organik gübre uygulamalarının etkileri Çizelge 3, 4, 5, 6, 7, 8, 9 ve 10’da sunulmuştur.

Biber bitkisinin fide boyu üzerine, EC, EC+mikoriza ve Mikoriza+vermikompost interaksiyonları arasında %5 düzeyinde önemli bir fark olduğu, EC+vermikompost ve EC+mikoriza+vermikompost uygulamalarında ise fide boyu üzerine farkın önemsiz olduğu görülmektedir (Çizelge 3). Uygulamalar arasında en yüksek fide boyu %0 vermikompost uygulamasında 7,22 cm iken, en düşük fide boyu ise %20 vermikompost uygulamasında 6,06 cm arasında değişim göstermiştir. Sönmez (2017), domates fidesi yetiştiriciliğinde, torf, atık mantar kompostu ve perlitten oluşan çeşitli karışımlar kullanılarak yapılan çalışmada, fide boyunda en yüksek değer (8,70 cm) ile %30 perlit+%70 atık mantar kompostu içeren karışımdan elde edilmiştir. Momirovic ve ark. (2000) tarafından yürütülen bir çalışmada, torf, kompost, kompost + torf ve torf+zeolit gibi farklı organik ortamların biberde fide gelişimi ve kalitesi üzerindeki etkileri araştırılmıştır. Çalışma sonucunda, fide boyu 14,62-13,79 cm arasında bulunmuştur. Durukan, (2004) bazı sebze türlerinde (domates-biber) fide yetiştirme ortamı olarak tütün tozu kompostunun saf ve farklı oranlarda kullanılabilirliği üzerine yaptığı çalışma sonucunda, fide boyu domates bitkisinde 22,49-5,73 cm arasında biber bitkisinde 16,40-5,74 cm arasında olduğu görülmüştür. Çelebi (2019) serada farklı yetiştirme ortamlarının kullandığı çalışmada ise fidelerin ortalama uzunluğu 4,5-9,8 cm arasında olduğunu bildirmiştir. Sonuçlar Verdonck (1991), Olympios (1992), Kreen ve ark. (2002) ve Şirin ve ark. (2010) tarafından bildirilen sonuçlar ile paralellik göstermektedir.

Biber bitkisinin hipokotil boyu üzerine EC+mikoriza uygulamasında %0,01, mikoriza+vermikompost uygulamasında ise %0,05 düzeyinde önemli bir fark olduğu, diğer uygulamalar arasındaki farkların ise önemsiz olduğu görülmüştür (Çizelge 4). Mikoriza+vermikompost uygulamasında ortalamalara göre en yüksek hipokotil uzunluğu 4,28 cm ile %0 mikoriza+vermikompost dozunun EC 1 uygulamasında, EC 0,5 dozunda ise 4,17 cm ile %10 mikoriza+vermikompost uygulamasından elde edilmiştir. EC+mikoriza+vermikompost uygulamasında ise %0 ve %10 vermikompost uygulamalarından en iyi sonuç elde edilmiştir.



Çizelge 3. Biber bitkisinde organik gübre uygulamalarının fide boyu (cm) üzerindeki etkileri

Table 3. Effects of organic fertilizer applications on seedling length (cm) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	*	-	+	öd	*	öd
0	6,13	6,83	6,48	6,75b	7,37	8,57	7,97	7,70a	7,22
10	6,37	6,43	6,40	7,35a	8,33	6,13	7,23	6,28b	6,82
20	5,73	5,97	5,85	6,63b	7,53	5,00	6,27	5,48c	6,06
(EC+mikoriza) *	6,08b	6,41a			7,74a	6,57b			
(EC) *	6,24 b				7,16 a				

(EC × MKRZ : \* EC × VRMKST: öd MKRZ × VRMKST : \* EC × MKRZ × VRMKST: öd ); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir, \* : Uygulamalar arasındaki farkın %5 düzeyinde önemli olduğunu gösterir (P≤0,05).

Çizelge 4. Biber bitkisinde organik gübre uygulamalarının hipokotil boyu (cm) üzerindeki etkileri

Table 4. Effects of organic fertilizer applications on hypocotyl length (cm) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	*	-	+	öd	*	öd
0	3,30	3,93	3,62	3,88b	4,47	4,63	4,55	4,28a	4,08
10	3,77	4,07	3,92	4,17a	4,57	3,40	3,98	3,73b	3,95
20	3,43	3,47	3,45	3,80b	4,17	2,67	3,42	3,07b	3,43
(EC+mikoriza) **	3,50b	3,82a			4,40a	3,57b			
(EC) öd	3,67				3,98				

(EC × MKRZ: \*\*EC × VRMKST: öd MKRZ × VRMKST: \* EC × MKRZ × VRMKST: öd ); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir, \* : Uygulamalar arasındaki farkın %5 düzeyinde önemli olduğunu gösterir (P≤0,05); \*\* : Uygulamalar arasındaki farkın %0.1 düzeyinde önemli olduğunu gösterir (P≤0,01).

Çizelge 5. Biber bitkisinde organik gübre uygulamalarının gövde çapı (mm) üzerindeki etkileri

Table 5. Effects of organic fertilizer applications on stem diameter (mm) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	öd	-	+	öd	öd	*
0	2,93	2,83	2,88	2,92	2,90	3,07	2,98	2,95	2,93 a
10	2,83	2,93	2,88	2,97	3,10	2,53	2,82	2,73	2,85 ab
20	2,63	2,57	2,60	2,65	2,67	2,93	2,80	2,75	2,70 b
(EC+mikoriza) öd	2,80	2,78			2,89	2,84			
(EC) öd	2,79				2,87				

(EC × MKRZ : ödEC × VRMKST : öd MKRZ × VRMKST : ödEC × MKRZ × VRMKST : \* ); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir, \* : Uygulamalar arasındaki farkın %0,5 düzeyinde önemli olduğunu gösterir (P≤0,05).

Biber fidelerinin gövde çapı açısından tüm uygulamalar arasındaki fark önemsiz bulunmuş, ancak EC+mikoriza+vermikompost uygulamasında istatistiksel olarak %5 düzeyinde önemli bir fark gözlenmiştir. EC+vermikompost uygulamasında ortalamalara göre en yüksek gövde çapı 2,98 mm (%0 EC+vermikompost dozunun EC 1 uygulamasından), EC 0,5 dozunda ise 2,97 mm (%10 mikoriza+vermikompost uygulamasından) elde edilmiştir. EC+mycorrhiza+vermikompost uygulamasında, %0 vermikompost dozunda 2,93 mm ile en iyi sonucu vermiştir (Çizelge 5).

Aydın ve Demirsoy (2020), vermikompost ve torf ile yaptıkları çalışmada, biber bitkisinde vermikompost uygulamasında 13,44 mm, torf uygulamasından 12,40 mm gövde çapı elde etmişlerdir. Roy ve ark. (2011) biber bitkisinde yaptıkları çalışmada, en yüksek gövde çapı 12,40 mm olarak vermikompost ortamında bulmuşlardır. Bir başka benzer çalışmada, Khan ve ark. (2019) biberde en yüksek gövde çapı 1,69 cm olarak vermikompost uygulanan ortamdaki, en düşük gövde çapının ise 1,33 cm ile kontrol uygulamasından tespit etmişlerdir. Abdel-Razzak ve ark. (2019) kompost ve torf ortamlarını acı biber fidesi yetiştiriciliğinde kullandıkları çalışmada, gövde çapı 1,39-1,91 mm olarak bulmuşlardır. Durukan, (2004) tütün

tozu kompostunun saf ve farklı oranlarda bazı sebze türlerinde (domates-biber) fide yetiştirme ortamı olarak kullanılması üzerine yapılan çalışmada, gövde çapı domates bitkilerinde 0,22 ile 0,52 cm, biber bitkilerinde ise 0,17 ile 0,47 cm arasında bulunmuştur. Farklı sebze türlerinde yapılan çalışmalarda, Sönmez (2017) domates bitkisinde yetiştirme ortamı olarak torf, perlit ve atık mantar kompostunu denediği çalışmada, en yüksek gövde çapı (2,91 cm) atık mantar kompostu ortamından elde etmiştir. Yılmaz ve ark. (2018) farklı organik ortamlarla domates fidesi ile yapılan çalışmada, %50 atık mantar kompostu+%50 torf ortamından en yüksek gövde çapı (3,55 mm), %100 perlit ortamı içeren uygulamada ise en düşük gövde çapı (1,49 mm) elde etmişlerdir.

Biber bitkisinde yaprak sayısı bakımından uygulamalar arasında fark istatistiksel olarak önemsiz bulunmuştur (Çizelge 6). Bütün uygulamalara bakıldığında ise yaprak sayısı 5,81-5,41 adet arasında olduğu görülmektedir. Durukan, (2004) tütün tozu kompostunun saf ve farklı oranlarda fide yetiştirme ortamı olarak domates ve biber bitkisinde kullanılabilirliği üzerine yaptığı çalışmada, yaprak sayısı domates bitkisinde 3,33-6,56 adet arasında, biber bitkisinde ise 2,67-8,90 adet arasında olduğunu bildirmiştir.

Çizelge 6. Biber bitkisinde organik gübre uygulamalarının yaprak sayısı (adet) üzerindeki etkileri

Table 6. Effects of organic fertilizer applications on the number of leaves in pepper plants.

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	öd	-	+	öd	öd	öd
0	5,77	6,03	5,90	5,80	5,83	5,53	5,68	5,78	5,79
10	6,03	6,03	6,03	6,12	6,20	4,97	5,58	5,50	5,81
20	5,67	5,37	5,52	5,57	5,47	5,13	5,30	5,25	5,41
(EC+mikoriza) öd	5,82	5,81			5,83	5,21			
(EC) öd		5,82				5,52			

(EC × MKRZ : öd EC × VRMKST : öd MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir.

Çizelge 7. Biber bitkisinde organik gübre uygulamalarının yaprak yaş ağırlığı (g) üzerindeki etkileri

Table 7. Effects of organic fertilizer applications on leaf fresh weight (g) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	öd	-	+	öd	öd	öd
0	19,33	20,00	19,67	20,33	21,33	26,67	24,00	23,33	21,83
10	20,00	18,00	19,00	23,67	27,33	20,33	23,83	19,17	21,42
20	16,67	16,67	16,67	19,83	23,00	25,33	24,17	21,00	21,41
(EC+mikoriza) öd	18,67	18,22			23,89	24,11			
(EC) **		18,44 b				24,00 a			

(EC × MKRZ : öd EC × VRMKST : öd MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir; \*\* : Uygulamalar arasındaki farkın %0,1 düzeyinde önemli olduğunu gösterir (P≤0,01).

Çizelge 8. Biber bitkisinde organik gübre uygulamalarının yaprak kuru ağırlığı (g) üzerindeki etkileri

Table 8. Effects of organic fertilizer applications on leaf dry weight (g) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	öd	-	+	öd	öd	öd
0	2,13	2,10	2,12	2,25	2,37	2,40	2,38	2,25	2,25
10	1,67	2,33	2,00	1,95	2,23	2,20	2,22	2,27	2,11
20	2,23	2,10	2,17	2,38	2,53	2,30	2,42	2,20	2,29
(EC+mikoriza) öd	2,01	2,18			2,38	2,30			
(EC) *		2,09 b				2,34 a			

(EC × MKRZ : öd EC × VRMKST : öd MKRZ × VRMKST : öd EC × MKRZ × VRMKST : öd); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir; \* : Uygulamalar arasındaki farkın %0,5 düzeyinde önemli olduğunu gösterir (P≤0,05).

Momirovic ve ark. (2000) tarafından farklı organik ortamların biberde fide büyümesi ve kalitesi üzerindeki etkisinin araştırıldığı çalışmada, yaprak sayısı 6,1 ile 6,7 adet arasında bulunmuştur. Abdel-Razzak ve ark. (2019) kompost ve torf ortamlarının acı biber fidesi yetiştiriciliğinde kullanıldığı çalışmada, yaprak sayısı 5,40-5,90 adet olarak bulunmuştur. John ve Prabha (2013) Biber fide kalitesini belirlemek amacıyla organik(vermikompost) ve inorganik (sentetik gübre) ortamları deneği çalışmasında, yaprak sayısı, kök uzunluğu ve sürgün uzunluğu bakımından en iyi sonucu vermikompost uygulamasından elde etmişlerdir.

Markoviç ve ark. (2000) tatlı biberde farklı (torf, kompost + torf, kompost, torf ve zeolit) yetiştirme ortamları üzerine yaptıkları çalışmada en yüksek yaprak sayısı (6,5 adet) 2:1 torf: zeolit ortamından elde edildiğini bildirmişlerdir. Farklı sebze türleri üzerine yapılan çalışmalarda, Akbaşak ve Koral (2014), çeltik kabuğu ve torf ortamının farklı kombinasyonlarını hıyar fideleri üzerinde deneği çalışmada, çeltik kabuğunun torf ile kombine edildiği uygulamalarda, fideler arasında yaprak sayısının 4,11 ile 5,33 adet arasında değiştiğini bildirmişlerdir. Sönmez (2017) domates bitkisinde yetiştirme ortamı olarak torf, perlit ve atık mantar

kompostunu deneği çalışmada, en fazla yaprak sayısı (5,03 adet) karışımında %70 mantar kompostu bulunduran ortamdan elde edilmiştir. Aktaş ve ark. (2013) Mantar kompostu, volkanik tuf, kokopit, perlit ve talaş bulunan çeşitli yetiştirme ortamlarının patlıcan bitkilerindeki yaprak sayısı üzerindeki etkisinin önemsiz olduğunu bildirmişlerdir.

Biber bitkisinin yaprak yaş ağırlığı üzerine sadece EC uygulamasında %1 düzeyinde önemli bir fark oluşmuş, diğer uygulamalar arasında istatistiksel olarak önemli bir fark oluşmamıştır (Çizelge 7). En yüksek yaprak yaş ağırlığı %0 vermikompost uygulamasında (21,83 g), en düşük yaprak yaş ağırlığı ise %20 vermikompost uygulamasında (21,41 g) gözlenmiştir. Markoviç ve ark. (2000) tatlı biberde farklı (torf, kompost + torf, kompost, torf ve zeolit) yetiştirme ortamları üzerine yaptıkları çalışmada en iyi sonuç 21,6 cm fide boyu, 2,5 g fide ağırlığı %16,3 kuru madde ile 2:1 torf: zeolit ortamından elde edilmiştir. Durukan, (2004) tütün tozu kompostunun saf ve farklı oranlarda bazı sebze türlerinde (domates-biber) fide yetiştirme ortamı olarak kullanılması üzerine yapılan çalışmada, yaş ağırlık domates bitkisinde 10,70-0,53 g arasında biber bitkisinde 12,46-0,52 g arasında olduğunu bildirmişlerdir.

Çizelge 9. Biber bitkisinde organik gübre uygulamalarının kök yaş ağırlığı (g) üzerindeki etkileri

Table 9. Effects of organic fertilizer applications on root fresh weight (g) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	öd	-	+	öd	öd	öd
0	11,33	11,67	11,50	11,17	11,00	14,33	12,67	13,00	12,08
10	12,67	13,67	13,17	13,17	13,67	12,33	13,00	13,00	13,08
20	13,33	12,00	12,67	13,67	14,00	16,67	15,33	14,33	14,00
(EC+mikoriza) öd	12,45	12,44			12,89	14,44			
(EC) öd		12,44				13,67			

(EC × MKRZ : ödEC × VRMKST : öd MKRZ × VRMKST : ödEC × MKRZ × VRMKST : öd); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir.

Çizelge 10. Biber bitkisinde organik gübre uygulamalarının kök kuru ağırlığı (g) üzerindeki etkileri

Table 10. Effects of organic fertilizer applications on root dry weight (g) in pepper plants

Vermikompost Oranları (%)	EC 0,5				EC 1				
	Mikoriza	Mikoriza	EC+V	M+V	Mikoriza	Mikoriza	EC+V	M+V	EC+M+V
	-	+	öd	öd	-	+	öd	öd	öd
0	0,98	0,94	0,96	1,00	1,02	1,02	1,02	0,98	0,99
10	0,99	0,93	0,97	0,99	1,00	1,02	1,01	0,97	0,98
20	0,94	0,93	0,94	0,97	1,01	1,01	1,01	0,97	0,97
(EC+mikoriza) öd	0,97	0,94			1,01	1,02			
(EC) **		0,95 b				1,01 a			

(EC × MKRZ : ödEC × VRMKST : öd MKRZ × VRMKST : ödEC × MKRZ × VRMKST : öd); öd : Uygulamalar arasındaki farkın önemsiz olduğunu gösterir; \*\* : Uygulamalar arasındaki farkın %0.1 düzeyinde önemli olduğunu gösterir (P≤0.01).

Biber bitkisinin yaprak kuru ağırlığı üzerine EC uygulamasında %5 düzeyinde önemli ancak diğer uygulamalar arasında farkın önemsiz olduğu gözlenmiştir (Çizelge 8). En yüksek yaprak kuru ağırlığı (2,29 g) %20 vermikompost uygulamasında, en düşük yaprak kuru ağırlığı (2,11 g) %10 vermikompost uygulamasında görülmüştür. Durukan (2004), saf ve farklı oranlarda tütün tozu kompostunun bazı sebze türlerinde (domates-biber) fide yetiştirme ortamı olarak kullanılabilirliği üzerine bir çalışma yürütmüştür. Domates bitkilerinde kuru ağırlık 1,46 ile 0,22 g arasında, biber bitkilerinde ise 1,54 ile 0,13 g arasında olduğunu bildirmiştir. Yılmaz ve ark. (2018) domates fidesi gelişimi üzerine farklı organik ortamların etkinliğinin değerlendirildiği çalışmada, en yüksek bitki kuru ağırlığı %25 atık mantar kompostu ve %75 torf ortamı içeren uygulamada (0,245 g), en düşük ise %100 perlit ortamından (0,006 g), elde edilmiştir.

Biber bitkisinde, kök yaş ağırlığı üzerine uygulamalar arasında önemli bir fark gözlenmemiştir (Çizelge 9). Ortamlar birbirleri ile karşılaştırıldığında en yüksek kök yaş ağırlık 14,00 g ile % 20 vermikompost uygulamasından en düşük ise 12,08 g ile %0 vermikompost uygulamasından elde edilmiştir (Çizelge 9). Atmaca, (2012) domates ve hıyar bitkileri üzerinde 2011 ve 2012 yıllarında vermikompostun fide yetiştirme ortamı olarak kullanıldığı çalışmada, domates üst aksam yaş ağırlık 2011 yılında ortalama 15 g, 2012 yılında ise 18,97 g arasında olduğu bildirilmiştir. Abdel-Razzak ve ark. (2019) kompost ve torf ortamlarının acı biber fide yetiştiriciliğinde kullanıldığı çalışmada, kök ağırlığı 3,40-6,78 g arasında olduğunu bildirmişlerdir. Farklı sebze türünde yapılan çalışmada, Akbaşak ve Koral, (2014) çeltik kavuzu ile torf ortamlarının farklı oranlarda kombinasyonlarını hıyar fidesi üzerinde denedikleri çalışmada, kök ağırlığını 23,33-56,78 g arasında bulmuşlardır.

Domates fidelerinin kök kuru ağırlığı üzerine sadece EC uygulamasında %1 düzeyinde istatistiksel olarak

önemli bir fark gözlenmiştir (Çizelge 10). Abdel-Razzak ve ark. (2019) kompost ve torf ortamlarının acı biber fide yetiştiriciliğinde kullanıldığı çalışmada, kök kuru ağırlık 0,43-1,03 g olarak bulunmuştur. Küçükyumuk ve ark. (2014) vermikompost ve mikorizanın biber bitkileri üzerindeki etkileri üzerine yapılan bir çalışmada, her iki (vermikompost-mikoriza) uygulamasında bitkinin yaş, kuru ve besin alımını artırdığı bildirilmiştir.

## Sonuç

Birçok araştırma, vermikompost ve mikoriza gibi organik gübrelerin kullanımının topraktan besin alımını artırdığı, bitki büyümesini desteklediği, topraktaki organik ve humik madde oranını artırdığı ve mikrobiyal aktivitenin yükselmesine katkı sağladığını göstermektedir. Günümüz fide üretiminde en yaygın kullanılan torf ve kokopit gibi ortamlar yurt dışından ithal edilmekte, bu da maliyetlerin yükselmesine ve dolayısıyla fide üretim maliyetinin artmasına neden olmaktadır. Fide üretiminde artan maliyeti azaltmanın yollarından biri fide harçlarına belli oranda organik materyaller ortamlara dahil edilerek bir nevi maliyetin azaltılması sağlanabilmektedir.

Bu çalışmanın bulguları, EC, mikoriza ve vermikompost dozlarının uygulamalar üzerindeki etkileri farklılık göstermektedir. Vermikompost+mikoriza+EC uygulamasının gövde çapı üzerinde daha iyi sonuç verdiği ancak diğer uygulamalar üzerinde vermikompost dozlarının herhangi bir etkisinin olmadığı görülmüştür. Vermikompost dozları arttıkça fide boyu ve hipokotil uzunluğunda bir artış olduğu ancak bu artışta EC ve EC+mikoriza uygulamalarının daha fazla etkisinin olduğu görülmektedir. Sonuç olarak fide harcına belirli oranlarda vermikompost ve mikoriza ilavesinin bitki gelişimini artırdığını ve organik kökenli materyallerin karışıma eklenmesi sonucunda sentetik gübrelerin etkisinin arttığını söyleyebiliriz.

**Beyanlar****Yazar Katkı Beyanı**

Yazarların makaleye eşit şekilde katkıları bulunmaktadır.

**Çıkar Çatışması**

Yazarlar, herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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## Evaluating the Efficacy of Organic and Inorganic Seed Priming Methods in Promoting Cucumber Germination and Growth

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Cucumber  
Germination  
Seed priming  
Seedling

### ABSTRACT

Seed priming is a promising pre-sowing physiological treatment that utilizes a high osmotic potential solution and stores reserves in seed material to enhance germination. This study is aimed to investigate the influence of different seed priming methods in the germination and early seedling stages of cucumber. A completely random design (CRD) was used for the experiment, including six priming treatments, i.e., T1 (control), T2 (hydropriming), T3 (halopriming 0.5% NaCl), T4 (osmopriming 0.5% PEG), T5 (buffalo milk), and lastly T6 (cow urine). There was a significant effect of seed priming; the highest water imbibition was observed on halopriming (53.71%) and buffalo milk (53.53%); however, the highest germination percentage was observed in cow urine (93.75%), with the least mean germination time (3.65). The highest seedling length, root lengths, and shoot length were also observed on cow urine priming. The dry weight and moisture content were also observed to be highest in cow urine priming. Different priming techniques significantly impact cucumber seed germination and growth. Cow urine priming is the most effective, cost-effective, and environmentally friendly pre-sowing method for cucumber seedlings, benefiting farmers and promoting better germination without harming the environment.

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### Introduction

Cucumber (*Cucumis sativus* L.) belongs to Cucurbitaceae Family which chromosome number is  $2n=14$  (J et al., 2013a). It is a summer vegetable crop. The worldwide production of cucumber is increased by 2.3% from 2021“List of Countries by Cucumber Production”. But fulfilling the demands of the increasing population is quite challenging. It is might due to low germination percentage or low quality of seed. Quality seeds are essential for achieving successful germination and crop growth. Germination is a crucial process during this period, various physiological and biochemical activities occur, and softening of the seed coat which influences the emergence of radicles and growth of the embryonic axis (Suksa-ard et al., 2024). . Numerous biotic and abiotic variables affect the germination of seeds. It could differ from seed to seed, therefore germination might not happen all at once or consistently. To overcome this type of problems different alternative methods are use and one of the most commonly used method is seed priming (Abdullahi et al., 2021). Seed invigoration is a powerful and widely used alternative strategy to enhance seed vigor which is economically viable, environment friendly, and cost effective (Budhathoki et al., 2024).

Seed priming is one of the most promising pre-sowing physiological treatment where high osmotic potential solution is use and seed material utilizes store reserve that can enhance seed germination and improve tolerance to environmental stresses (Dar & Amir, 2024). Different types of priming are used such as hydropriming, halopriming, osmopriming, hormonal priming, biopriming and nutrient priming. At present, different priming techniques are use so they are categorized as traditional and advanced methods. Traditional priming includes hydropriming, halopriming, chemical priming, osmopriming and biopriming. Using any physical agents like microwaves, magnetic fields ultraviolet and other laser radiation falls under advanced seed priming (Lazim & Ramadhan, 2023).

Hydropriming is one of the most widely used and cost effective among others. Seeds are soaked in tap water for the different time periods and redrying seeds it also activates the metabolic activities (Adhikari et al., 2021a). Due to high water potential water enters the seed ad is absorbed (J et al., 2013b). Hydropriming application of different duration caused positive effects such as 18-h period increased corn germination and seedling growth

(Dawadi et al., 2023). It helps to faster germination and stronger seedling growth of crops. In halopriming seeds are soaked in inorganic salts such as sodium chloride (NaCl), potassium nitrate (KNO<sub>3</sub>) and calcium chloride (CaCl) solution before sowing. Initially, halopriming lowers the electrical conductivity (EC) of leachates from seeds, decreasing the amount of salt released. This decrease in salt content lessens the detrimental effects of salinity and enhances the seed germination and seedling growth even in saline soil condition (Pandey & Bhanuprakash & Umesha, 2017). Osmopriming means high water potential that rapidly enters water into the seed and leads to seed imbibition. For this, seeds are soaked in an osmotic solution like; sugar, polyethylene glycol (PEG), glycerol and mannitol (Jarrar et al., 2024). Osmosis prevents the intake of excess water into seeds during imbibition, which reduces the accumulation of reactive oxygen species and protects the cell from oxidative damage (Y. Wang et al., 2023). When seeds are osmotically primed, their solute concentration can be raised, which lowers their water potential and increases the rate of germination uniformly. PEG is widely using as an osmotic agent as compare to other and it boosts the antioxidant enzyme activities, helps to stabilize the cell members, increases compatible solutes like proline and enhance the germination without triggering radicle emergence (Ekeruo et al., 2024). Nutrient priming is a technique where seeds are soaked in different nutrient containing solutions like vit, potassium, zinc and other micronutrient and enhances the nutrient content and its efficiency. In biopriming, seeds are soaked in different microbial solution which enable to entry of beneficial microorganisms like *Bacillus spp*, *Trichoderma spp* and *Pseudomonas spp* which help to nutrient uptake and fasten the germination of seed. Bio priming not only enhance the germination also protect from biotic and abiotic stresses. It makes resilience to different adverse environmental factors (Quality, 2024) It also helps to production of plant hormone (Phooi et al., 2023). Hormonal priming is also another technique in which different plant growth regulator (PGR) use like GA<sub>3</sub>, abscisic acid, auxin and salicylic acid (Tan, 2024). It helps to improve the harmful effect of abiotic stress and also maintains the male and female flower ratio that makes the increase in germination and crop production (G. Wang et al., 2024). Cow urine and milk priming may revolutionary method in the current situation and a great substitute for other nutritional and biopriming. Cow urine contains approximately 1.0% nitrogen, residues of P<sub>2</sub>O<sub>5</sub> and roughly 1.0% K<sub>2</sub>O, ammonia, vitamin, enzymes and other nutrients (Raj Joshi & Adhikari, 2019). Moreover, cow milk is easily available, accessible and fresh milk consistency helps to break the physiological dormancy of seed and enhance the seed germination (Shreevastav et al., 2023). Cow urine is a universal cure-all for ailments and cows are mobile pharmacies. The inhibitory response to seed emergence, shoot growth and seedling vigor index all are influence by the presence of Fe, urea, estrogen and progesterone in cow urine (Choudhary et al., 2023).

There are difficulties in timely seed germination which makes late production of vegetable crops eventually reduction in yield. Poor seed quality, susceptibility to insect pest and diseases, and lack of synchronized seed germination at sub-optimal condition obstructs

germination and growth of plants (Anwar et al., 2020). There is a presence of high male and female flower ratio that makes less production. There is a huge understanding gap about benefit of priming in farmer's fields. There is lack of sustainable and easily accessible priming method. There is always debate in which one is the ideal priming method. From this study cucumber producers, retailer and other marketing aspects get benefited. Priming is one of the promising methods of pre germination treatment. This study was aimed to assess the effect of different priming methods on germination and early seedling growth stage of cucumber. Also, to find the most effective, economical and accessible method of priming to the farmers.

## Materials and Methods

### Experimental Setup

A completely randomized design (CRD) was used to carry out the experiment. Six treatments in all, each replicated four times, were randomized and allocated to twenty-four Petri plates. For the purpose of guaranteeing randomization and removing bias, each Petri plate served as a single experimental unit and was handled independently of the others. There were four replications and a total of six treatments (Table 1) in the completely randomized block design experiment. Treatments were; T1 (control), T2 (hydropriming), T3 (halopriming), T4 (osmopriming), T5 (buffalo milk) and last T6 (cow urine). The experiment employed a total of 24 Petri plates, each of which contained 20 seeds and was considered a separate treatment that was replicated four times. The study was carried out in the IAAS, Gokuleshwor Agriculture and Animal Science Campus laboratory Situated in the mid-hill region.

### Seed Materials

*Cucumis sativus* L. seeds of a local variety called Bhaktapur local were used. The seeds had a minimum germination rate of 70%, and 97 % physical purity.

### Sterilization

In order to prevent contamination, Petri-plates, wash bottles, experimental trays, and all other necessary equipment were surface sterilized using ethanol and formaldehyde solution to avoid the contamination.

### Preparation of Priming Solution

A 1000 mL measuring cylinder was used to measure 100 mL of tap water, which was then added to each beaker for hydropriming. Before usage, 0.5 g of sodium chloride (NaCl) was thoroughly dissolved in 100 mL of tap water to create the 0.5% NaCl solution. Likewise, 100 mL of tap water was thoroughly combined with 1.5 g of polyethylene glycol (PEG 6000) to create the 0.5% PEG 6000 solution. Each beaker was filled with 100 mL of pure, undiluted cow pee for the purpose of priming. Similarly, 100 milliliters of pure buffalo milk were used in each beaker for buffalo milk priming. For a full 24-hour period, the seeds were immersed in their corresponding priming solutions to ensure proper treatment and absorption. To preserve uniformity throughout the experimental setting, care was taken to guarantee that all treatments received the same priming conditions.

### Germination Medium

The substrate that covers the Petri-plate bases is Lokta paper. In sterile Petri dishes, seeds were evenly distributed in a linear fashion to conduct germination tests.

### Data collection

#### Water imbibition (WI)

After weighing the seeds, each treatment was primed with 100ml of room temperature tap water in a separate beaker. In order to determine the proportion of water uptake (imbibition) after priming, the seed weight was once more measured after the surface water was removed (Adhikari et al., 2021a).

$$WI\% = \frac{(WSAP - WSBP)}{WSBP} \times 100\% \quad (1)$$

WSAP: Weight of seed after priming

WSBP: Weight of seed before priming

#### Germination Percentage (GP)

A population of seeds' viability is estimated by the germination percentage. At least 2 mm of radicle length was required for a seed to be deemed germination. Regular observations were made on Petri plates at 12-hour intervals, and the total number of seeds that germinated was noted. The formula used to determine GP was,

$$GP\% = \frac{\text{Total no. of seed germinated}}{\text{Total no. of seed sown}} \times 100 \quad (2)$$

#### Mean germination time (MGT)

MGT is a reliable indicator of how long it takes for a lot to germinate, however it does not provide a good correlation with the uniformity or time spread of germination. A population of seeds germinates faster when the MGT is lower (Orchard, 1977). The MGT as embraced by,

$$MGT = \frac{\sum(n \times d)}{\sum N} \quad (3)$$

Where n= number of seeds germinated on each day, d= number of days from the beginning of the research and N= total number of seeds germinated.

#### Measurement of shoot length

Using a ruler scale (in centimeters), five samples were randomly chosen from each Petri-plate to determine the total length of the shoot length. After 21 days following seed germination, measurements of radicle were made.

#### Measurement of root length

Using a ruler scale (in centimeters), five samples were randomly chosen from each Petri- dishes to determine the length of root length. After 21 days of following seed germination, measurements of radicle length are made.

#### Measurement of seedling length

Five randomly chosen seedlings had their plumule and radical length with great care detached from their radical and measured in centimeters with a ruler.

#### Fresh weight (FW)

When they are uprooted the seedlings were washed with clear water, let to dry out in the shade, and then put on a weighing balance to be weighed.

#### Dry Weight (DW)

Following the measurement of the length of the roots and shoots, the seedlings from each dish were placed inside an envelope, labelled, and left for a 12-hour oven dry at 90°C in a hot air oven. Subsequently, the dry weight of each envelope's seedlings was determined by weighing them.

#### Moisture content

After an hour at 130± 20 C in a hot air oven, two grams of seeds were placed in a petri plate and cooled down in room temperature. Using this formula, the seed moisture content was stated in percentage on wet weight basis (Bereded Sheferie, 2023),

$$MC(\%) = (W2 - W3) / (W2 - W1) \times 100 \quad (4)$$

Where,

W1 = weight of the empty aluminum container

W2 = weight of the empty aluminum container + seeds before drying

W3 = weight of the empty container + seeds after drying

#### Seed vigor index (SV)

The following formula is used to calculate the Seed vigor index (Parvin et al., 2024);

$$SV = (SL \times \text{Germination percentage}) / 100 \quad (4)$$

Where,

SL: Seedling length = Root length + Shoot length

#### Statistical Analysis

The collected data were subjected to Microsoft Excel and analyzed by using R-studio version 4.3.2. The analysis of variance (ANOVA) was used to examine the significant difference for each parameter. Fisher's least significant difference (LSD) was used to establish the comparisons of mean values. The mean values were considered at a 5% significant level.

## Results and Discussion

### Water Imbibition (WI)

This study revealed that, the effect of different priming treatments was found significant difference ( $p \leq 0.001$ ) on water imbibition. Halopriming had the highest response among all treatments with (53.71%) which is statistically similar to buffalo milk priming (53.53%), followed by hydro priming showed moderately effective in water imbibition with (49.95%) which is statistically at par with osmopriming. Without priming (control) showed no any effect however treatment with cow urine (46.69%) is effective but statistically lower than both halopriming and buffalo milk priming which indicates a less potent effect but notable (Table 1). This showed that the treatments like halopriming and buffalo milk were most effective also markedly improved performance in comparison to the control and cow urine.



Table 1. Effect of seed priming on germination and early seedling parameters of cucumber seed (*Cucumis sativus*).

Treatments	WI %	G %	MGT (Days)	SVI	SDL (cm)	RL (cm)	SL (cm)	FW(g)	DW(g)	MC
Control	0.00 <sup>c</sup>	70.50 <sup>c</sup>	6.84 <sup>a</sup>	914.20 <sup>a</sup>	12.54 <sup>ab</sup>	7.74 <sup>ab</sup>	4.77 <sup>ab</sup>	831.95 <sup>b</sup>	92.60 <sup>ab</sup>	88.76 <sup>ab</sup>
Hydropriming	49.95 <sup>ab</sup>	80.50 <sup>b</sup>	4.97 <sup>b</sup>	762.76 <sup>a</sup>	11.40 <sup>b</sup>	6.72 <sup>b</sup>	4.69 <sup>ab</sup>	752.77 <sup>b</sup>	91.00 <sup>ab</sup>	87.94 <sup>b</sup>
Halopriming	53.71 <sup>a</sup>	79.75 <sup>b</sup>	5.32 <sup>b</sup>	839.94 <sup>a</sup>	12.00 <sup>ab</sup>	7.52 <sup>ab</sup>	4.48 <sup>b</sup>	853.17 <sup>b</sup>	86.75 <sup>b</sup>	89.76 <sup>ab</sup>
Osmopriming	48.43 <sup>ab</sup>	85.50 <sup>b</sup>	5.18 <sup>b</sup>	940.98 <sup>a</sup>	13.54 <sup>a</sup>	8.56 <sup>a</sup>	5.04 <sup>ab</sup>	841.42 <sup>b</sup>	96.65 <sup>ab</sup>	88.39 <sup>ab</sup>
Buffalo Milk	53.53 <sup>a</sup>	71.00 <sup>c</sup>	4.83 <sup>bc</sup>	757.68 <sup>a</sup>	11.14 <sup>b</sup>	6.73 <sup>b</sup>	4.40 <sup>b</sup>	852.50 <sup>b</sup>	94.52 <sup>ab</sup>	88.74 <sup>ab</sup>
Cow Urine	46.69 <sup>b</sup>	93.75 <sup>a</sup>	3.65 <sup>c</sup>	959.24 <sup>a</sup>	13.93 <sup>a</sup>	8.76 <sup>a</sup>	5.22 <sup>a</sup>	1063.15 <sup>a</sup>	103.50 <sup>a</sup>	90.21 <sup>a</sup>
Mean	42.06	80.16	5.13	862.46	12.42	7.67	4.76	865.82	94.17	88.97
Sem (±)	13.3	27.47	0.73	26118	1.74	1.04	0.19	12021	91.52	1.65
CV	8.66	6.53	16.68	18.73	10.63	13.29	9.31	12.66	10.15	1.44
LSD	5.41	7.78	1.27	240.08	1.96	1.51	0.65	162.87	14.21	1.90
F-Test	***	***	**	NS	*	*	NS	*	NS	NS

\*\*\*significant at  $p \leq 0.05$ , \*\*Significant at  $p \leq 0.01$  level of significant, \*\*\*Significant at  $p \leq 0.001$  level of significant, NS Non-significant, Water imbibition percentage (WI%) Germination percentage (G%), Mean germination time (MGT), Seed vigor index (SVI), Shoot length (SL), Root length (RL), Seedling's length (SDL), Fresh weight (FW), Dry weight (DW), and Moisture content (MC), LSD: Least significant difference and CV: Coefficient of variation, and Standard error mean (SEM). Means with the same letter denotes the treatment are not significantly different.

Through the activation of  $\beta$ -1, 3-glucanase, chitinase, and thaumatin-like protein genes, hydropriming and osmotic priming provide resistance against *Aspergillus niger* in wheat (*Triticum aestivum* L.), hydropriming and osmopriming also performed well but were slightly less effective (Gul et al., 2022). Research shows that seed priming treatments, like hydropriming, can significantly enhance seed performance by triggering metabolic pathways and promoting water absorption. Halopriming optimizes moisture content and nutrient availability for better seedling establishment, while buffalo milk priming may enhance seed vigor through organic additives in seed treatment (Adhikari et al., 2021b).

#### Germination Percentage (GP)

During this study, ANOVA demonstrated significant results ( $p \leq 0.001$ ) in germination percentage among different priming methods. Priming with cow urine had the highest germination percentage with (93%) outperformed all other treatments followed by priming with osmotic solutions (85.50%) which significantly improves seed germination. The lowest GP was recorded in control (70.50%) which resulted in poor seed germination. The effects of Hydropriming (80.50%) and halopriming (79.75%) were statistically similar which means both had improved germination rates compared to control which revealed that water and salt-based priming can enhance the seed viability. However, priming with buffalo milk (71.00%) showed no substantial improvement over control. Studies showed that, cow urine priming aids in breaking down macromolecules, enhances enzymatic action and makes the availability of nitrogen,  $K_2O$ ,  $P_2O_5$ , Iron, Urea, uric acid and other minerals makes faster in seed germination and growth of seedling. This result was supported by (Deeksha et al., 2023; Damalas et al., 2019). Hydropriming and halopriming showed positive results, but buffalo milk showed minimal improvement, suggesting different organic ingredients' effectiveness in seed germination, necessitating further research.

#### Mean Germination Time (MGT)

Analysis of variance showed that, seed priming technique has a considerable impact ( $p \leq 0.01$ ) on the mean germination time (MGT) of cucumber seeds. The highest

mean germination time resulted with no priming (6.84 days) which means cucumber seeds took the longest time without priming. The germination period was greatly shortened by hydropriming (4.97 days), halopriming (5.32 days) and osmopriming (5.18 days) when compared to the control nevertheless, there were no significant differences across the treatments. Priming with buffalo milk also reduced the MGT, performing slightly better than above mentioned treatments but is not statistically different from hydro, halo and osmopriming. The shortest mean germination time (3.65days) was obtained in priming with cow urine compared to all other treatments due to its unique chemical properties, and showing the most effective method followed by buffalo milk which also performed well (Mekonnen et al., 2024). This study highlights the importance of seed priming techniques in boosting cucumber seed germination rates and reducing mean germination time. Cow urine priming showed a significant reduction in MGT, suggesting its nutrient-rich composition accelerates metabolic processes. Other methods, like hydropriming, halopriming, and osmopriming, shortened MGT but did not offer distinct advantages (Cifuentes et al., 2023).

#### Seed Vigor Index (SVI)

The statistical analysis findings showed that non-significant ( $p > 0.05$ ) influence on the seed vigor index of different priming treatments on cucumber seed which means statistically, there is no significant difference between the treatments at the level of confidence used. The highest SVI was found in priming with cow urine (959.24) and osmopriming (940.98). This means these treatments might improve cucumber seed vigor and the least SVI was found in hydro priming (762.76). The treatment with water, osmotic agent and buffalo milk had less or no positive impact on seed vigor. High SVI in treatments like cow urine and buffalo milk may enhance seedling development, while hydropriming's lower SVI suggests limited benefits. Impact of Purification Methods Using Cow's Milk and Urine on Kupeelu's Strychnine and Brucine Content (*Strychnos nuxvomica* Linn.) Seeds, Buffalo milk's lack of impact highlights different organic materials' seed performance benefits (Mitra et al., 2011).

**Seedling length (SL)**

Analysis of the result showed that there was a significant effect ( $p \leq 0.05$ ) of priming on the seedling length of cucumber. The result depicted the longest seedling length in cow urine pre-treatment (13.93cm), which is statistically at par with osmopriming (13.54cm). Followed by control (12.54 cm), halopriming (12.00 cm) and hydropriming (11.40cm). Priming with buffalo milk showed the shortest seedling length (11.14cm) among all the treatments. Compared to the control, while hydropriming, halopriming and buffalo milk did not show a significant benefit. Cow urine and osmopriming are effective priming agents in sustainable agriculture, improving crop performance. Hydropriming, halopriming, and buffalo milk may not provide sufficient advantages for cucumber seedlings, suggesting that nutrient-rich treatments are more effective (Debta et al., 2023).

**Root Length (RL)**

Data analysis of ANOVA showed significant variation ( $p \leq 0.05$ ) in root length among different seed priming treatments. The highest root length (8.76cm) was observed in cow urine priming which was statistically at par with osmopriming (8.56cm), followed by control (7.74 cm) and halopriming (7.52cm). The shortest root length (6.72 cm) was found in Hydropriming followed by priming with buffalo milk (6.73 cm) but these were not statistically different. Cow urine when compared to other treatments and they promote the root development of cucumber seeds this finding was supported by (R. K. Kumar et al., 2022). These treatments provide better growth conditions and physiological responses, highlighting their potential as sustainable agricultural inputs (SHARMA et al., 2021).

**Shoot Length (SL)**

This study revealed that no significant difference ( $p > 0.05$ ) was found in the shoot length of cucumber seed under different priming treatments. The longest shoot length (5.22cm) found treatment with cow urine followed by osmopriming (5.04cm), and control (4.77cm) which was statistically similar with hydropriming (4.69 cm). The shortest shoot length was found in priming with buffalo milk (4.40cm) however halopriming resulted in slightly longer shoot length (4.48cm) than buffalo milk treatment. Nevertheless, cow urine had a significantly positive effect on cucumber shoot growth compared to halopriming and buffalo milk treatments (Krishnaveni & Mamatha, 2021). Osmopriming positively impacts cucumber shoot length, but environmental conditions or seed genetics may influence growth outcomes (Tarchoun et al., 2024). Hydropriming, control, and halopriming methods may not significantly improve shoot length.

**Fresh weight (FW)**

The table demonstrated that the priming techniques had a substantial impact ( $p \leq 0.05$ ) on the fresh weight of cucumber. The maximum fresh weight was recorded by priming with cow urine (1063.15g) followed by halopriming (853.17g) which was statistically similar to buffalo milk priming (852.50 g) and osmopriming (841.42 g). Cucumber seed with no treatment was the lowest FW among all the treatments with a mean value of (831.95 g) and even lower than control that means least result was

found in hydro priming with (752.77g). This indicates these methods were less effective in promoting biomass accumulation than halopriming, buffalo milk, and osmopriming and more favorable for biomass production. Among all the treatments cow urine stands out as the most effective treatment for improving the fresh weight of cucumber seedlings, showed that significant increase compared to other treatments Zhang et al. (2021) found that priming techniques significantly impact the fresh weight of cucumber seedlings, with cow urine being the most effective treatment for enhancing biomass accumulation. This is due to its rich nutrient profile, which supports plant growth. Other treatments like halopriming and buffalo milk also showed strong results in promoting fresh weight. However, osmopriming did not offer comprehensive benefits (ANWAR et al., 2020).

**Dry weight (DW)**

The priming strategy has no significant ( $p > 0.05$ ) impact on the dry weight of cucumber. The highest DW was found in treatment with cow urine (103g) followed by osmopriming (96.65g) statistically similar with buffalo milk priming (94.52g) whereas halopriming (86.75g) had a lowest dry weight. However, cucumber seed with no treatment (92.60 g) and treatment with water (91.00 g) had slightly higher DW than halopriming which means these treatments performed moderately in terms of DW but not best. Cow urine may provide essential nutrients that significantly enhance solid biomass production which made this most effective treatment for improving DW among all other treatments. The study found cow urine as the most effective strategy for enhancing biomass accumulation in cucumber seedlings, attributed to its nutrient-rich composition (Ambigalakshmi N et al., 2023). Other treatments like osmopriming and buffalo milk priming also showed promising results, but halopriming and hydropriming may not provide sufficient benefits for biomass accumulation (V. K. Kumar & Rajalekshmi, 2021).

**Moisture Content (MC)**

Non significant result ( $p > 0.05$ ) was found in the moisture content of cucumber under different priming treatments. The highest MC was exhibited by cow urine priming (90.21%) whereas hydropriming resulted in the lowest moisture content (87.94%) among all other treatments. This revealed that seedling's ability to retain moisture may be slightly reduced by hydropriming, possibly as a result of a lower water absorption efficiency. However, cow urine primed with higher water retention may have resulted from increased nutrient availability, which improved biomass accumulation and overall growth than all other treatments (Muhammad et al., 2015). The study found that cow urine priming may improve moisture retention in cucumber seedlings, possibly due to its nutrient-rich composition, which enhances growth and biomass accumulation. However, hydropriming's lower moisture content may not be as effective in promoting water retention, potentially hindering the seedlings' growth potential (S. & Aliero, 2004).

**Conclusion**

This study concludes that cucumber seeds show significant impact over the unprimed for various germination and early seedlings parameters. Based on the

present findings, reveal that different priming techniques have a substantial effect on germination and growth of cucumber seedlings. From this experiment, it was found that the highest water imbibition was obtained in halopriming followed by buffalo milk priming. Cow urine priming shows better results over germination percentage, seedling length, root length, and fresh weight in successive days but in the case of seedling length and root length result was statistically at par with osmopriming. Also, the mean germination time of cucumber found better in cow urine priming. This research widened the possibility of cow urine priming of seeds before sowing may result in faster seed germination in high germination percentage and better growth and development of cucumber seedlings through organic, cost-effective, easily access and sustainable way. Hence, seed priming with cow urine seems most effective over other priming techniques. Also, it is cost effective, easily available, environment friendly, organic and sustainable techniques of pre-sowing method. From this finding, most of the farmers benefited by use of cow urine priming with better germination of seeds in lower cost without harming the environment.

## Declarations

### **Ethics approval and consent to participate**

Not applicable

### **Consent for publication**

Not applicable.

### **Author contribution statement**

All author contributed equally.

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### **Data availability statement**

Data will be made available on request

### **Declaration of interest's statement**

The authors declare no conflict of interest.

### **Additional information**

No additional information is available for this paper.

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## Effect of Manure Addition After Reclamation of Saline-Sodic Soils on Soil Properties and Barley Yield

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 24.10.2024 Accepted : 18.11.2024</p> <p><b>Keywords:</b> Saline-sodic soil Farm manure Soil hydraulic conductivity Porosity Sodium exchange capacity</p>	<p>This study was conducted to determine the effect of farm manure applied after reclamation to saline-sodic soils on some important soil properties of the soil and barley yield. In the analyzes made after the plant harvest, it was determined a decrease in the pH, CaCO<sub>3</sub>, exchangeable Ca, and Na, CEC, ESP, bulk and particle densities of the soils farm manure applied and barley grown. There was an increase in organic matter, porosity (%), aggregate stability, hydraulic conductivity, field capacity, wilting point and available water content values were detected. It has been determined that manure addition to soils cause a decrease in pH values, a significant increase in the amount of organic matter and a slight increase in EC values. ESP values in the soil also decreased significantly by the increase in the amount of manure applied. It was seen that high doses of manure application, in both types of manure, cause a decrease in the cation exchange capacity, while an increase in hydraulic conductivity, porosity, aggregate stability and water holding capacity. It was determined that an improvement of barley plant grown in soils farm manure applied at high doses after soil reclamation, the plant height, number of grains and weight per spike, spike number per m<sup>2</sup> and the rate of crude protein.</p>

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## Tuzlu-Sodyumlu Toprakların Islahı Sonrasında Çiftlik Gübresi İlavesinin Toprak Özellikleri ile Arpa Verimine Etkisi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 24.10.2024 Kabul : 18.11.2024</p> <p><b>Anahtar Kelimeler:</b> Tuzlu-sodyumlu toprak Çiftlik gübresi Hidrolik iletkenlik Porozite Değişebilir sodyum yüzdesi</p>	<p>Bu çalışma; ıslah sonrasında tuzlu - sodyumlu topraklara çiftlik gübresi uygulanmasının toprakların bazı önemli özellikleri ile arpa verimine etkisini belirlemek amacıyla yürütülmüştür. Hasat sonrası yapılan analizlerde çiftlik gübresi uygulanmış ve arpa yetiştirilmiş toprakların pH, CaCO<sub>3</sub>, değişebilir Na ve Ca, KDK, ESP, kütle ve tane yoğunluğunda azalma olduğu görülmüştür. Organik madde, porozite; agregat stabilitesi, hidrolik iletkenlik, tarla kapasitesi, solma noktası ve yarayışlı nem değerleri artmıştır. Gübre uygulamalarının pH değerinde düşüşe, organik madde miktarında önemli bir artışa ve EC değeri ise çok az bir artışa neden olduğu tespit edilmiştir. Uygulanan gübre dozunun artışıyla topraklardaki ESP değerlerinin önemli oranda azaldığı belirlenmiştir. Her iki gübre türünde de yüksek dozda gübre uygulamasının toprakların katyon değişim kapasitesi değerlerinde azalmaya, buna karşılık hidrolik iletkenlik, porozite, agregat stabilitesi ve toprağın su tutma kapasitesi değerlerinde artış görülmüştür. İslahtan sonrası gübre uygulanmış topraklarda yetiştirilen arpa bitkisinin 1000 dane ağırlığı, birim alan başına çıkış yapan bitki sayısı ve boyu, başaktaki dane sayısı ve ham protein miktarlarında özellikle yüksek gübre dozlarında iyileşme olmuştur.</p>

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## Giriş

Tarım topraklarından kaynaklı tuzluluk - sodyumluluk sorunu bitkisel üretim açısından verimsizliğe ve çevre sorunlarının ortaya çıkmasına neden olmaktadır. Ülkemizde de tarımsal üretimi kısıtlayan, ürün verim ve kalitesini düşürerek gıda güvenliği üzerine olumsuz etkisi olan bu sorun büyük alanlarda kendisini göstermektedir. FAO (2000) verilerine göre ülkemizde topraklarının 2,8 milyon hektarlık kısmında drenaj, 1,5 milyon hektarlık kısmında tuzluluk ve sodyumluluk, 0,8 milyon hektarlık kısmında ise tuzluluk ve drenaj sorunu bulunmaktadır. Yani 8,5 milyon hektar olan sulamaya uygun arazi varlığımızın %30'dan fazlasında tuzluluk, alkalilik ve drenaj sorunları yaşanmaktadır.

Verimsizliğe neden olan çözünebilir tuzların yıkanarak topraktan uzaklaştırılması, yani topraktaki tuz yükünün azaltılarak eşik değerlerin altına getirilmesi toprakların verimliliğini korumak ve geliştirmek bakımından önemlidir. Sulamada kalitesi iyi suların kullanımı, doğru yöntem kullanılarak sulama yapılması, sulama sahalarına etkin çalışan drenaj sistemi kurulması ile toprağın fiziksel özelliklerini iyileştirilmesi için organik gübre kullanımı toprakların iyileştirilmesi konuları başlıkları arasındadır (Abrol ve ark., 1988).

Toprakta çözünebilir tuzlar, ozmotik basıncı artırarak bitkilerin su alımını güçleştirmekte, yani fizyolojik kuraklığa neden olmaktadır. Toprakta mevcut çözünebilir tuzlar ile değişebilir sodyum bitkiler üzerinde özel iyon etkisi yapmaktadır (Marcelis and Hooijdonk 1999). Öte yandan NaCl ve B gibi diğer iyonlar da bitkilere toksik etki yapmakta olup tuzlu topraklarda bitki yetiştirilmesi güçleşmektedir (Konukcu ve Akbuğa 2006). Tuz stresindeki mısır bitkisinde bitki boyu, bitkilerin toplam yaş ve kuru ağırlıkları ve su içeriğinde düşüş olurken Na, Na/K ve prolin oranı değerleri yükselmiştir (Çiçek ve Çakırlar; 2002),

Ünlükara ve ark. (2010); toprakta tuzluluk oranının bir birim artışıyla patlıcan bitkisinin su tüketiminin %2,1 azaldığı ve bitki veriminin %4,4 düştüğünü belirlemişlerdir. Yine Ünlükara ve ark. (2008) toprakta eşik değeri sayılan 3,48 dS/m üzerindeki tuzluluğun bamyada veriminde %4,2 düşüşe neden olduğunu, Kurunc ve ark. (2011) toprak tuzluluğunun eşik değeri olan 1,2 dS/m'nin üstünde olması durumunda dolmalık biberin veriminde %10,9 azalma olacağını söylemektedirler.

Malkoç ve Aydın (2003), fasulye bitkisinin tuza karşı tepkisini araştırmışlar, uygulanan tuz oranının yükselişiyle bitki gelişiminde yavaşlama olduğunu, ayrıca Cl<sup>-</sup> tuzlarının SO<sub>4</sub><sup>-</sup> tuzlarının verim kaybına etkisinin daha yüksek olduğunu ifade etmişlerdir. Toprak profilinde biriken tuzun bitkilerin tuzluluğa karşı tolerans seviyesinin altına düşünceye kadar yıkanması gerektiği sonucuna varmışlardır.

Yüksek miktarlarda tuz ve sodyum varlığı toprakların kimyasal ve fiziksel özelliklerine olumsuz etki yaparak toprakların verim kabiliyetini azaltmaktadır. Değiştirilebilir sodyum toprağı dispers ederek hidrolik iletkenliğini düşürmektedir (Abrol ve ark. 1988). Sodyumun artışı ise fiziksel yapıyı bozmakta, özellikle ağır bünyeli toprakların infiltrasyon hızını düşürerek bitki gelişimini yavaşlatmaktadır. Tuzluluktaki artışla paralel olarak, sodyumlu toprakların sodyum karbonat ve

bikarbonat düzeyindeki artışla pH değerleri yükselmektedir (Gupta ve ark. 1989).

Sodyumlu toprakların ıslahı; tuzlu toprakların ıslahından daha farklı yöntemlerle yapılmaktadır. Bu yöntemlerde değişebilir sodyum ile kalsiyum iyonlarının yer değiştirme esasına yönelik kimyasal ıslah yöntemlerinden yararlanılmakta olup bunların başında ise sodyumlu topraklara jips uygulamak gelmektedir. Jips (CaSO<sub>4</sub> • 2H<sub>2</sub>O) doğadan daha kolay sağlanabilen bir materyal olup çözülebilir kalsiyum kaynağı (Dorivar ve DeAnn, 2017) olup sodyumlu toprakların yeniden tarıma kazandırılması ve ıslah edilmesi için yaygın olarak kullanılan bir materyaldir.

Üretimde başarılı bitkisel gelişimini sağlayabilmek topraklarının fiziksel ve kimyasal özelliklerinin iyileştirilmesi ile mümkündür. Toprağın fiziksel özellikleri iyileştirilerek yetiştirme ortamının gelişimi ve bitkisel üretimin sağlanabilir (Bender ve ark. 1998). Toprakların fiziksel özelliklerinin iyileştirilmesi organik madde içeriğinin iyileştirilmesi ile sağlanabilir. Bir toprak düzenleyici organik madde ilave edilerek toprakların havalanma, agregat stabilitesi, su tutma kapasiteleri, toplam gözenek miktarları ve hidrolik iletkenlikleri gibi fiziksel özelliklerin geliştirilmesi amaçlanır (Sort ve Alcaniz, 1999; Aggelides ve Londra, 2000).

Angın ve Yağanoğlu (2009) yürüttükleri bir araştırmada; tuzlu-sodyumlu toprakların ıslahında kent çamuru kullanmışlar ve sonuçta toprakların fiziksel ve kimyasal yapısının iyileştiği ve organik materyal ilavesinin ıslahın etkinliğini artırdığını belirlemişlerdir.

Anaç ve Aksoy (2000), toprakları fiziksel özellik bakımından yaptıkları bir ıslah çalışmasında; topraktaki organik madde miktarını artıran çiftlik gübresi uygulaması yaygın olmakla beraber, bu materyalin sınırlı olması nedeniyle yüksek organik madde içeriğine sahip çöp kompostunun da kullanılabileceğini vurgulamışlardır.

Leonardit, çiftlik gübresi, kent çamuru ve kentsel atıklar gibi organik materyaller tarımsal üretimde toprak düzenleyici olarak kullanılmaktadır. Çiftlik gübresinin toprak verimliliğine katkısı; gübrenin mineralizasyon süresi ve olgunlaşma ortamı türüne, gübre türü ve yaşına, hayvaların beslendikleri rasyona, barınaklarda kullanılan yataklık materyal özelliğine bağlı olarak değişmektedir. Organik madde ilavesiyle toprağın strüktürel yapısının iyileşmesini, su tutma kapasitesini artırması, toprağın katı, sıvı ve gaz faz oranlarında dengeleme sağlayarak fiziksel özellikleri sağlanabilmektedir (Canbolat, 1992). Organik materyaller ve organik kökenli gübrelerin kullanımı yaygınlaşma, kimyasal gübrelerin kullanımında düşüşler başlamıştır (Hanay ve ark 2004). Bu durum iyi tarım uygulamalarının başarı ile uygulanması açısından uygun bir gelişmedir.

Toprak ıslahını müteakip diğer kültür bitkileri üretimine geçilmeden önce tuza toleransı yüksek olan buğday gibi bitkiler kullanılmaktadır. Dünyada olduğu gibi ülkemizde de arpa; buğday, mısır ve çeltik büyük alanlarda üretimi yapılmaktadır (Anonim, 2000). Özellikle hayvancılıkta kesif yem hammaddesi olarak kullanılan arpa günümüzde bira sanayisinde ve insan beslenmesinde de kullanılmaktadır (Sarı ve İmamoğlu, 2009). Ülkemizin arpa alanı ve üretim miktarı incelendiğinde; buğdayı takip

etmekte olup ikinci sıradadır (Anonim, 2000). Arpa bitkisinin tuza karşı toleransları çeşitlere göre farklılık gösterse de genelde yüksektir (Özkan ve Topçu, 2017).

Toprak ıslahı organik madde eksikliğini giderilmesine yönelik yürütülen bu çalışmada; tuzun yıkanması ve jips uygulaması ile tuzlu-sodyumlu toprakların ıslahı sonrası çiftlik gübresi ilave edilerek arpa ekimi yapılmış toprakların verimliliği ile fiziksel ve kimyasal özelliklerindeki değişiklikler araştırılmıştır.

## Materyal ve Yöntem

### Materyal

Deneme toprakları; tuzlu sodyumlu alanların büyük olduğu Iğdır Ovasının doğusundan alınmıştır, Aras havzasında yer alan ovanın denizden ortalama yüksekliği 850 m ve alanı 83211 hektardır. Bölgede yıllık ortalama yağış toplamı 260 mm, sıcaklık 12,1°C ve buharlaşma miktarı 1116,3 mm'dir (Temel ve Şimşek, 2011),

Laboratuvarda hava kurusu duruma getirilinceye kadar kurutulmuş ve 2 mm açıklığa sahip elekten elenmiş tuzlu sodyumlu toprak örneklerinin bazı önemli özellikleri belirlenmiştir (Çizelge 1).

Organik madde olarak kullanılan küçükbaş ve büyükbaş hayvan gübrelere Atatürk Üniversitesi Hayvancılık Araştırma Merkezinden alınmıştır. Bu çiftlik gübrelere ilişkin çalışılmış bazı özellikleri Çizelge 2'de sunulmuştur. Araştırmada T2A1 sınıfı sulama suyu kullanılmıştır.

Tuzlu-sodyumlu toprakların ıslahında; doğadan temini daha kolay ve kullanımı yaygınlaşmış etkin bir kimyasal ıslah maddesi olan ve araştırmacılar tarafından da önerilen çözünebilir kalsiyum kaynağı özelliğindeki jips ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) kullanılmıştır (Seenivasan et al., 2016; Dorivar ve DeAnn, 2017), Safiyeti %100'e yakın olan bu materyal öğütülerek toz haline getirilmiş, daha sonra ölçülü olarak topraklara ilave edilmiştir (Hanay vd., 1990), Denemede kullanılan Tokak 157/37 arpa çeşidinin denemenin yürütüldüğü Erzurum'da yaygın üretimi yapılmaktadır (Öztürk ve Akkuş, 2015).

### Yöntem

Deneme; tam şansa bağlı desende 3 tekerrürlü kurulmuş ve yürütülmüştür. Tuzlu sodyumlu topraklar laboratuvarda kurutulup 2,00 mm'lik elekten elenerek elek altında geçen kısım denemede kullanılmıştır. Daha sonra bu topraklar tabanına drenajı ağılayabilecek zarf malzemesi serilmiş 30 cm çaplı plastik deneme kaplarına 20 cm derinliğe yerleştirilmiştir. Uygulanacak jips miktarı Kovda eşitliği kullanılarak 15 cm derinliğindeki toprakların değişebilir sodyum yüzdesini 15'e indirgeyebilecek miktar hesaplanmıştır (Kanber ve Ünlü 2010). Jips uygulanmış topraklar 21 adet drenaj tipi deneme kabına doldurulmuş ve bu kaplar 2,50 cm yüksekten 20 kez düşülerek homojen karışımı sağlanmıştır. Bu işlemi müteakip deneme saksılarına eşit aralıklarla, eşit miktarda su uygulanarak yıkama yapılmıştır. Deneme kaplarının alt kısmına sızan suların EC değeri 4 dS/m düzeyinin altına düşüncüye kadar yıkama yapılmıştır. Toplam 60 günlük sürede deney kaplarına 420 cm yıkama suyu uygulanmıştır (Anapalı, 1994; Hanay, 1990). Bu işlemden sonra deneme kapların yüzeyinden 5 cm derinliğine homojen olarak 20, 40 ve 60 ton/ha düzeyinde gübre ilave edilerek olgunlaşmaya bırakılmıştır. Jips uygulanmış bir deneme kabı (a), yıkama uygulaması yapılmış denem kapları (b) ve gübre ilave edilmiş deneme kapları (c) Şekil 1' de görülmektedir.

Islah işlemi tamamlanan sonra seraya taşınan deneme kaplarına; yazlık olarak sulu koşullarda yetiştirilen arpa çeşidi olan Tokak 157/37 ekilmiştir. Arpa ekimi 20 Şubat, hasadı 20 Temmuzda yapılmıştır. Bitki ekim yapılırken 575 adet / m<sup>2</sup> bitki sıklığı hesabıyla her deneme kabına 44 tohum ekilmiştir (Öztürk ve Akkuş, 2015).

Bitki su tüketimleri, ağırlık esasına göre hesaplanmış, 4-5 gün aralıklarla deneme kapları tartımı yapılmış, eksilen toprak nemli tarla kapasitesine ulaşınca kadar sulama yapılmıştır (Safi, 2012).

Hasat işlemi manuel olarak yapılarak, bitkilerin boyu (cm), çıkış yapan bitki sayısı, ortalama başak dane sayısı, arpanın bin tane ağırlığı (gr) ile ham protein oranı (%) Akkaya ve Akten (1990)'in kullandıkları yöntemlerle belirlenmiştir.

Çizelge 1. Deneme topraklarının ıslah öncesi bazı özellikleri

Table 1. Some characteristics of trial soils before reclamation

Parametre	Değer	Parametre	Değer
pH (1:2,5)	9,09	Değişebilir sodyum yüzdesi (ESP), (%)	81,77
Elektrik iletkenlik (dS/m)	14,26	Bünye	Kumlu Killi Tın
Organik madde (%)	1,53	Tarla kapasitesi, %	29,60
Kireç, CaCO <sub>3</sub> (%)	8,90	Solma noktası, %	17,40
Kasyon değ. kap,(KDK) (me/100 gr)	51,18	Tane Yoğunluğu (g/cm <sup>3</sup> )	2,68
Değişebilir Na (me/100 gr)	41,85	Kütle Yoğunluğu (g/cm <sup>3</sup> )	1,22
Değişebilir Ca+Mg (me/100 gr)	8,83	Porozite (%)	54,47
Değişebilir K (me/100 gr)	0,50	Agregat stabilitesi, (%)	58,77

Çizelge 2. Deneme materyali olan çiftlik gübrelere bazı özellikleri

Table 2. Some properties of farm manure used in the study

Türü	pH	EC	OM	N, %	C/N	P, %	K, %
Sığır gübresi	7,54	3,27	46,10	1,57	17,03	1,0	2,0
Koyun gübresi	7,15	3,65	48,3	1,95	14,37	0,60	2,9



Jips uygulanması (a)

Yıkama işlemi (b)

Gübre uygulanması (c)

Şekil 1. Jips uygulama, yıkama ve gübre ilavesi aşamaları  
Figure 1. Gypsum application, washing and fertilizer addition stagesÇizelge 3. Çiftlik gübresi uygulanmış toprakların organik madde, pH, EC ve CaCO<sub>3</sub> (%) değerleriTable 3. Organic matter, pH, EC, and CaCO<sub>3</sub> values of farm manure applied soils

Gübre türü	Gübre dozu (ton/da)	pH	EC (dS/m)	Organik madde (%)	CaCO <sub>3</sub> (%)
Koyun	20	8,25	3,44	2,19	8,49
	40	7,81	3,58	2,72	8,20
	60	7,06	3,72	3,23	7,70
	Ortalama	8,11	3,58	2,71	8,13
Sığır	20	8,21	3,43	2,18	8,29
	40	7,54	3,54	2,64	8,13
	60	7,04	3,74	3,35	7,57
	Ortalama	7,60	3,57	2,72	8,06
Kontrol		8,42	3,37	1,54	9,92

Bouyoucus Hidrometre yöntemiyle toprak tekstürü, bünye sınıfı da tekstür üçgeni kullanılarak (Gee ve Bauder, 1986) belirlenmiş, saturasyon ekstraktından pH-metre ile toprak reaksiyonu (McLean, 1982), EC metre ile elektriksel iletkenliği ölçülmüştür (FAO 2000). Toprakların kation değişim kapasiteleri Rhoades (1982), değişebilir sodyum (ESP) yüzdesi Kanber ve Ünlü (2010)'ye göre, organik madde içeriği ise Smith-Weldon yöntemiyle belirlenmiştir (Nelson and Sommers 1982). Örneklerin kireç içeriği Scheibler kalsimetresiyle ölçülmüş (Nelson, 1982), değişebilir kation (Ca, Mg, Na, K) miktarları, amonyum asetat ile Flame Fotometrede okunmuştur (Rhoades 1982).

Tarla kapasitesi ve solma noktası; basınçlı membranda 1/3 atm ve 15 atm basınç uygulanarak belirlenmiştir (Cassel ve Nielsen, 1986). Örneklerin tane yoğunluğu Piknometre, toprak örneklerinin etüvde 105°C'de 24 saat kurutulduktan sonraki ağırlığının toplam hacme oranlanmasıyla (Blake and Hartge, 1986), porozite ise kütle ve tane yoğunlukları değerleri kullanılarak (Danielson and Sutherland, 1986)'a göre hesaplanmıştır.

Yarayışlı nem miktarı, tarla kapasitesi ile solma noktası farkından (Cassel and Nielsen, 1986) hesaplanmış, agregat stabilitesi ise ıslak eleme yöntemi (Kemper and Rosenau, 1986) ile belirlenmiştir. Toprakların hidrolik iletkenlikleri sabit seviyeli su yükü altında 2 saatlik süreyle satüre durumda ölçülmüş ve Darcy metodu ile hesaplanmıştır (Sahin ve ark. 2020).

Bitkilerdeki ham protein (%) oranı Kjeldahl yöntemiyle belirlenmiş olan azot miktarının 6,25 ile çarpılmasıyla, bitki boyu toprak yüzeyi ile kılçıklara kadar yüksekliğin

ölçülmesiyle, bin dane ağırlığı numune ağırlığının sayılan dane sayısına bölümünün 100 ile çarpılması ve bitki sayısı sayılarak tespit edilmiştir (Öztürk ve Akkuş, 2015).

Sulama sularının pH değeri pH-metre, EC değeri de EC-metre ile ölçülmüştür (Tüzüner 1990). Ca ve Mg miktarları EDTA titrasyon yöntemi, Na ve K fleymfotometrik yöntem CO<sub>3</sub> ve HCO<sub>3</sub> sülfirik asitle titrasyon yöntemi, Cl gümüş nitrat yöntemi belirlenmiştir. SO<sub>4</sub> baryum klorür çözeltisi ile spektrofotometrede, sodyum adsorbsiyon oranı (SAR), sodyum miktarı kalsiyum ve magnezyum toplamının yarısının karekökünü alınmasıyla bulunmuştur (Richards, 1954).

#### İstatistiksel Analizler

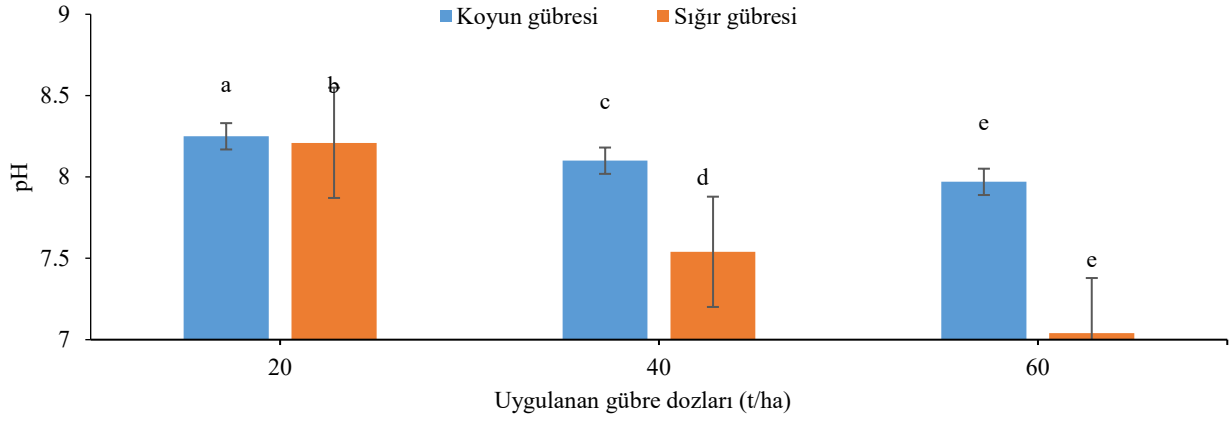
Araştırma sonuçlarına ilişkin varyans analizleri SPSS paket programında yapılmış, daha sonra ortalamaların farklılıkları Duncan çoklu karşılaştırma testi ile belirlenmiştir (Yurtsever, 1984).

#### Araştırma Bulguları

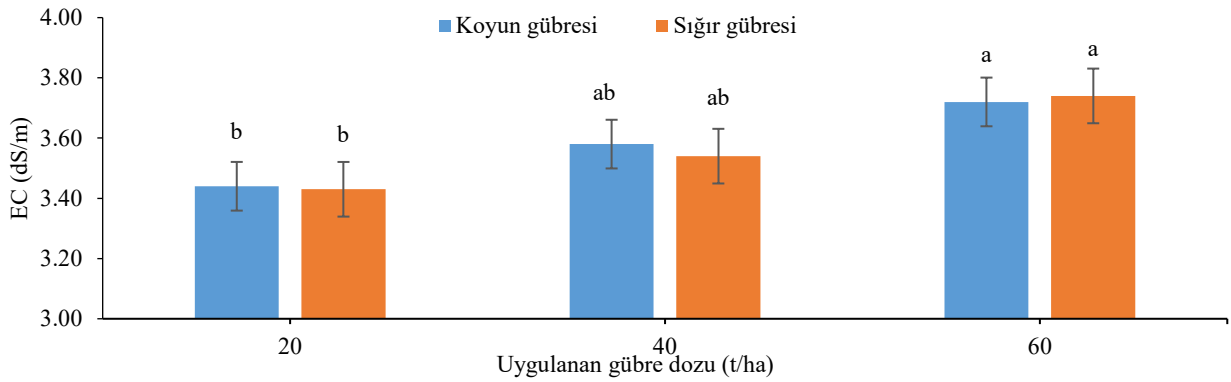
Toprakların çiftlik gübresi ilavesi sonrasında pH, EC, organik madde (%) ve CaCO<sub>3</sub> (%) değerlerindeki değişimler Çizelge 3'ten izlenebilmektedir.

Çizelge 3'te de görülebileceği gibi gübre türlerinde uygulanan gübre miktarının artışıyla toprakların pH değerlerinde düşüş (Şekil 2), EC değerlerinde ise az bir yükseliş görülmüştür (Şekil 3). pH ve EC değerlerindeki değişim bakımından sığır ve koyun gübresi arasındaki fark çok önemli (p<0,01) çıkmıştır.

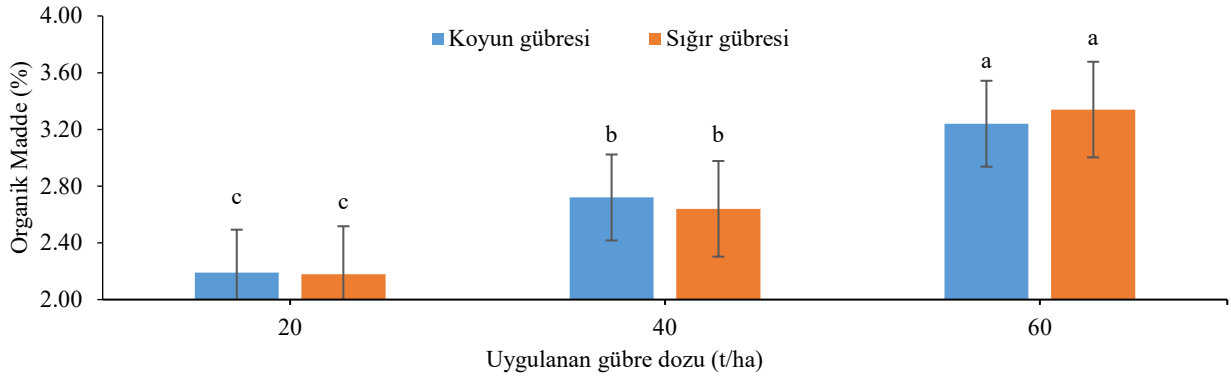




Şekil 2. Uygulanan gübre türü ve dozunda pH değerlerindeki değişim  
Figure 2. Changes in pH values depending on the type and dose of manure applied



Şekil 3. Uygulanan gübre türleri ve dozlarına göre EC değerlerindeki değişim  
Figure 3. Changes in EC values depend on the type and dose of manure applied



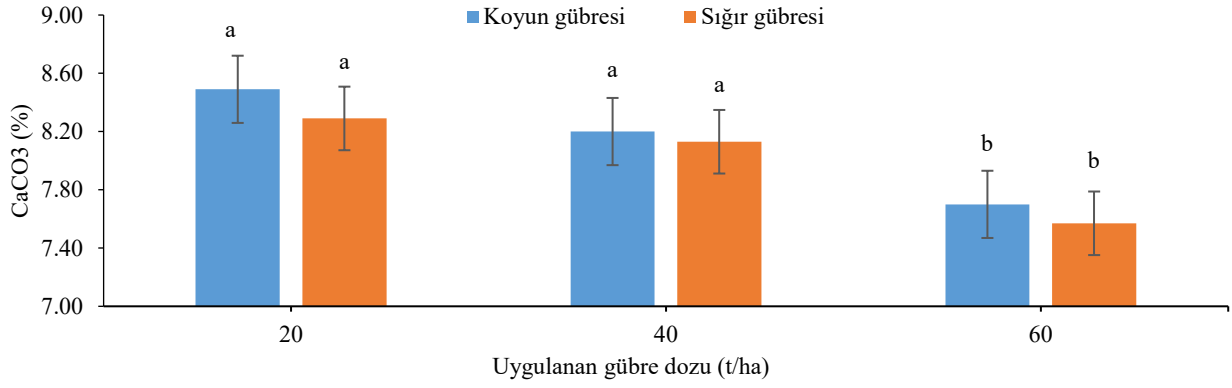
Şekil 4. Uygulanan gübre dozlarının toprak organik madde içeriğine etkisi  
Figure 4. Effect of applied manure doses on soil organic matter content

EC değerlerindeki bu yükselişin toprak organik maddesinin içeriği ile değiştiğini birçok araştırmacı tarafından ortaya konulmuş topraklara uygulanan organik maddelerin EC değerini artırdığını belirtmişlerdir (Ferreira ve ark., 2011).

Organik madde miktarı kontrol grubunda %1,54 olup gübre dozu artışlarıyla yükselerek ortalama %2,72 değerine ulaşmıştır. Denemeye başlamadan önce ve sonra koyun gübresinin uygulanmış topraklarının organik madde miktarları 20 ton/ha, 40 ton/ha ve 60 ton/ha dozlarında sırasıyla %42,2; %76,6 ve %109,7 artış, sığır gübresinin aynı dozlarda uygulanması bu artışların % 41,6; % 71,4 ve % 117,5 yükseliş olduğu belirlenmiştir. Gübre dozlarındaki değişimin organik madde artışına etkisinin

çok önemli ( $p < 0,01$ ), gübre türleri arasındaki farkın önemsiz olduğu belirlenmiştir (Şekil 4).

Toprakların %  $\text{CaCO}_3$  içerikleri; her iki gübre türlerinde doz artışıyla azalış göstermiştir. Topraklara 20 ton/ha, 40 ton/ha ve 60 ton/ha dozundaki koyun gübresi % $\text{CaCO}_3$  değerlerini sırasıyla %7,92; %11,06 ve %16,49; sığır gübresi de %10,09; %11,82 ve %17,90 oranında düşürmüştür. İstatistiksel olarak gübre dozlarının %  $\text{CaCO}_3$  değerlerindeki düşüşe etkisi çok önemli ( $p < 0,01$ ) iken gübre türleri arasındaki farkın önemsiz olduğu belirlenmiştir (Şekil 5). Toprakların  $\text{CaCO}_3$  ile pH değerleri arasında doğrusal ilişki varken gübre dozundaki artışıyla pH ve  $\text{CaCO}_3$  değerlerindeki azalış ilişkileri uyumlu bulunmuştur.



Şekil 5. Gübre tür ve dozlarına göre toprakların CaCO<sub>3</sub> içerikleri  
Figure 5 CaCO<sub>3</sub> contents of soils according to manure type and dose

Çizelge 4. Gübre uygulamalarının toprak Ca, Na, K, Mg, KDK ve ESP içeriğine etkisi  
Table 4. Effects of manure applications on soil Ca, Na, K, Mg, KDK and ESP contents

Gübre türü	Dozu (ton/da)	Ca	Na	K (me/100 g)	Mg	KDK	ESP (%)
Koyun	20	37,39	6,87	2,36	3,09	49,71	13,82
	40	37,33	5,16	2,43	4,87	49,38	10,46
	60	36,99	4,05	2,57	5,41	48,84	8,30
	Ortalama	37,24	5,36	2,45	4,46	49,31	10,86
Sığır	20	37,65	6,76	2,37	2,98	49,76	13,58
	40	36,83	5,00	2,45	5,08	49,36	10,12
	60	36,69	3,86	2,65	5,60	48,81	7,91
	Ortalama	37,06	5,21	2,47	4,55	49,31	10,54
Kontrol		38,47	7,48	2,22	1,76	49,93	14,97

Torakların CaCO<sub>3</sub> ve pH değerlerinin değişimler arasında doğrusal ilişki görülmüştür. Sabtow (2019) tuzlu sodyumlu topraklara stabilize kent çamuru uygulamasıyla inkubasyon süresi sonunda organik fraksiyonunun biyo-bozunmasıyla pH değerinin yükseldiğini, bu esnada organik asitlerin etkisiyle asitleşmeye olduğunu belirtmiştir. Araştırmacı uygulanan gübre dozundaki artışla pH ve CaCO<sub>3</sub> azalışının uyumlu olduğunu belirtmiştir.

#### Gübre Uygulamalarının Ca, Na, K, Mg, KDK ve ESP Üzerine Etkisi

Gübre uygulaması sonrası toprakların her iki gübre türü ve dozlarının artışıyla paralel olarak Ca ve Na içeriklerinde düşüşler gözlenirken Mg ve K içeriğinde artışlar olduğu belirlenmiştir (Çizelge 4).

Her iki gübre çeşidinde de uygulama dozu artışıyla toprakların değişebilir Ca ve Na içeriklerinde çok önemli (p<0,01) azalışlar olmuştur. Koyun gübresi uygulanan toprakların 60 ton/ha dozlarında kontrol konusuna mukayese yapıldığında sodyumun %45,86, aynı dozda uygulanan sığır gübresinin de %48,40 azalışa neden olduğu belirlenmiştir. Her iki çeşit gübrenin 60 ton/ha dozlarının uygulanmasıyla değişebilir kalsiyum miktarının kontrol konusuna göre sırasıyla %3,85 ve %4,63 düzeyinde azaldığı belirlenmiştir. Uygulanan her iki gübre çeşidinde de doz artışına karşılık toprakların değişebilir Mg ve K içeriklerindeki artış farkı çok önemli (p<0,01) bulunmuştur. Değişebilir K miktarındaki artışlar koyun gübresinin 60 ton/ha düzeyinde uygulandığı konularda %15,77 iken aynı doz düzeyinde sığır gübresi uygulanan konularda %19,37 olarak gerçekleşmiştir. Kontrol konusuna karşılaştırıldığında, koyun ve sığır gübresi uygulanmış toprakların değişebilir magnezyum

içeriklerinde 60 ton/ha düzeylerinde sırasıyla 3,07 ve 3,18 kat artış olduğu saptanmıştır. Değişebilir Ca ve Na düzeylerindeki düşüş bakımından gübre dozları arasındaki farklar istatistiksel olarak (p<0,05) önemli, K içeriğindeki azalma önemsiz, Mg miktarındaki azalma çok önemli (p<0,01) bulunmuştur.

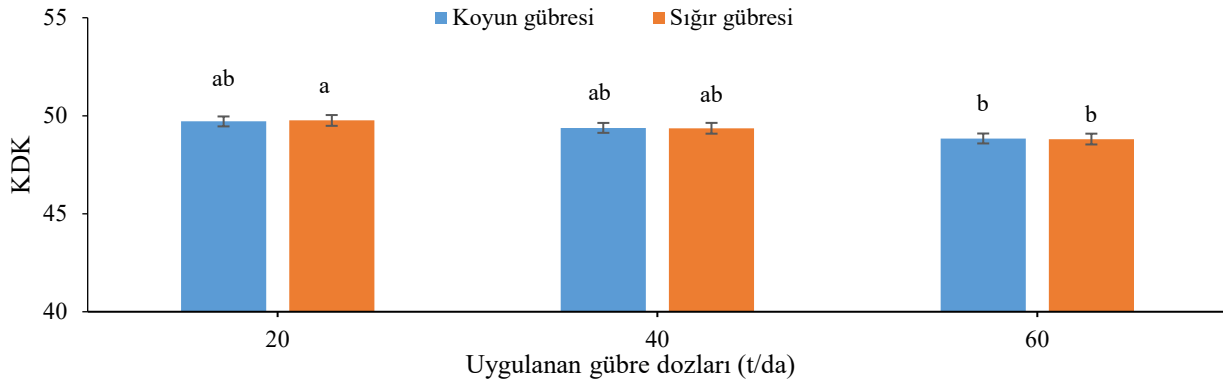
#### Deneme Sonrası Topraklarının Fiziksel Özellikleri

Deneme topraklarının uygulanan gübre sonrasında gübre türü ve dozları bakımından toprak fiziksel özelliklerinde meydana gelen değişim Çizelge 5'te sunulmuştur. Buradan görülebileceği gibi gübre uygulanması sonucunda toprakların kütle yoğunluğu değerlerinde azalma olmuştur. Kütle yoğunluğundaki düşüş açısından bakıldığında gübre çeşidi ve uygulama dozları arasındaki farklılık çok önemli (p<0,01) bulunmuştur. Uygulanan gübrenin toprak parçacıklarının birleştirici etkisi kütle yoğunluklarında azalmalara neden olmuştur (Outhman 2016). Uygulanan 20 ton/ha, 40 ton/ha ve 60ton/ha dozlarındaki koyun ve sığır gübrelere kontrol konusuna kıyasla kütle yoğunluğunda sırasıyla %0,82; %1,64 ve %2,46 düşüşe neden olduğu belirlenmiştir. Önceki çalışmaların birçoğunda da organik madde ilavesiyle toprakların kütle yoğunluklarında azalmalar gözlemlenmiştir (Mondal ve ark. 2015). Yine gübrenin 20 ton /ha düzeyinde bir değişim olmamışken, gübre dozu artışıyla tane yoğunluğu değerlerinde de azalmalar olmuştur (Çizelge 5). Koyun ve sığır gübrelere 40 ton/ha ile 60 ton/ha dozlarında uygulamaları tane yoğunluğunu % 0,37 düşürmüştür. Gübre miktarlarının tane yoğunluğundaki düşüşe etkisi p<0,05 önemli çıkmıştır.

Çizelge 5. Gübre uygulaması sonrası toprakların fiziksel özellikleri

Table 5. Physical properties of soils after fertilizer application

Gübre türü	Gübre dozu (ton/da)	Kütle yoğunluğu (g/cm <sup>3</sup> )	Tane yoğunluğu (g/cm <sup>3</sup> )	Porozite (%)	Agregat stabilitesi (%)
Koyun	20	1,21	2,68	54,84	62,99
	40	1,20	2,67	55,02	65,08
	60	1,19	2,67	55,40	68,03
	Ortalama	1,20	2,67	55,09	65,37
Sığır	20	1,21	2,68	54,79	63,59
	40	1,20	2,67	55,11	68,78
	60	1,19	2,67	55,31	70,10
	Ortalama	1,20	2,67	55,08	66,43
	Kontrol	1,22	2,68	54,47	58,84
Gübre türü	Gübre dozu (ton/da)	Kütle yoğunluğu (g/cm <sup>3</sup> )	Tane yoğunluğu (g/cm <sup>3</sup> )	Porozite (%)	Agregat stabilitesi (%)
Koyun	20	0,015	30,41	17,64	12,77
	40	0,019	30,90	17,85	13,05
	60	0,028	31,48	18,24	13,24
	Ortalama	0,021	30,93	17,91	13,02
Sığır	20	0,016	30,23	17,46	12,78
	40	0,021	30,78	17,86	12,92
	60	0,029	31,24	18,03	13,21
	Ortalama	0,022	30,75	17,78	12,97
	Kontrol	0,015	29,69	17,37	12,32



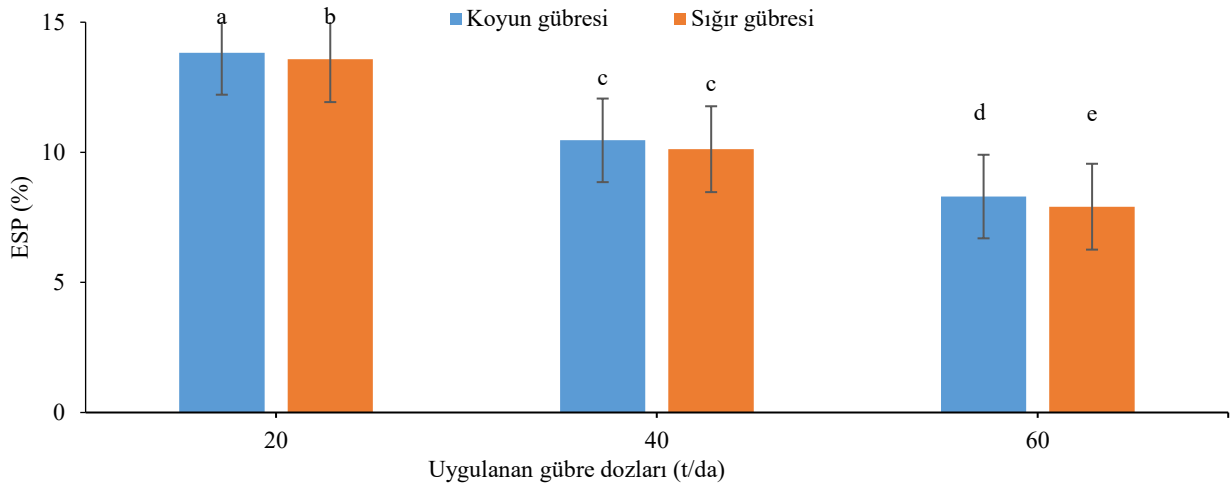
Şekil 6. Gübre tür ve uygulama dozuna bağlı olarak toprakların KDK değerleri  
Figure 6. Changes in the CEC of soils depend on fertilizer type and applied doses

Gübre uygulamaları toprakların porozite değerlerinde artışlara neden olmuştur. Bu artış miktarları 20 ton/ha, 40 ton/ha ve 60 ton/ha dozlu koyun gübresi uygulanan konularda sırasıyla %0,31; %0,64 ve %1,34 iken sığır gübresi uygulananlarda sırasıyla %0,22; %0,81 ve %1,17 olarak gerçekleşmiştir. Uygulanan gübre miktarları ile toprak porozitesi değerleri arasındaki fark çok önemli ( $p < 0,01$ ) bulunmuştur. Gübre dozlarındaki artışla toprakların KDK değerlerinde azalmalar olmuş; bu azalma koyun gübresinin en düşük dozunda %0,44 en yüksek dozda ise %2,18 iken sığır gübresi için bu değerler %0,34 ve %2,24 olarak gerçekleşmiştir. Her iki gübre çeşidinde de KDK değerindeki azalmaları, aslında artan dozlarda düşen pH değerlerini de doğrulamaktadır (Çizelge 3 ve Şekil 6). Gübre uygulama dolarına bağlı toprakların pH ve KDK değerleri arasında pozitif korelasyon oluşmuş, yani toprak pH'sı ile KDK değerlerindeki değişimin benzer olduğu belirlenmiştir (Jaramillo and Restrepo, 2017; Sabtow 2019).

Organik madde düzeyinin iyileşmesi sonucunda gelişen agregasyonun bir sonucu porozite değerlerinde de

iyileşme olmuştur. Gübre uygulamaları toprakların ıslak agregat stabilitesini artırmıştır. Koyun gübresi uygulanan topraklarda agregat stabilitesindeki artış oranları 20, 40 ve 60 ton/ha seviyelerinde sırasıyla %7,05; %10,61 ve %15,62 iken sığır gübresi uygulananlarda %8,07; %16,90 ve %19,13 olmuştur. Uygulanan gübre miktarları ile agregat stabilitesindeki artışa etkisi ( $p < 0,01$ ) düzeyinde çok önemli olup gübre türleri arasındaki fark önemli bulunmamıştır.

Gübre çeşidi ve dozlarındaki değişimin ESP üzerine etkisi çok önemli ( $p < 0,01$ ) bulunmuştur. Gübre dozu artışıyla ESP değerlerinde genel bir azalma görülmüştür. Her iki gübre türünde de ESP 60,0 ton/ha uygulama dozunda en düşük değerine ulaşmıştır. Azalma miktarları uygulanan 20 ton/ha, 40 ton/ha ve 60 ton/ha dozlarında koyun gübresi için sırasıyla %7,68, %30,13 ve %44,56 oranlarında iken sığır gübresi sırasıyla %9,29, %32,40 ve %47,16 oranlarında olmuştur (Şekil 7).



Şekil 7. Uygulanan gübre dozlarına göre ESP değerlerindeki değişim  
Figure 7. Changes in ESP values depending on the type and dose of fertilizer applied

Uygulanan gübre toprakların hidrolik iletkenlik değerlerinde iyileşmeyi sağlamıştır. Ancak hidrolik iletkenlik değerlerindeki değişim ile gübre dozu ve çeşidi arasındaki farklılıklar istatistiksel olarak önemsiz çıkmıştır. Tuzlu-sodyumlu, özellikle sodyumlu toprakların en önemli sorunu serbest drenaj koşullarının iyi olmamasıdır. Bu durumda hidrolik iletkenlikleri düştüğünden toprakların üretim kapasiteleri de düşmektedir (Sabtow, 2019). Tuzlu-sodyumlu toprakların hidrolik iletkenlik değerleri şişme ve dağılma nedeniyle azalmaktadır. Organik maddeler toprak agregasyonunu geliştirerek hidrolik iletkenliğini yükseltmektedir (Armstrong ve Tanton, 1992).

Gübre uygulamaları toprakların; tarla kapasitesini, solma noktasını ve dolayısıyla yarayışlı nem kapasitelerini yükseltmiştir. Koyun gübresinin 20 ton/da, 40 ton/da ve 60 ton/ha uygulama düzeyleri tarla kapasitesini sırasıyla % 2,43; %4,08; %6,03 artırmıştır. Sığır gübresinin aynı uygulama düzeyinde uygulanmasıyla bu artış değerleri sırasıyla %1,82; %3,67; %5,22 olmuştur. Tarla kapasitesindeki yükseliş ile gübre dozları arasındaki fark çok önemli ( $p<0,01$ ) iken gübre türleri farkı önemsiz bulunmuştur (Şekil 7).

Toprakların solma noktası değerlerinde de yükseliş olmuş, 20 ton/ha, 40 ton/ha ve 60 ton/da düzeyinde uygulanan koyun gübresi toprakların solma noktası değerlerini sırasıyla %1,55; %2,76; ve %5,04 artırmıştır. Aynı dozda uygulanan sığır gübresi için bu artış değerleri sırasıyla %0,52; %2,82; %3,80 olmuştur. Kontrol konusu ile karşılaştırıldığında; istatistiksel olarak solma noktası değerlerindeki yükseliş ile gübre dozu arasındaki farklılıklar çok önemli ( $p<0,01$ ), gübre çeşitleri arasındaki fark önemsiz çıkmıştır.

Fiziksel ıslah amaçlı kullanılan toprak düzenleyiciler yani organik maddeler, topraklarda su hava ve katı madde miktarlarının hacimsel olarak dengelenmesini, ıslak agregat stabilitesinin iyileşerek toprağın su tutma kapasitesinin artmasını sağlamakta, ayrıca porozite ve hidrolik iletkenlik gibi özellikleri de geliştirmektedir (Aggelides ve Londra, 2000; Demir ve ark., 2022). Yarayışlı su tutma kapasitelerindeki artış 20 ton/ha, 40 ton/ha ve 60 ton/ha koyun gübresi uygulanan topraklarda sırasıyla %3,65; %5,93 ve %7,47 iken aynı dozlardaki sığır gübresi için bu değerler sırasıyla %3,73; %4,87 ve %7,22

düzeylerindedir. İstatistiksel olarak yarayışlı nem artışı bakımından gübre türleri ve dozları arasındaki fark önemsiz çıkmıştır. Ahır gübresi uygulanmış toprakların su tutma kapasitesindeki değişimi araştıran Er ve ark., (2020), ağırlık olarak %1, %2, %4 oranında uygulanan çiftlik gübresi yüksek dozunun yarayışlı su tutma kapasitesini %9,47 artırdığını belirlemişlerdir.

Hasat sonrası yapılan ölçümlerde uygulanan gübre türlerinin; artan dozlarının saksıdaki bitki sayısına, ortalama bitki boyuna, başaktaki dane sayısı, bin dane ağırlığına ve ham protein oranına yükseliş yönünde etkisinin olduğu belirlenmiştir.

## Sonuç

Araştırmadan sonucunda; tuzlu sodyumlu topraklara çiftlik gübresi uygulamakla toprakların fiziksel ve kimyasal özelliklerinin iyileştirilebileceğini göstermiştir. Özellikle yüksek dozda çiftlik gübresi uygulaması ile toprak özelliklerinin iyileştirilmesi akabinde arpa yetiştiriciliğinin ıslah sürecine katkı sağlayabileceğini de göstermiştir. Yönetimi açısından önemli sorunların yaşandığı tuzlu sodyumlu topraklara çiftlik gübresi uygulanmasının atık yönetimi ve bertrafi bakımından uygun bir yöntem olabileceği görülmüştür.

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## Morphological Characterization and Multivariate Analysis of Rain Fed Rice (*Oryza sativa* L) Genotypes in Northwest Ethiopia

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### ABSTRACT

Over the years, more than 3336 rice genotypes were introduced into Ethiopia from different countries and evaluated for diseases reaction and agronomic traits. However, morphological characteristics and relationships were not well investigated among genotypes. Hence, the objective of this study was to characterize 100 rice genotypes morphologically for better identification and to classify genotypes relationships. The experiment was done using triple lattice design with 10×10 in 2021/22 main cropping season. Twenty-five qualitative morphological traits were used to characterize and 13 quantitative traits were used to categorize relationships of the studied rice genotypes. The Shannon Weaver diversity index of qualitative traits were varied from 0 to 1. Moreover, based on quantitative traits the studied rain fed rice genotypes were grouped into four distinct clusters and four principal components with Eigen value >1 were contributed 78.99% of the total variation. We recommend crossing cluster I with IV genotypes to get early-maturing besides moderate grain yield variety for short rainfall season areas. And cross cluster III with IV genotypes to get medium matured, high filled spikelet per panicle, augmented harvest index, biomass and grain yield variety for long rainfall season areas.

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## Introduction

Rice is the world's most essential cereal crop next to wheat by its total production and after maize by its productivity (FAOSTAT, 2020). It is also the staple food for over half of the world's population (Loitongbam et al., 2017).

In Ethiopia, rice is a recent introduced crop that the government has given emphasis to ensuring food security in the country. Rice import is consistently increasing while the volume of domestic production is increased mainly due to productivity increase, area expansion and increasing number of farmers (CSA, 2018).

Hence rice is recently introduced crop in Ethiopia, the source of genotypes until now is via introduction. Since the inception of formal rice research system many genotypes are introduced from different countries. The major source of Ethiopian genotypes are Africa Rice, International Rice Research Institute (IRRI), IRRI-ESA, China and Japan (Asmelash, 2014; Dessie et al., 2019). Between 2007 and 2018 a total of 3336 rice genotypes were introduced and pass through national evaluation procedures for variety release (Dessie et al., 2019). During evaluation stages to release variety the main concern was yield and yield related traits. However, the qualitative morphological

characteristics and relationships of the introduced rice genotypes in different times were not studied and documented in detail.

Morphological characterization is essential tool for classification, evaluation of a given genotypes and for selecting lines/varieties (Sarawgi & Ojha, 2016). Qualitative characteristics are known as morphological markers for identification of a given germplasm and less influenced by environment than quantitative traits (Rebeira et al., 2014; Pachauri et al., 2020). Therefore, morphological characterization using qualitative trait is a simple accessed marker to identify and maintain distinct and uniform genotypes.

Clustering and genetic divergence is a multivariate statistical analysis system which partitioning a set of individuals into groups, so that individuals within a group are more similar and individuals in different groups are more dissimilar (Mahalanobis, 1936). Clustering is the process of organizing individuals into groups whose members are similar in some ways (Chahal & Gosal, 2002). There are two types of clustering methods which are distance and model based method clusters (Johnson & Wichern, 1992).

Principal component analysis is the oldest and most popular multivariate technique (Pearson, 1901). Principal component analysis reveals the most contributing traits that accounted for the total genetic variability at each axis of differentiation (Noirot et al., 1996; Sharma, 1998). It reduces a large data set in to correlated variables which aims to identify principal components, combinations of variables which best characterize a data set (Jolliffe, 2002; Akbar et al., 2011). The principal components have both direction and magnitude; the direction represents across which principal axis the data is mostly distributed and the magnitude signifies the amount of variance (Jolliffe, 2002). Eigen values measure the amount of the variation explained by each principal component, an Eigen value  $>1$  is considered for a significant amount of information from the original variables (Kaiser, 1958; Akbar et al., 2011). Traits having closer to  $|1|$  within the first principal component influence the clustering more than those with a lower absolute value closer to zero (Chahal & Gosal, 2002).

Therefore, this research was conducted to characterize 100 rain fed rice genotypes morphologically using 25 qualitative traits and categorize relationships of the studied rice genotypes via 13 quantitative traits.

## Materials and Methods

### Description of the Study Area

The experiment was conducted at Fogera National Rice Research and Training Center (FNRRTC) experiment site in 2021/22 main cropping season. The site is located 57km away from Bahir Dar, capital city of Amhara National Regional state and 607km away from Addis Ababa, Ethiopia, in the north-western part of Ethiopia (Figure 1). The site altitude is located at an altitude 1810m above sea level with geographic coordinates of  $37^{\circ} 41'$  E longitude and  $11^{\circ} 58'$  N latitude. Based on ten years' average meteorological data, the annual rainfall is 1300mm and the annual minimum is  $11.5^{\circ}\text{C}$  and maximum temperatures is  $27.9^{\circ}\text{C}$ . The soil type is Vertisol (black) with pH of 5.90 (Abebe, 2016).

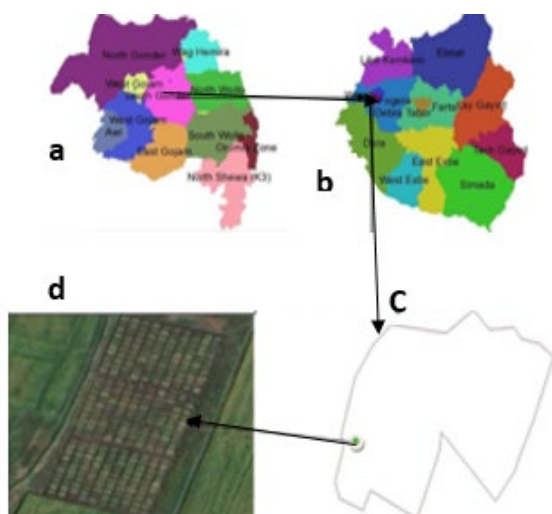


Figure 1. Map locating the study area (a = map of Amhara region, b = map of South Gondar zone, c = map of FNRRTC experiment station and d = Location of our experiment and its satellite photo)

### Experimental Materials

It was composed of 100 rain fed rice genotypes including 10 released varieties (Table 1). All genotypes were obtained from FNRRTC which was sourced from different rice research organization mainly from IRRI and Africa Rice Center. These genotypes were maintained for future breeding work.

### Experimental Design

The experiment was laid out in  $10 \times 10$  triple lattice designs. The plot size was  $7.5\text{m}^2$  and the net harvestable plot size was  $6\text{m}^2$ , excluding two boarder rows. The space between rows, plots and blocks were 0.25m, 0.5m and 1m, respectively. The plot size, space between rows, plots and blocks were done based on the national rice research protocol for breeding activities of rain fed rice (Zewdu et al., 2020).

### Agronomic Practices

A seed rate of  $60\text{kg ha}^{-1}$  was applied with direct seeding methods in a row. Fertilizer application and weeding were done based on the local recommendations.

### Data Collected

It was collected on plot and plant basis according to standard evaluation systems of rice (Bioversity International, IRRI and WARDA, 2007; IRRI, 2013). The total of 25 morphological traits were collected and the details are summarized in Table 2. Moreover, the details of 13 quantitative traits that were collected either on plant or plot bases and used for multivariate analysis are described as follows:

#### On plant basis

**Plant height (cm):** It was measured by taking five plants randomly (by 'X' sampling) in the harvestable plot and measured starting from the base of the main stem to the tip of the main panicle at physiological maturity.

**Panicle length (cm):** It was measured from 5 randomly taken rice plants of the main tiller panicle at physiological maturity.

**Number of filled and unfilled spikelets /panicle:** The number of filled and unfilled spikelets per panicle was counted from 5 randomly selected plants at maturity.

**Tillering ability, number of fertile and non- fertile tillers per plant:** Three time counting was done since the sowing method was drilling. First, number of seedlings were counted before tillering at 50cm row length from two sampled harvestable rows and tagged. Second, maximum tillers were counted at maximum tillering stage from the pre tagged samples. Third, Fertile tillers were counted at maximum tillering from the pre tagged samples too. Then, tillering ability was calculated by dividing the number of tillers at maximum tillering stage by the number of seedlings and the number of non-fertile tillers were obtained by calculating the difference between tillering ability and fertile tillers.

#### On plot basis

**Days to 50% heading:** It was recorded by visual judgment in whole plot, which was number of days counted from sowing to 50% heading.



Table 1. List of the one hundred rice genotypes tested at Fogera district, Ethiopia

Genotype #	Genotype Designations	Genotype #	Genotype Designations
G1	GSR IR1-17-Y16-Y3-Y2	G51	MET-HE-17-10
G2	GSR IR1-15-D4-D1-Y1	G52	MET-HE-17-14
G3	GSR IR1-5-D1-D1	G53	MET-HE-17-16
G4	GSR IR1-12-Y4-Y1-D1	G54	MET-HE-17-23
G5	GSR IR1-8-S9-D2-Y2	G55	MET-HE-17-25
G6	GSR IR1-12-S2-Y3-Y2	G56	Aromatic-1
G7	GSR IR1-5-D20-D2-D1	G57	Edirne
G8	GSR IR1-5-S10-D3-Y2	G58	Trakya
G9	GSR IR1-12-S8-Y1-S1	G59	Halilibey
G10	GSR IR1-8-S14-S1-SU1	G60	Osmancik-97
G11	GSR IR1-12-D10-S1-D1	G61	Tunca
G12	GSR IR1-12-Y4-D1-Y2	G62	Condai
G13	GSR IR1-12-S8-Y1-Y2	G63	Hangamchal
G14	GSR IR1-5-S10-D1-D1	G64	Hawaghaelo-2
G15	GSR IR1-8-S6-S3-S1	G65	Namcheobyeo
G16	GSR IR1-5-S12-D3-Y2	G66	Samgangbyeo
G17	GSR IR1-5-S8-D2-S1	G67	Suitou Chuukanbohon Nou 11
G18	ROJOMENA271/10	G68	PCT-11\0\0\2, Bo\2\1>487-1-6-2-3-3-M
G19	IRGA370-38-1-1F-B1-1	G69	CT11231-2-2-1-3-M-5-2-M-3-M
G20	PSBRC92	G70	PCT-11\0\0\2, Bo\2\1>1-M-3-1-2-M
G21	FKRS	G71	PCT-4\0\0\1>161-3-2-1-M
G22	IR75502-5-1-1-B	G72	PCT-4\0\0\1>204-1-3-3-M-3-M
G23	WAB95-B-B-40-HB	G73	PCT-4\0\0\1>295-2-3-1-3-3-M
G24	IR76999-52-1-3-2	G74	PCT-4\0\0\1>295-2-6-1-3-3-M
G25	WAB502-8-5-1	G75	IR 84639-7-76-3-2-1-2-4-2-2-B-Tsukuba
G26	WABC165(IAC165)	G76	IR 84633-9-16-5-11-2-2-4-3-2-2-B-Tsukuba
G27	Yungeng 45	G77	SCRID091-10-1-3-2-5
G28	SR35239-HB3403-27	G78	SCRID091-24-3-2-2-3
G29	MwuR4	G79	SCRID090-60-1-1-2-4
G30	Pakistan	G80	SCRID090-72-3-1-3-5
G31	SIM2 SUMADEL	G81	SCRID090-177-2-4-3-4
G32	WAS 127-12-1-2-1	G82	SCRID090-18-1-2-2-1
G33	IR-63275-B-1-1-13-2	G83	SCRID091-20-3-1-3-4
G34	IR-72593-B-B-2-3-14P1	G84	SCRID122-5-2-1-1-3
G35	HHZ 12-Y4-DT1-Y2	G85	SCRID122-13-1-1-4-3
G36	ARS755-5-B-B	G86	SCRID186-72-1-1-2
G37	CT18973-1-7-1-4SR-1P	G87	SCRID198-73-5-1-3
G38	HHZ12-SAL2-Y3-Y2	G88	Kb-2
G39	ARS 775-1-B-B	G89	Zongeng
G40	ARS 105-2-2-B	G90	Yuukeng
G41	Sahel134	G91	Selam(released variety)
G42	ARS-755-4-B-B	G92	Shaga(released variety)
G43	IR95786-9-2-1-2	G93	Wanzaye(released variety)
G44	IR98976-20-1-2-1	G94	Erib(released variety)
G45	IR92521-147-3-1-2	G95	Abay(released variety)
G46	IR98816-10-2-2-2	G96	Fogera-1(released variety)
G47	IR99648-59-1-1	G97	Ediget(released variety)
G48	IR106358-B-B-AJY6	G98	NERICA-12(released variety)
G49	IR97044-1-2-1-2	G99	X-jigina(The most cultivated cultivar)
G50	IR99637-6-1-1	G100	NERICA- 4(released variety)

*Days to 85% maturity:* Maturity was recorded by visual judgment of the whole plot, which was number of days from sowing to 85% physiological maturity.

*Above ground biomass yield (kg/ha):* It was measured in each harvestable plot after two days sundry.

*Grain yield (Kg/ha):* Cleaned paddy rice yield and moisture were measured. Then, the grain yield was adjusted at 14% moisture content as follows:

$$\text{Grain yield} = \frac{Akg(100 - B)}{(100 - mc)}$$

Where, A=actual measured weight, B=Measured moisture content, mc= moisture content.

*Harvest index (HI %):* It was calculated as follows:

$$HI \% = \frac{(\text{Economic grain yield per plot})}{(\text{Biological yield per plot})} * 100$$

*Thousand grain weights (g):* It was counted by seed counter by taking whole grains randomly from clean and sun dried for each of harvestable plot and then weighed by using sensitive balance and adjusted at 14% moisture content.

### Statistical Analysis

#### Statistical analysis for qualitative morphological traits

Shannon Weaver diversity index formula was used (Shannon and Weaver, 1949) and computed by excels as follows:

$$H' = \left[ \sum \left( \frac{n}{N} \right) * \{ \log_2 \left( \frac{n}{N} \right) * (-1) \} \right] / \log_2(k)$$

Where, H' stands for standardized Shannon Weaver diversity index, k stands for number of phenotypic classes for a character, n stands for frequency of a phenotypic class of that character and N stands for total number of observations for that character.

#### Cluster and genetic divergence analysis

The data was standardized to have a mean of zero and variance of one before cluster analysis by using paired group algorithm. Clustering of genotypes was performed using the proc cluster procedure of SAS software version 9.4 (SAS Institute Inc., 2013). The appropriate number of clusters was determined based on Cubic clustering criterion (CCC), Pseudo F and Pseudo  $t^2$  values (Figure 19). The points where local peaks of the CCC and pseudo F value join with small value of the pseudo- $t^2$  statistic followed by a larger pseudo- $t^2$  value for the next cluster fusion (Mohammadi & Prasanna, 2003).

#### Principal Component Analysis

It is used to complement cluster analysis (Lombard et al., 2000) and to explore patterns of genetic diversity (Mohammadi & Prasanna 2003). Eigen values greater than or equal to 1 were considered important to explain the observed variability (Jeffers, 1967). The principal component analysis was computed by SAS software version 9.4 (SAS Institute Inc., 2013) and the bi-plot illustration was computed by Genstat (2015).

## Results and Discussion

### Morphological Characteristics

#### Leaf senescence

As indicated in Figure 2 and Table 2, among the tested genotypes, 35% was very early in leaf senescence at harvesting and all leaves were lost their green color before grain maturity. The other 57% of the genotypes were early (all leaves were lost their green color at harvest), 7% of the rice genotypes intermediate (one leaf were still green at harvest) and 1% of the studied genotypes were late. The result of leaf senescence showed polymorphic with Shannon Weaver diversity index of 0.57 (Table 2). In harmony with the present result, Mondal et al. (2014) reported leaf senescence as polymorphic. This wide variability in the current report is an opportunity for future breeding works. Accordingly, G33 (IR-63275-B-1-1-13-2) can be used to improve early leaf senescence for future breeding works (Table S1). Hence, late leaf senescence has significant impact on photosynthesis, nutrient remobilization, stress responses and productivity (Guo et al., 2021). In addition, the study area beneficiaries prefer stay green rice to use the straw for animal feed.

#### Panicle exertion

As showed in Table 2, the rice genotypes scored 18% of enclosed, 12% of partly exerted, 16% of just exerted, 42% of moderately well exerted and 12% of well exerted.

The result of panicle exertion indicated polymorphic with Shannon Weaver diversity index of 0.92 (Figure 3; Table 2). In agreement, Ghimire et al. (2018) reported polymorphic with the index of 0.75. Differently, Mondal et al. (2014) and Pathak (2020) reported dimorphic panicle exertion. Therefore, the present study had better genetic variability on panicle exertion. The more exerted is better, because decreased panicle exertion as a causal factor of spikelets sterility in moisture stressed rice (O' Toole & Namuc, 1983).

#### Panicle threshability

As indicated in Table 2, panicle threshability of the rice materials were polymorphic with Shannon Weaver diversity index of 0.80. Similarly, Pathak (2020) reported polymorphic of panicle threshability.

#### Leaf blade color and pubescence

As showed in Table 2, leaf blade color of the studied rice genotypes were polymorphic with Shannon Weaver diversity index of 0.45. While the leaf blade pubescence showed dimorphic with the index of 0.56. Similarly, Rawte & Saxena (2018) reported polymorphic for leaf blade color. Differently, Sinha & Mishra (2013) showed polymorphic of leaf blade pubescence and also Pathak (2020) reported non polymorphic of leaf blade pubescence (all glabrous).

#### Basal leaf sheath anthocyanin coloration

Ninety eight percent of the rice genotypes had no basal leaf sheath anthocyanin coloration, only 1% (G31 = SIM2 SUMADEL) showed medium and 1% (G18 = ROJOMENA271/10) showed strong basal leaf sheath anthocyanin coloration (Figure 4; Table 2, 3). Its result indicated very small evenness of polymorphic with Shannon Weaver diversity index of 0.08.

#### Leaf angle

As indicated in Figure 5 & Table 2, 66% the genotypes were erect and 34% were intermediate. Leaf angle was dimorphic with Shannon Weaver diversity index of 0.58. In different result to this study, Ghimire et al. (2018) reported polymorphic with the index of 0.69. This implies that the present study genotypes are more advanced than Ghimire et al. (2018) cold tolerant rice accessions because the more erect leaf angle are better for effective light interception.

#### Flag leaf angle

As indicated in Table 2, flag leaf angle was polymorphic with Shannon Weaver diversity index of 0.67. Moreover, among studied rice genotypes 58% were erect, 31% were intermediate and 11 % were horizontal (Figure 6). Similar to with the current study, Ghimire et al. (2018) & Pathak (2020) reported polymorphic of the flag leaf angle.

#### Ligules color

Among the studied rice genotypes, 99% showed white and only 1 % (G18 = ROJOMENA271/10) showed purple lines ligules color which showed dimorphic with small evenness (Figure 7; Table 2, 3). Similarly, Mondal et al. (2014) reported dimorphic of the ligules color.

#### Culm Angle and ligules shape

All the tested rice genotypes had same erected culm angle and truncate ligules shape with Shannon Weaver diversity index of 0 (Figure 8; Table 2). This implies that the studied genotypes didn't show polymorphic for these two traits.

Table 2a. Phenotypic classes, proportion and diversity index for 100 rice genotypes using qualitative traits

S. No.	Traits	Measuring time	Number of phenotypic classes	Scale	Number of genotypes	Proportion of a phenotypic class	Shannon Weaver diversity index (H')
1	Leaf senescence	At harvest	Very early	1	35	35	0.57
			Early	3	57	57	
			Intermediate	5	7	7	
			Late	7	1	1	
			Very late	9	0	0	
2	Panicle exertion	Dough growth stage	Enclosed	1	18	18	0.92
			Partly exerted	3	12	12	
			Just exerted	5	16	16	
			Moderately well exerted	7	42	42	
			Well exerted	9	12	12	
3	Panicle Threshability	At harvest	Difficult (less than 1%)	1	11	11	0.80
			Moderately difficult (1-5%)	3	3	3	
			Intermediate (6-15%)	5	8	8	
			Loose (26-50%)	7	36	36	
			Easy (51-100%)	9	42	42	
4	Leaf blade pubescence	Heading stage	Glabrous	1	31	31	0.56
			Intermediate	2	69	69	
			Pubescent	3	0	0	
5	Leaf blade color	Booting stage	Light green	1	19	19	0.45
			Green	2	72	72	
			Dark green	3	8	8	
			Purple tips	4	0	0	
			Purple margins	5	1	1	
			Purple mixed with green	6	0	0	
			Purple	7	0	0	
6	Basal leaf sheath anthocyanin coloration	Stem elongation stage	Absent	1	98	98	0.08
			Weak	3	0	0	
			Medium	5	1	1	
			Strong	7	1	1	
7	Leaf angle	Booting stage	Erect	1	66	66	0.58
			Intermediate	3	34	34	
			Horizontal	5	0	0	
8	Flag leaf angle	Booting stage	Erect	1	58	58	0.67
			Intermediate	3	31	31	
			Horizontal	5	11	11	
			Descending	7	0	0	
9	Ligules color	Booting stage	Absent	0	0	0	0.04
			White	1	99	99	
			Purple lines	2	1	1	
			Purple	3	0	0	
10	Culm angle	Dough growth stage	Erect (<30°)	1	100	100	0.00
			Intermediate (~45°)	3	0	0	
			Open (~60°)	5	0	0	
			Spreading (>60°)	7	0	0	
			Procumbent	9	0	0	
11	Ligules Shape	Stem elongation stage	Absent	0	0	0	0.00
			Acute to acuminate	1	0	0	
			Cleft	2	100	100	
			Truncate	3	0	0	
12	Collar color	Booting stage	Absent (collarless)	0	0	0	0.04
			Light green	1	99	99	
			Green	2	0	0	
			Purple	3	1	1	
13	Auricle Color	Stem elongation stage	Absent (no auricles)	0	0	0	0.05
			Light green	1	99	99	
			Purple	2	1	1	
14	Culm internodes color	Dough growth stage	Green	1	31	31	0.48
			Light gold	2	68	68	
			Purple lines	3	1	1	
			Purple	4	0	0	

Table 2b. Phenotypic classes, proportion and diversity index for 100 rice genotypes using qualitative traits

S. No.	Traits	Measuring time	Number of phenotypic classes	Scale	Number of genotypes	Proportion of a phenotypic class	Shannon Weaver diversity index (H')
15	Panicle type	Dough growth stage	Compact	1	32	32	1.00
			Intermediate	2	34	34	
			Open	3	34	34	
16	Panicle curvature of main axis	Maturity stage	Upright	1	1	1	0.54
			Semi-upright	3	33	33	
			Slightly drooping	5	65	65	
			Strongly drooping	7	1	1	
17	Awning	Dough growth stage	Absent	0	88	88	0.27
			Short and partly awned	1	10	10	
			Short and fully awned	5	1	1	
			Long and partly awned	7	0	0	
			Long and fully awned	9	1	1	
18	Awn color	Dough growth stage	Awnless	0	88	88	0.25
			Straw	1	8	8	
			Gold	2	0	0	
			Brown (tawny)	3	0	0	
			Red	4	4	4	
			Purple	5	0	0	
19	Apiculus color	Dough growth stage	Black	6	0	0	0.4
			White	1	0	0	
			Straw	2	68	68	
			Brown (tawny)	3	0	0	
			Red	4	0	0	
			Red apex	5	28	28	
			Purple	6	1	1	
20	Lemma and Palea Color	Maturity stage	Purple apex	7	3	3	0.37
			Straw	0	0	0	
			Gold and gold furrows on straw background	1	50	50	
			Brown spots on straw	2	0	0	
			Brown furrows on straw	3	0	0	
			Brown (tawny)	4	46	46	
			Reddish to light purple	5	1	1	
			Purple spots on straw	6	0	0	
			Purple furrows on straw	7	3	3	
21	Lemma and Palea Pubescence	Maturity stage	Purple	8	0	0	0.58
			Black	9	0	0	
			Glabrous	1	14	14	
			Hairs on lemma keel	2	0	0	
			Hairs on upper portion	3	1	1	
22	Chalkiness of endosperm	After harvest	Short hairs	4	64	64	0.93
			Long hairs (velvety)	5	21	21	
			None	0	32	32	
			Small (less than 10%)	1	40	40	
23	Brown rice length	After harvest	Medium (11% to 20%)	5	15	15	0.75
			Large (more than 20%)	9	13	13	
			Extra-long (>7.5 mm)	1	3	3	
			Long (6.61 to 7.5 mm)	3	27	27	
24	Brown rice shape	After harvest	Medium (5.51 to 6.6 mm)	5	57	57	0.69
			Short (5.5mm or less)	7	13	13	
			Slender – ratio (Over 3.0)	1	25	25	
			Medium – ratio (2.1 to 3.0)	3	59	59	
25	Seed coat (bran) color	After harvest	Bold – ratio(1.1 to 2.0)	5	16	16	0.16
			Round – ratio(Less than 1.1)	7	0	0	
			White	1	91	91	
			Light brown	2	0	0	
			Speckled brown	3	0	0	
			Brown	4	0	0	
			Red	5	9	9	
Variable purple	6	0	0				
Purple	7	0	0				

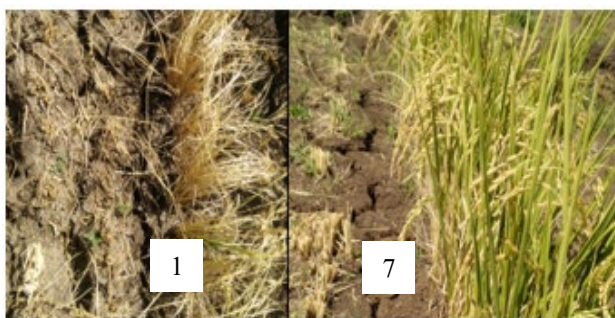


Figure 2. Leaf senescence scale 1 and 7 (Table 2): photos were taken in our experiment in 2021



Figure 3. Panicle exertion scale 1, 5 and 7 (Table 2): photos were taken in our experiment in 2021



Figure 4. Basal Leaf Sheath anthocyanin coloration scale 1, 5 and 7 (Table 2): photos were taken in our experiment in 2021

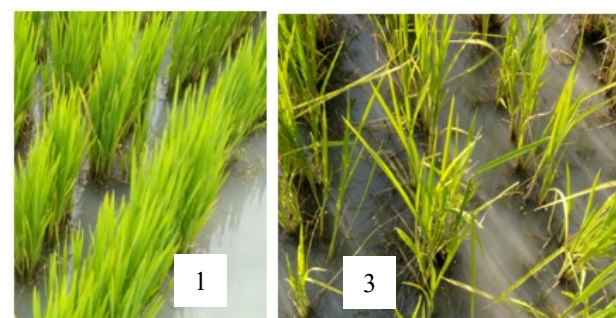


Figure 5. Leaf angle scale 1 and 3 (Table 2): photos were taken in our experiment in 2021



Figure 6. Flag leaf angle scale 3 and 5 (Table 2): photos were taken in our experiment in 2021

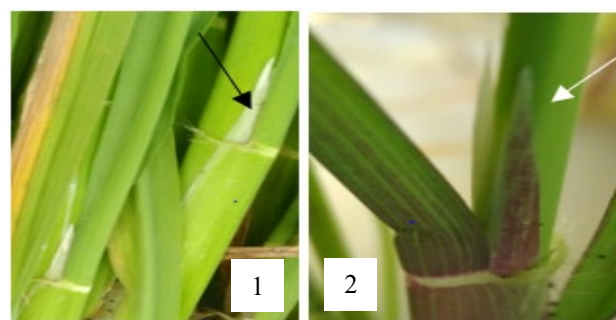


Figure 7. Ligules color scale 1 and 2 (Table 2): photos were taken in our experiment in 2021

Therefore, culm angle and ligules shape are not important traits to characterize the FNRRTC genotypes. Similarly, Mondal et al. (2014) reported that ligules shape were non-polymorphic. Similarly, Sinha & Mishra (2013) reported the non-polymorphic of ligules shape; while, they reported polymorphic for culm angle.

#### Collar and auricle color

Both collar and auricle color showed dimorphic with very small evenness of Shannon Weaver diversity index of 0.04 and 0.05, respectively (Table 2). Moreover, 99% the studied genotypes scored light green and 1% (G31 = SIM2 SUMADEL) scored purple collar color (Figure 9; Table 2, 3). In different to the present study result, Pathak (2020) reported polymorphic for collar color.

#### Culm internodes color

It revealed polymorphic with Shannon Weaver diversity index of 0.48 (Table 2). Among 100 rice genotypes considered 31% were green, 68% were light gold and 1% was purple lines (Figure 10; Table 2). In different to the present study, Ghimire et al. (2018) reported dimorphic of culm internodes with Shannon Weaver diversity index of 0.14 and 95.3% of their studied rice accessions were light gold and the other 4.7% were of purple lines. The other scholars Nascimento et al. (2011)

and Pathak (2020) also reported different results to the present findings that their studied rice genotypes showed non-polymorphic for culm internodes color. This indicates that the genotypes in our study had appreciable amount of variation on culm internode color.

#### Panicle type and curvature of main axis

The Shannon Weaver diversity index of panicle type was 1, perfect evenness which implies the studied genotypes were fairly distributed in the three phenotypic classes (Table 2). In contrary, Nascimento et al. (2011) reported non-polymorphic of panicle type (all 146 accessions were intermediate). For panicle curvature of main axis of the studied genotypes which were upright (1%), semi-upright (33%), slightly drooping (65%) and strongly drooping (1%) with the index of 0.53 (Figure 11; Table 2). In line with the present study, Mondal et al. (2014) reported polymorphic of panicle curvature of main axis and similarly Pathak (2020) reported polymorphic of panicle type.

#### Awning

As indicated in Figure 12 & Table 2, awning showed polymorphic with small evenness and Shannon Weaver diversity index of 0.27 which awning absent (88%), short and partly awned (10%), short and fully awned (1%) plus

long and fully awned (1%). Similarly, Sinha & Mishra (2013) reported polymorphic of awning. On the other hand, Mondal et al. (2014) reported dimorphic for awning. In the present study, 1% of genotypes were long and fully awned (Table 2). Sahu et al. (2018) reported that expression of long and fully awned trait advocated the presence of two independent genes; one dominant and another one recessive. As well as 10% of the present study genotypes were short and partly awned. Sahu et al. (2018) reported that partially awned trait was expressed by three gene interaction of two dominant genes either of which being capable to complement with another dominant gene.

*Awn color*

It indicated polymorphic with small evenness and Shannon Weaver diversity index of 0.25 (Table 2). Among 100 studied rice genotypes, 88%, 8% and 4% were Awnless, Straw and red awn color, respectively (Figure 13; Table 2). In agreement with the present study, Sinha & Mishra (2013) and Mondal et al. (2014) reported polymorphic for awn color.

*Apiculus color*

As indicated in Table 2, apiculus color confirmed polymorphic by small evenness with Shannon Weaver diversity index of 0.4. Apiculus color that indicated straw (68%), red apex (28%), purple (1%) and purple apex (3%) are illustrated in Figure 14. In agreement to the present study, Nethra et al. (2005) reported polymorphic for apiculus color. The result the present studied genotypes on apiculus color can be used as phenological markers for future breeding work to identify distinctiveness and uniformity of a given genotypes or variety at dough growth stage.

*Lemma and palea Color*

As indicated in Table 2, lemma and palea color revealed polymorphic with small evenness and Shannon

Weaver diversity index of 0.37. Lemma and palea color that scored gold (50%), brown (tawny) (46%), reddish to light purple (1%) and purple furrows on straw (3%) are illustrated in Figure 15. In agreement to the present result, Mondal et al. (2014) & Pathak (2020) reported polymorphic for lemma and palea color.

*Lemma and palea pubescence*

It showed polymorphic with Shannon Weaver diversity index of 0.58 (Table 2). Among 100 studied rice genotypes 14% were glabrous, 1% were hairs on upper portion, 64% were short hairs and 21% were long hairs (Figure 16; Table 2). In line with the present study, Mondal et al. (2014) demonstrated polymorphic for lemma and palea pubescence.

*Chalkiness of endosperm*

It showed polymorphic with Shannon Weaver diversity index of 0.93. Among 100 studied rain fed rice genotypes, 32%, 40%, 15%, and 13% were translucent (no chalkiness), small chalkiness, medium chalkiness and large chalkiness, respectively (Figure 17; Table 2). Those 32% of studied rain fed rice genotypes are opportunities for breeders for selection as well as for hybridization because as translucent increases rice quality also increases (Sharma & Khanna, 2019).

*Brown rice length and shape*

Both brown rice length and shape showed polymorphic with Shannon Weaver diversity index of 0.75 and 0.69, respectively. Among the studied rice genotypes, slender (25%), medium (59%), and bold (16%) brown rice shape (Table 2). In the study area, people who consume rice in the form of 'Injera' may not concern for rice shape while people who live in urban areas that used for table rice prefer slender shape.

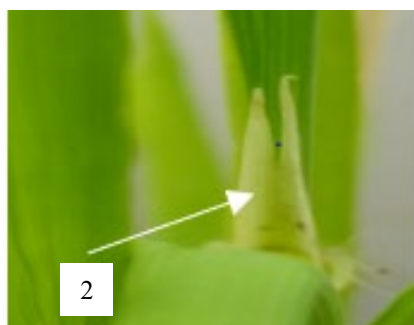


Figure 8. Ligules shape scale 2 (Table 2): photo was taken in our experiment in 2021



Figure 9. Collar color scale 1 and 3 (Table 2): photos were taken in our experiment in 2021

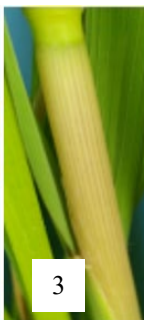


Figure 10. Culm internodes color scale 1, 2 and 3 (Table 2): photos were taken in our experiment in 2021

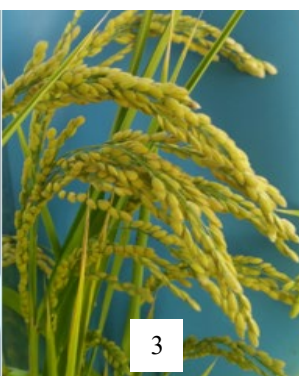


Figure 11. Panicle curvature of main axis scale 1, 3, 5 and 7 (Table 2): photos were taken in our experiment in 2021

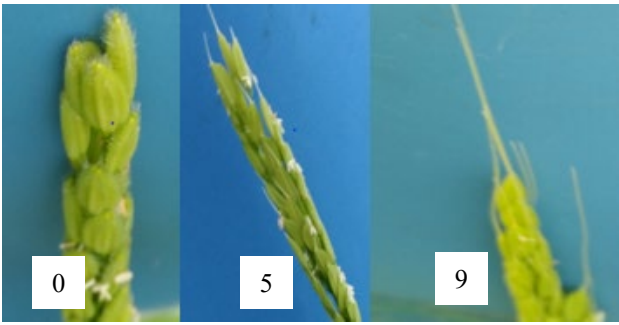


Figure 12. Awning scale 0, 5 and 9 (Table 2): photos were taken in our experiment in 2021

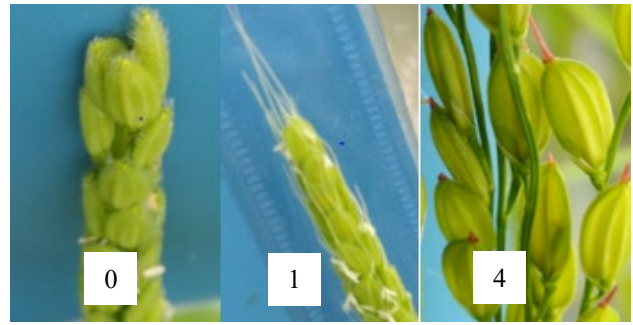


Figure 13. Awn color scale 0, 1 and 4 (Table 2): photos were taken in our experiment in 2021



Figure 14. Apiculus color scale 2, 5, 6 and 7 (Table 2): photos were taken in our experiment in 2021



Figure 15. Lemma and palea color scale 1, 4, 5 and 7 (Table 2): photos were taken in our experiment in 2021



Figure 16. Lemma and palea pubescence scale 1, 3, 4 and 5 (Table 2): photos were taken in our experiment in 2021

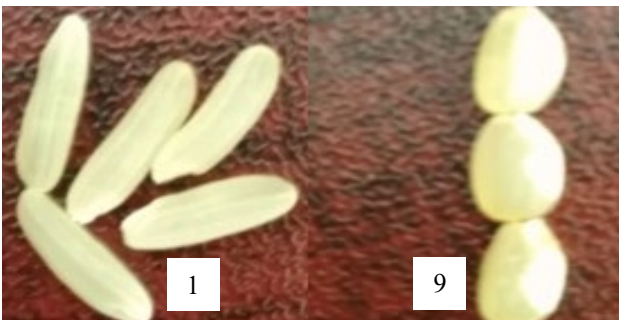


Figure 17. Chalkiness of endosperm scale 1 and 9 (Table 2): photos were taken in our experiment in 2021



Figure 18. Seed coat color scale 1 and 5 (Table 2): photos were taken in our experiment in 2021

#### Seed coat color

It showed very small evenness with Shannon Weaver diversity index of 0.16 (Table 2). Among 100 studied genotypes, 91% had white and 9% had red seed coat color (Figure 18; Table 2). These 91% of studied genotypes that

showed white seed coat color are opportunities for breeders because farmers of the study area prefer white seed coat color. In general the detail of each 100 genotypes for each 23 di/polymorphic qualitative morphological characteristics are presented in Supplementary Table 1.

### Multivariate Analysis

#### Clustering analysis

As illustrated in Figure 20 and Table 3, the current studied 100 rain fed rice genotypes were grouped into four clusters based on their similarities on 13 quantitative morphological traits with the cutting point of 0.97 distances. Cluster I was the largest cluster which consisted of 58 genotypes followed by clusters II, III and IV with 28, 12 and 2 genotypes, respectively. This result indicated that the existence of genetic divergence among the studied genotypes which implies that genotypes within a cluster are genetically less different from each other than genotypes found in different clusters.

Similar to the present results, many researchers reported the existence of genetic diversity in their studied genotypes. Worede et al. (2014) reported two hierarchical clusters with additional subgroups in each groups for 24 rice genotypes that were done in Thailand. Tejaswini et al. (2016) studied 121 rice genotypes and reported 12 clusters. The other researchers Rashid et al. (2013) and Dhakal et al. (2020) reported 6 clusters for 20 and 30 rice landraces, respectively. In addition, Fentie et al. (2021) reported 4 clusters for 30 studied rain fed rice genotypes.

#### Cluster distance and mean analysis

As presented in Table 4, the maximum average inter cluster distance was found between clusters II and IV ( $D^2 = 49.96$ ) followed by between clusters III and IV ( $D^2 = 32.76$ ) which showed that the genotypes found in these clusters are genetically more diversified than other clusters. These results are opportunities for breeders to hybridize and would bring maximum heterosis to generate promising segregantes for grain yield and other important traits. In line with the present study, many researchers found maximum average inter cluster distances between their reported clusters. Dhakal et al. (2020) reported maximum average inter cluster distances for their studied genotypes.

On the other hand, the minimum average inter cluster distances were found among cluster I and III, while that distance was big as compared with intra cluster distances. Crossing of genotypes from cluster I and III might not give higher heterotic value in F1 generation. From 10 released

and commonly cultivated varieties, nine were in cluster I and only one (selam) was in cluster III. Therefore, according to the present results, hybridization between these 10 released varieties would not give heterosis in F1 generation. Therefore to get heterosis F1 generation of these 10 released varieties, it is better to cross with genotypes that found in cluster II and IV. The chi-square test among all inter clusters were significant ( $p < 0.01$ ), which implies that categorical traits for clustering were highly correlated for each genotypes under each of those significant inter clusters. While, the chi-square test between clusters I and III were not significant which revealed that the traits used for clustering of genotypes were not correlated in cluster I and III based on Chi-square test of independence interpretation by Turhan (2020).

Cluster mean of 13 quantitative of the studied rice genotypes are presented in Table 5. Considerable differences were found among 13 quantitative traits of inter-clusters.

Cluster I comprised 58 genotypes (Table 3). This cluster had characteristics of low number of fertile tillers, short panicle length, low number of unfilled spikelets, early heading, early mature, low adjusted grain yield, low harvest index and heavy 1000 seed weight as compared to other clusters (Table 5). In agreement to the present findings, Abebe (2016) reported same characteristics of early heading, relatively early maturing period, lowest biomass yield and heavy 1000 grain weight in the same cluster. The other researchers Dejen Bekis et al. (2021) reported similar short panicle length, low number of filled spikelets and high thousand grain weight in one cluster. Besides, the present study of cluster I had moderate maximum tillering ability, fertile tillers per panicle, plant height, filled spikelets per panicle and biomass yield. Similar to the present study, Abebe (2016) found same characteristics of moderate number of filled spikelets and fertile tillers in the same cluster. In agreement to the current study, Fentie et al. (2021) also reported similar characteristics of moderate fertile tiller per plant, plant height, panicle length and biomass yield in the same cluster.

Table 3. Distribution of 100 rice genotypes into 4 clusters based on genetic divergence analysis

Cluster	No. of Genotypes	Proportion in (%)	Genotypes
I	58	58	G2, G7, G8, G9, G12, G16, G17, G19, G20, G21, G22, G23, G25, G26, G28, G29, G30, G51, G52, G53, G56, G57, G58, G59, G60, G61, G62, G63, G64, G65, G66, G67, G69, G70, G71, G72, G73, G74, G77, G78, G79, G80, G81, G82, G83, G84, G85, G86, G87, G92, G93, G94, G95, G96, G97, G98, G99 and G100
II	28	28	G5, G11, G13, G14, G15, G24, G31, G32, G33, G34, G35, G36, G37, G38, G39, G40, G41, G42, G43, G44, G46, G47, G48, G49, G50, G54, G75 and G76
III	12	12	G1, G3, G4, G6, G10, G27, G45, G55, G88, G89, G90 and G91
IV	2	2	G18 and G68

Table 4. Pair-wise generalized intra - (bolded diagonal) and inter-cluster distances (off-diagonal)

Cluster	1	2	3	4
1	<b>4.33</b>			
2	27.79**	<b>3.64</b>		
3	19.72 <sup>ns</sup>	30.04**	<b>3.77</b>	
4	30.34**	49.96**	32.76**	<b>4.36</b>

\*\* Significant  $> \chi^2$  value 26.22 ( $p < 0.01$ ) and ns = non-significant  $< \chi^2$  value 21.03 ( $p < 0.05$ )



Table 5. Four cluster mean values of 13 quantitative traits

Traits	Clusters			
	I	II	III	IV
MTA	2.10	3.22 <sup>H</sup>	2.04 <sup>L</sup>	2.69
FTP	1.61	2.22 <sup>H</sup>	1.52 <sup>L</sup>	1.92
NFT	0.57 <sup>L</sup>	1.09 <sup>H</sup>	0.58	0.83
PH	78.31	61.66 <sup>L</sup>	82.42	111.35 <sup>H</sup>
PL	17.87 <sup>L</sup>	18.77	19.11	22.63 <sup>H</sup>
FSP	81.81	81.70 <sup>L</sup>	109.16 <sup>H</sup>	104.23
UFS	7.21 <sup>L</sup>	13.02 <sup>H</sup>	9.07	8.53
DM	136.57 <sup>L</sup>	148.37 <sup>H</sup>	145.92	143.33
DH	102.76 <sup>L</sup>	114.50 <sup>H</sup>	109.92	109.33
BYD	8.67	8.58	8.55 <sup>L</sup>	10.89 <sup>H</sup>
AGY	2.85 <sup>L</sup>	3.61	4.00	4.05 <sup>H</sup>
HI	33.52 <sup>L</sup>	42.28	46.94 <sup>H</sup>	37.37
ATW	29.10 <sup>H</sup>	22.96 <sup>L</sup>	25.55	24.36

'L' and 'H' are the lowest and highest value of cluster mean, respectively. MTA = Maximum tillering ability; FTP = Number of fertile tillers per plant; NFT = Number of Non-fertile tillers per plant; PH = Plant height (cm); PL = Panicle length; FSP = Number of filled spikelets per panicle; UFS = Number of unfilled spikelets per panicle; DM = Days to 85% maturity; DH = Days to 50% heading; BYD = Above ground biomass yield (ton/ha); AGY = Adjusted grain yield (kg/ha) at 14% moisture content; HI = Harvest Index %; ATW = Adjusted thousand grain weight (g) at 14% moisture content

Table 6. Eigenvectors, variance explained and Eigen values of the first four PCs

Traits	Eigen vectors			
	PC-1	PC-2	PC-3	PC-4
MTA	0.36	-0.10	0.38	-0.15
FTP	0.30	-0.05	0.49	-0.13
NFT	0.35	-0.14	0.20	-0.08
PH	-0.18	0.47	0.09	0.00
PL	0.19	0.40	-0.03	0.29
FSP	0.06	0.48	-0.27	0.04
UFS	0.29	-0.13	-0.23	0.27
DM	0.36	0.01	-0.18	0.30
DH	0.36	0.00	-0.11	0.34
BYD	-0.03	0.39	0.51	0.29
AGY	0.26	0.40	0.02	-0.39
HI	0.28	0.16	-0.31	-0.59
ATW	-0.32	0.03	0.16	-0.09
EV	5.38	2.52	1.34	1.03
EXV	41.38	19.38	10.31	7.92
CV	41.38	60.76	71.07	78.99

MTA = Maximum tillering ability; FTP = Number of fertile tillers per plant; NFT = Number of Non-fertile tillers per plant; PH = Plant height (cm); PL = Panicle length; FSP = Number of filled spikelets per panicle; UFS = Number of unfilled spikelets per panicle; DM = Days to 85% maturity; DH = Days to 50% heading; BYD = Above ground biomass yield (ton/ha); AGY = Adjusted grain yield (kg/ha) at 14% moisture content; HI = Harvest Index %; ATW = Adjusted thousand grain weight (g) at 14% moisture content; EV = Eigen value; EXV = Explained variance; CV = Cumulative variance (%) (%)

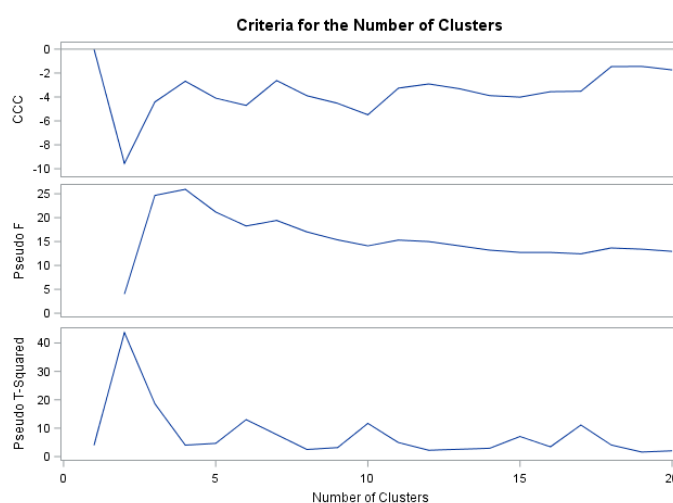


Figure 19. Criteria for the number of clusters

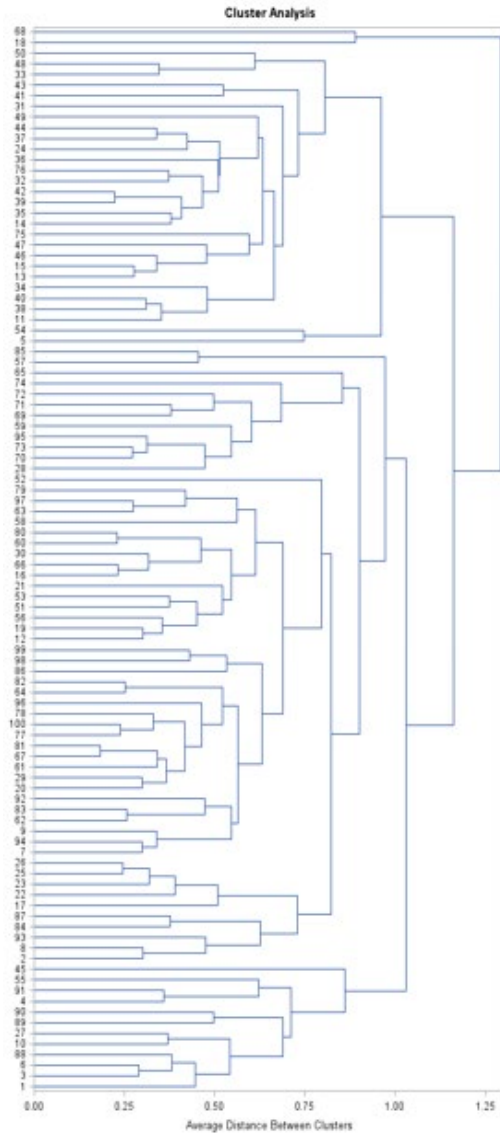


Figure 20. Dendrogram that showing relationships among 100 rain fed rice genotypes

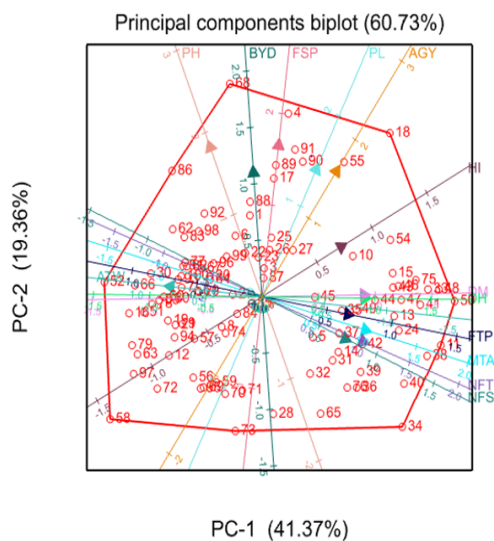


Figure 21. Biplot of PC1 and PC2 showing the relationships of genotypes by traits. The traits abbreviations are expanded in Table 6 and genotype designations are depicted in Table 1.

Cluster II included 28 genotypes (Table 3). It had features of high maximum tillering ability, fertile tiller per plant and unfilled spikelets per panicle, late heading and maturing. Moreover, cluster II had characteristics of short plant height, low filled spikelets per panicle and 1000 seed weight. In addition, cluster II had characteristics of relatively moderate panicle length, biomass yield, grain yield and harvest index (Table 5). In agreement to this study, Tirunch et al. (2019) reported high tillering ability, fertile tillers/plant and number of unfilled grains/panicle in the same cluster. The other researchers Alamir Ayenew et al. (2020) reported short plant height, moderate panicle length, late heading and maturing in the same cluster.

Cluster III consisted of 12 genotypes (Table 3). This cluster had characteristics of low maximum tillering ability, fertile tiller/plant and biomass yield. Moreover, cluster III had high filled spikelets per panicle and harvest index. In addition, cluster III had feature of moderate plant height, panicle length, non - filled spikelets per panicle, heading, maturing, grain yield and 1000 seed weight (Table 5). In corresponding to the present study, Abebe (2016) reported similar high filled spikelets/panicle and harvest index in the same cluster.

Cluster IV comprised only two genotypes (G18 = ROJOMENA271/10 and G68 = PCT-11\0\0\2, Bo\2\1>487-1-6-2-3-3-M). It had also its own characteristics of long plant height and panicle length, high biomass yield and grain yield (Table 5). Moreover, the other traits of this cluster had moderate mean values. Similarly, Tirunch et al. (2019) reported long plant height and panicle length, high biomass yield and grain yield in the one cluster. The other researcher Birhanu (2020) scored high biomass yield and grain yield in the same cluster.

*Principal component analysis*

The result of the principal component analysis (PCA) is represented in Figure 21 and Table 6. The study was carried out using 100 rain fed rice genotypes with 13 quantitative traits. Among 13 principal components (PC), the first four PC with Eigen value >1 were maintained. These 4 PC contributed to 78.99% of the total variance. PC1 accounted for the highest variance (41.38%) followed by PC2 (19.38%), PC3 (10.31%) and PC4 (7.92%). Similarly, many researchers reported that most of the total variation was explained by the first three to five PC. Ayenew et al. (2020) reported that 64.16% variability was described by the first three PC. The other researchers Abebe (2016) reported that 79.23% variation was explained by the first four PC. Moreover, Worede et al., (2014), Tirunch et al. (2019), Birhanu (2020), Dhakal et al. (2020), and Kashyap & Yadav (2020) reported 89.68%, 75.20%, 75.56%, 84.67% and 80.03% of the total variation was explained by the first five PC, respectively; among different studied rice genotypes.

According to Raji (2003), traits having coefficients of the Eigen vectors value greater than 0.3 were considered as important effects on the overall variation. Moreover, the direction of Eigen vectors value ( $\pm$ ) represents across which principal axis the data is mostly distributed and the magnitude signifies the amount of variance (Jolliffe, 2002). Based on these evidences, the first four PC of the present study are discussed as follows:

Supplementary Table 1a. Qualitative characterization for 100 rice genotypes in Ethiopia

GEN	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	R	S	T	U	V	Y	Z
G1	3	7	1	2	2	1	1	3	1	1	1	2	1	5	0	0	2	4	4	5	5	5	1
G2	3	7	7	1	3	1	1	1	1	1	1	2	1	3	0	0	2	4	4	1	7	5	1
G3	3	7	1	1	2	1	1	1	1	1	1	2	1	3	0	0	2	4	4	1	7	5	1
G4	3	9	7	2	2	1	1	3	1	1	1	2	1	3	0	0	2	4	4	5	7	5	1
G5	1	7	1	1	2	1	1	1	1	1	1	2	2	3	0	0	2	4	4	5	7	5	1
G6	3	7	3	2	2	1	1	1	1	1	1	1	1	3	0	0	2	4	4	1	7	5	1
G7	3	7	1	2	2	1	1	1	1	1	1	2	1	3	0	0	2	4	5	0	5	3	1
G8	3	7	7	1	2	1	1	1	1	1	1	2	1	3	0	0	2	4	4	5	7	5	1
G9	3	7	1	1	2	1	1	1	1	1	1	2	1	3	0	0	2	4	4	0	7	5	1
G10	3	3	9	1	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	1	5	3	1
G11	3	1	9	2	2	1	1	1	1	1	1	1	2	3	0	0	2	1	4	0	5	1	1
G12	3	5	9	2	2	1	3	3	1	1	1	2	1	3	0	0	2	4	5	5	5	3	1
G13	3	3	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G14	3	3	9	1	2	1	3	3	1	1	1	2	3	5	0	0	2	1	4	1	3	3	1
G15	3	3	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G16	3	7	9	2	2	1	3	3	1	1	1	2	1	3	0	0	2	4	5	5	5	3	1
G17	1	7	3	2	1	1	3	1	1	1	1	2	2	3	0	0	2	1	4	1	3	1	1
G18	1	9	7	2	5	7	1	1	2	1	1	3	3	3	0	0	7	1	4	1	5	3	1
G19	1	7	9	2	3	1	1	3	1	1	1	2	2	5	1	1	2	4	4	9	5	3	1
G20	3	7	5	1	3	1	1	1	1	1	1	2	2	5	0	0	5	1	1	1	3	3	1
G21	5	5	9	2	2	1	3	1	1	1	1	2	1	5	1	1	2	4	5	9	3	3	1
G22	3	7	9	2	1	1	3	1	1	1	1	2	3	3	0	0	2	4	4	1	5	3	5
G23	1	7	7	2	1	1	3	1	1	1	1	2	3	3	0	0	2	4	4	1	5	3	5
G24	1	1	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	3	1	1
G25	3	7	7	2	1	1	3	1	1	1	1	2	3	3	0	0	2	4	4	1	5	3	5
G26	1	7	7	2	1	1	3	1	1	1	1	2	3	3	0	0	2	4	4	1	5	3	5
G27	3	7	1	1	2	1	3	1	1	1	1	1	1	3	0	0	2	4	4	5	7	5	1
G28	3	5	1	1	2	1	1	1	1	1	1	1	2	3	0	0	2	4	4	1	5	3	1
G29	5	7	7	1	3	1	1	1	1	1	1	2	2	5	0	0	5	1	1	0	3	1	1
G30	3	7	7	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	9	5	3	1
G31	1	3	9	2	2	5	1	1	1	3	2	2	3	5	0	0	7	4	4	0	5	1	1
G32	3	3	7	2	1	1	1	1	1	1	1	1	3	5	1	1	2	1	4	1	3	3	1
G33	7	1	7	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	3	1
G34	1	1	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	3	1
G35	1	1	9	2	1	1	1	1	1	1	1	2	3	5	0	0	2	1	4	0	3	1	1
G36	1	1	7	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G37	1	3	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	1	5	3	1
G38	1	1	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G39	1	1	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G40	1	1	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	1	5	1	1
G41	1	1	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G42	1	1	9	2	1	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	5	1	1
G43	1	3	9	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	3	1	1
G44	1	1	9	2	2	1	1	1	1	1	1	1	3	5	1	1	2	1	4	1	5	3	1
G45	3	5	9	1	3	1	1	1	1	1	1	1	3	3	0	0	2	1	4	0	5	3	1
G46	1	3	9	2	2	1	1	1	1	1	1	2	3	5	0	0	2	1	4	1	3	1	1
G47	3	1	7	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	3	1	1
G48	1	1	9	2	2	1	1	3	1	1	1	1	3	5	0	0	2	1	4	0	3	1	1
G49	1	1	9	2	3	1	1	1	1	1	1	1	3	5	0	0	2	1	4	1	5	1	1
G50	1	1	9	2	1	1	1	1	1	1	1	1	3	5	1	1	2	1	4	0	3	1	1
G51	3	9	5	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	9	5	5	1
G52	3	7	5	2	1	1	3	3	1	1	1	2	1	5	0	0	5	4	5	9	5	5	1
G53	3	9	5	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	9	5	3	1
G54	1	7	7	2	1	1	1	1	1	1	1	2	3	5	0	0	2	1	4	1	5	3	5
G55	1	7	9	2	1	1	1	1	1	1	1	2	3	3	0	0	2	1	4	5	5	3	1
G56	1	7	9	1	2	1	1	3	1	1	1	2	2	5	1	1	2	4	4	5	5	3	1
G57	3	9	9	1	2	1	3	5	1	1	1	2	2	5	0	0	5	1	1	1	5	3	1
G58	1	5	5	2	1	1	3	3	1	1	1	2	1	3	0	0	2	4	5	5	3	3	1
G59	3	5	7	2	2	1	3	3	1	1	1	2	1	1	0	0	2	4	4	5	5	3	1
G60	3	7	7	2	2	1	1	3	1	1	1	1	1	5	0	0	5	4	4	5	3	3	1
G61	3	5	7	1	3	1	1	3	1	1	1	2	2	5	1	4	5	1	1	0	1	1	1
G62	3	7	7	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	1	5	3	1
G63	3	7	7	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	9	5	3	1
G64	3	7	7	2	1	1	3	3	1	1	1	2	1	5	0	0	5	4	5	5	5	3	1
G65	1	1	7	1	2	1	1	1	1	1	1	1	2	3	0	0	2	4	4	0	7	5	1
G66	3	7	9	2	1	1	3	3	1	1	1	2	1	3	0	0	2	4	5	1	5	3	1
G67	3	7	7	2	2	1	1	1	1	1	1	2	2	5	0	0	5	4	5	0	3	3	1
G68	1	9	1	2	1	1	3	5	1	1	1	2	2	7	0	0	2	5	3	1	5	3	1
G69	3	5	9	1	2	1	1	1	1	1	1	1	2	5	0	0	5	1	1	0	3	3	1
G70	3	5	9	2	2	1	1	3	1	1	1	2	2	3	0	0	2	1	4	1	3	3	1
G71	3	5	9	1	2	1	1	5	1	1	1	2	2	3	0	0	2	1	1	1	5	3	1
G72	3	5	9	2	2	1	1	3	1	1	1	1	2	5	0	0	2	1	4	1	5	3	1
G73	5	5	9	2	2	1	1	5	1	1	1	2	2	3	0	0	2	1	4	0	5	3	1
G74	5	3	7	2	2	1	1	5	1	1	1	2	2	3	0	0	2	1	4	1	3	3	1
G75	1	3	7	2	2	1	1	1	1	1	1	1	3	5	0	0	2	1	4	0	1	1	1
G76	1	1	9	2	1	1	1	1	1	1	1	1	3	5	0	0	2	1	4	1	3	3	1

Supplementary Table 1b. Qualitative characterization for 100 rice genotypes in Ethiopia

GEN	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	R	S	T	U	V	Y	Z
G77	3	7	7	2	2	1	1	1	1	1	1	2	2	5	0	0	5	4	5	1	5	3	1
G78	3	3	9	2	2	1	3	3	1	1	1	2	2	5	0	0	5	4	4	1	5	3	1
G79	3	5	7	2	2	1	3	1	1	1	1	2	1	3	0	0	5	4	5	9	5	3	1
G80	3	7	1	1	2	1	1	3	1	1	1	2	1	5	9	1	2	4	4	5	3	3	1
G81	3	7	7	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	4	1	3	3	1
G82	3	7	7	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	1	5	3	1
G83	3	7	7	2	2	1	1	1	1	1	1	2	1	5	0	0	5	4	5	1	5	3	1
G84	1	7	9	2	2	1	1	5	1	1	1	2	2	5	1	4	5	1	4	1	3	3	5
G85	3	9	9	1	2	1	3	5	1	1	1	2	2	5	1	4	5	1	1	0	5	3	1
G86	5	7	7	2	2	1	3	3	1	1	1	2	1	5	0	0	5	4	5	0	3	3	1
G87	1	7	7	1	2	1	3	5	1	1	1	2	2	5	1	4	5	1	4	1	5	3	5
G88	1	9	7	1	2	1	1	1	1	1	1	2	1	5	0	0	2	4	4	9	7	5	1
G89	3	7	1	1	2	1	1	3	1	1	1	2	2	3	0	0	2	7	4	5	7	5	1
G90	3	7	1	2	2	1	1	3	1	1	1	2	2	3	0	0	2	7	4	9	5	3	1
G91	3	9	7	1	2	1	1	1	1	1	1	2	1	5	0	0	2	4	4	9	7	5	1
G92	3	9	3	1	2	1	1	5	1	1	1	2	2	5	0	0	7	1	1	1	7	3	5
G93	1	9	5	1	2	1	1	5	1	1	1	2	2	5	0	0	6	7	1	1	5	3	5
G94	3	7	5	1	2	1	3	1	1	1	1	2	2	5	0	0	5	1	1	1	1	1	1
G95	5	5	5	1	1	1	3	3	1	1	1	2	2	3	5	1	2	1	1	0	3	3	1
G96	3	7	7	1	2	1	1	1	1	1	1	2	2	5	0	0	5	1	1	1	3	1	1
G97	3	5	9	2	2	1	3	3	1	1	1	2	1	3	0	0	2	4	5	9	5	3	1
G98	5	5	7	1	2	1	3	1	1	1	1	2	2	5	0	0	2	1	1	0	3	1	1
G99	3	9	9	2	1	1	3	5	1	1	1	2	2	5	0	0	5	4	5	9	5	5	1
G100	3	7	7	1	3	1	1	1	1	1	1	2	2	5	0	0	5	1	1	0	5	3	1

GEN: Genotypes, A: Leaf Senescence, B: Panicle Exertion, C: Panicle Threshability, D: Leaf Blade Pubescence, E: Leaf Blade Color, F: Basal Leaf Sheath Anthocyanin Coloration, G: Leaf Angle, H: Flag Leaf Angle, I: Ligules Color, J: Collar Color, K: Auricle Color, L: Culm Internodes Color, M: Panicle Type, N: Curvature of Main Axis, O: Awning, P: Awn Color, R: Apiculus Color, S: Lemma and Palea Color, T: Lemma and Palea Pubescence, U: Chalkiness of Endosperm, V: Brown Rice Length, Y: Brown Rice Shape and Z: Seed Coat Color

The first principal component (PC1), major contributor traits were maximum tillering ability (0.36), days of maturity (0.36), days of heading (0.36), number of non-fertile tillers (0.35), thousand grain weight (-0.32) and number of fertile tillers (0.30). In corresponding to the current study, Abebe (2016) found same traits of days of maturity, days of heading, number of fertile tillers contributed positively and 1000 seed weight contributed negatively in PC1. Similarly, Kashyap & Yadav (2020) reported same traits of days of heading contributed positively and 1000 seed contributed negatively in PC1.

The second principal component (PC2), main provider traits were number of filled spikelets per panicle (0.48), plant height (0.47), grain yield (0.40), panicle length (0.40), and biomass yield (0.39) in the respective order. In agreement, Abebe (2016) found plant height and panicle length that contribute positively in PC2. The other researchers Mahendran et al., (2015) also reported similar traits, biomass yield and grain yield that contribute positively in PC2. Differently, Worede et al., (2014) reported plant height and panicle length were important traits in PC1.

The third principal component (PC3), major provider traits were biomass yield (0.51), number of fertile tillers (0.49), maximum tillering ability (0.38), and harvest index (-0.31) in the respective order. In agreement, Tiruneh et al. (2019) reported biomass yield and harvest index that contribute positively in PC3. Moreover, Kashyap & Yadav (2020) reported similar positive contribution of number of fertile tillers in PC3.

In fourth principal component (PC4), most important contributor traits were harvest index (-0.59), grain yield (-0.39), days to 50% heading (0.34), and days of 85% maturity (0.30). Differently, Tiruneh et al. (2019) and Birhanu (2020) found that harvest index contributed positively in PC4.

As illustrated in Figure 21, the bi-plot represents more than 60.73% of the total variance and gave more opportunity to assess which genotypes were good for which traits. Convex of the hull showing the outliers was occupied by G18

(ROJOMENA271/10), G50 (IR99637-6-1-1), G11 (GSR IR1-12-D10-S1-D1), G34 (IR-72593-B-B-2-3-14P1), G73 (PCT-40\0\1>295-2-3-1-3-3-M), G58 (Trakya), G52 (MET-HE-17-14), G86 (SCRID186-72-1-1-2) and G68 (PCT-11\0\0\2, Bo\2\1>487-1-6-2-3-3-M). Those outlier genotypes could be due to their unique gene expression than other genotypes and give opportunities for future variety development through selection as well as through hybridization. Especially for hybridization, those nine genotypes are the most recommended in 13 studied quantitative traits of rice genotypes; because heterosis in F1 generation might be generated between those outlier genotypes. According to Dehghani et al. (2008), the correlation among two traits is approached by the cosine of the angle between their vectors. Accordingly, the present study traits of panicle length, harvest index and number of filled spikelets/panicle were positively correlated with grain yield.

In general, among four principal components of major contributing traits, maximum tillering ability, days of maturity, days of heading, thousand grains weight and number of fertile tillers in PC1 were major influential for clustering than traits found in other components. In agreement to Chahal and Gosal (2002) those traits having the highest  $|\sim 1|$  for the first principal component influence clustering more than those with a lower absolute value closer to zero.

### Conclusions and Recommendations

The Shannon Weaver diversity index of the present studied traits varied from 0 to 1. Among 25 qualitative traits, 23 were showed polymorphic except culm angle and ligules shape. Based on breeders' objective, these 23 qualitative traits had unique genotypes. For example, if breeder's interest is to have late leaf senescence, G33 (IR-63275-B-1-1-13-2) is the recommended donor parent for future crossing work to improve early leaf senescence genotypes.

Based on the clustering result, the studied 100 genotypes were grouped into four distinct clusters. According to genetic divergence, it is better to cross cluster I and IV genotypes to get early maturing and moderate grain yield variety for moisture stress area. Moreover, it is better to cross cluster III and IV genotypes to get medium matured, high filled spikelet/panicle, optimized harvest index, biomass and grain yield variety for rain fed with irrigation supplemented areas.

According to principal component result, nine genotypes {G18 (ROJOMENA271/10), G50 (IR99637-6-1-1), G11 (GSR IR1-12-D10-S1-D1), G34 (IR-72593-B-B-2-3-14P1), G73 (PCT-4\0\0\1>295-2-3-1-3-3-M), G58 (Trakya), G52 (MET-HE-17-14), G86 (SCRID186-72-1-1-2) and G68 (PCT-11\0\0\2, Bo\2\1>487-1-6-2-3-3-M)} are recommended for hybridization parents because those genotypes are genetically divergent to have heterosis F1s.

## Declarations

### Author Contributions

Assaye Berie; initiated the research idea, collected, organized, analyzed and interpreted the data and wrote the manuscript. Tiegist Dejene; supervised the research, suggested the research methods, structured the paper and edited the manuscript.

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### Competing Interests

The authors declare that there is no competing of interests

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## Modelling of Rheological Behaviour of Persimmon Puree

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 31.10.2024 Accepted : 05.12.2024</p> <p>Keywords: Persimmon puree Rheology Mizhari-Berk Herschel-Bulkley Türkiye</p>	<p>In this study, the rheological properties of persimmon (<i>Diospyros kaki L.</i>) puree were investigated at various pH (4.0, 5.5 and 7.0), concentrations (15%, 17.5% and 20%), temperatures (25, 50 and 75°C) and shear rates (8.4–28 s<sup>-1</sup>) to determine the rheological model that best describes the flow behaviour of persimmon (<i>Diospyros kaki L.</i>) puree. Experimental data were applied to Power Law, Herschel-Bulkley, Casson and Mizhari-Berk models. In order to evaluate the goodness of fit, three statistical criteria including the coefficient of determination (<math>R^2</math>), reduced chi-squared (<math>\chi^2</math>) and the root mean squared error (RMSE) were used. Herschel-Bulkley and Mizhari-Berk were the models that provided the best fit to the experimental data under all processing conditions of persimmon puree. However, Mizhari-Berk model was the model that best described the flow behavior of persimmon puree with statistical parameter values of <math>R^2 \geq 0.983</math>, <math>RMSE \leq 0.0683</math> and <math>\chi^2 \leq 0.0160</math>. Persimmon puree exhibited a non-Newtonian behavior (<math>n &lt; 1</math>) which was pseudoplastic (shear thinning).</p>

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## Trabzon Hurma Püresinin Reolojik Davranışının Modellenmesi

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 31.10.2024 Kabul : 05.12.2024</p> <p>Anahtar Kelimeler: Trabzon hurması püresi Reoloji Mizhari-Berk Herschel-Bulkley Türkiye</p>	<p>Bu çalışmada Trabzon hurması (<i>Diospyros kaki L.</i>) püresinin akış davranışını en iyi tanımlayan reolojik modelin belirlenmesi amacıyla pürenin reolojik özellikleri çeşitli pH'larda (4,0, 5,5 ve 7,0), konsantrasyonlarda (%15, %17,5 ve %20), sıcaklıklarda (25, 50 ve 75°C) ve kayma hızında (8,4–28 s<sup>-1</sup>) araştırılmıştır. Deneysel veriler Power Law, Herschel-Bulkley, Casson ve Mizhari-Berk modellerine uygulanmıştır. Reolojik modellerin akış davranışını açıklamadaki uygunluğunu doğrulamak için belirleme katsayısı (<math>R^2</math>), hata kareleri ortalamasının kare kökü (RMSE) ve indirgenmiş ki- kare (<math>\chi^2</math>) olmak üzere üç istatistiksel ölçüt kullanılmıştır. Herschel-Bulkley ve Mizhari-Berk, hurma püresinin tüm işlem koşullarında deneysel verilere en iyi uyum sağlayan modeller olduğu görülmüştür. Ancak Mizhari-Berk modeli <math>R^2 \geq 0.983</math>, <math>RMSE \leq 0.0683</math> ve <math>\chi^2 \leq 0.0160</math> istatistiksel parametre değerleriyle hurma püresinin akış davranışını en iyi tanımlayan model olmuştur. Trabzon hurması püresi psödoplastik (kayma incelmesi) ve Newtonyen olmayan bir davranış (<math>n &lt; 1</math>) sergilemiştir.</p>

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## Giriş

Meyve püreleri, meyvenin eti, kabuğu ve yenebilen kısımlarından oluşan, yumuşak, pürüzsüz ve yarı katı akışkanlardır. Meyve ve sebzelere bulunduğu sezon dışında da ulaşılabilmesi için püreye işlenmektedir. Ticari olarak elma, armut, şeftali, kayısı, havuç ve domates gibi çok sayıda meyve ve sebze püresi üretilmektedir (Barbieri ve ark., 2018). Püreler zengin bir besin kaynağı olmasının yanı sıra tüketiciler tarafından duyuşal açıdan kabul edilebilir olmalıdır. Bu nedenle pürelerin akış davranışı, ürünün yeme özellikleri açısından önemlidir. Püreler doğrudan tüketilebildiği gibi, birçok üründe bileşen ve ara ürün olarak kullanılmaktadır. Reçeller, marmelatlar, pulplar ve meyve suları gibi gıda ürünlerinin en önemli bileşenlerindedir. Pürelerin reolojik özellikleri, meyvelerin endüstriyel işlenmesinde ve ürünlerin elde edilmesinde önemli rol oynar.

Reoloji, gıda maddelerinin yapısında meydana gelen akış ve deformasyonu inceler. Deformasyon ve akış ise, gıda maddelerinin üretiminden tüketimine kadar her aşamasında ve farklı biçimlerde yer aldığı için gıda sanayi açısından çok önemlidir (Salehi, 2020). Deformasyona ve akmaya sebep olan mekanik özelliklere, reolojik özellikler denir. Gıdayı karakterize etmenin temel bir parçasıdır ve yeni bir gıda ürünü geliştirirken reolojik özelliklerin belirlenmesi gerekir. Çünkü akışkan gıdaların üretim süreçlerinin tasarımı ve proses optimizasyonu (pomplar, boru hatları ve ekipman), ürün geliştirme için ısı ve kütle transfer katsayıları, paketleme ve depolama stratejileri gibi mühendislik parametrelerinin tahmin edilmesine katkı sağlarlar (Diamante ve Umamoto, 2015).

Pürelerin akış davranışı, kayma gerilimi, kayma hızı ve viskozite gibi reolojik parametrelerle karakterize edilir. Meyve püreleri, bileşenleri arasındaki karmaşık etkileşimleri nedeniyle Newtonyen olmayan akış davranışı gösterirler. Bu tür akışkanların kayma gerilimine karşı kayma hızı grafiği doğrusal değildir (Lukhmana ve ark., 2018). Meyve pürelerinin, çözünür şekerler, pektin maddeleri, lifler ve askıda katılar arasındaki etkileşimler, pürelerin Newtonyen olmayan akış davranışına katkıda bulunurlar. Meyve ve sebze pürelerinin yapısı, çözünmeyen (hücre duvarlarının malzemeleri, lifler) ve çözünen (şekerler, mineraller, proteinler ve polisakaritler) bileşenlerden oluşur. Bu oran meyve ve sebzelere göre değişiklik gösterir. Pürelerin reolojik davranışı, pürenin bileşenlerinin oranı, pürenin parçacık şekli ve boyutu gibi birçok faktörden etkilenmektedir. Bu nedenle her meyve püresinin akış davranışını tanımlayan reolojik model farklıdır. Meyve ve sebze pürelerinin akış davranışını ve deformasyonunu tanımlamak amacıyla, reolojik testlerden elde edilen deneysel veriler reolojik modellere uygulanır (Martínez-Padilla, 2024). Bu modellerden en yaygın kullanılanlar Power Law, Casson, Mizhari-Berk ve Herschel-Bulkley modelleridir. Kıvam katsayısı ( $k$ ), akma gerilimi ( $\tau_0$ ) ve akış davranış indeksi ( $n$ ) gibi modellerin reolojik parametreleri, incelenen pürenin reolojik davranış tipini belirler (Milani ve ark., 2019).

Trabzon hurma meyvesi (*Diospyros kaki L.*), duyuşal özellikleri, biyoaktif fitokimyasalları ve zengin besin içeriği ile birçok ülkede sevilerek tüketilen değerli bir meyvedir. Hurma, biyoaktiviteye sahip birçok makro ve mikro besin kaynağıdır (Kaur ve ark., 2022). Bu bağlamda,

karbonhidratlar, diyet lifleri, mineraller, vitaminler, karotenoidler, organik asitler ve fenolik bileşikler meyvenin ana besin maddelerini oluşturur (Matheus ve ark., 2022; Tardugno ve ark., 2022). Bu besin içerikleriyle antioksidan, anti-inflamatuar, sitotoksik, kardiyoprotektif ve nöroprotektif aktivitelere sahip olduğu bilinmektedir. Yapılan araştırmalar, Trabzon hurma tüketimini çeşitli hastalık risklerinin azalmasıyla ilişkilendirilmiş ve terapötik özellikleri nedeniyle biyoaktif fenolik bileşiklerin katkısı vurgulanmıştır (Tardugno ve ark., 2022). Bu fonksiyonel bileşikler, diyabet, hiperkolesterolemi ve kanser gibi çeşitli rahatsızlıkların önlenmesinde ve tedavisinde önemli bir rol oynamaktadır (Yaqub ve ark., 2016). Hurma meyveleri geleneksel olarak öksürük, hipertansiyon, donma, yanıklar ve kanama gibi birçok tıbbi amaç için de kullanılmıştır (Tardugno ve ark., 2022).

Trabzon hurması mevsimlik bir meyvedir ve her yıl sınırlı bir süre (Ekim-Aralık) taze olarak tüketilir (Murali ve ark., 2023). Olgunlaşmış Trabzon hurmasının raf ömrü kısadır ve çabuk bozulur. Meyvenin besin bileşenlerinin korunması ve daha uzun süre tüketilebilir olması için işlenerek farklı hurma ürünlerine dönüştürülmelidir. Hurma püresi gıda işleme endüstrisi tarafından jelatin, kek, jöle, turta, yoğurt, meyve suyu, sirke, alkol, dondurma, puding, nektar gibi çeşitli gıda ürünlerinin üretiminde kullanılabilir (Murali ve ark., 2023). Meyvenin püreye işlenebilmesi için reolojik özelliklerinin belirlenmesi gerekir. Meyve pürelerinin reolojik özellikleri üzerine çok sayıda çalışma yapılmıştır. Bunlara, yaban mersini püresi (Nindo ve ark., 2007), macaiba posası (Brasileiro ve ark., 2022), Hint hurması posası (Patel ve ark., 2022) ve jambolan posası (Costa ve ark., 2024) gibi çalışmalar örnek olarak verilebilir. Endüstriyel işleme için gerekli olmasına rağmen, Trabzon hurma püresinin reolojik karakterizasyonu ile ilgili çalışmalara literatürde rastlanmamıştır. Trabzon hurması püresinin akış davranışının ve reolojik özelliklerinin belirlenmesi, püre üretimine önemli katkı sağlayacaktır. Bu nedenle pH, sıcaklık ve konsantrasyon gibi çalışma koşullarında Trabzon hurması püresinin akış davranışını en iyi tanımlayan reolojik modelin belirlenmesi amaçlanmıştır.

## Materyal ve Yöntem

### Materyal

Bu çalışmada kullanılan Trabzon hurmaları Burdur'da bir meyve bahçesinden Ekim ayı sonunda temin edilmiştir. Olgunlaşan meyveler +4°C'de depolanmıştır. Çalışmada kullanılan tüm reaktifler ve çözücüler analitik saflıktadır (Merck, Darmstadt, Almanya).

### Metot

#### Pürenin Hazırlanması

Trabzon hurması meyveleri tam olgunlaşmanın ardından yıkanıp temizlenmiş ve ev tipi bir blender (Braun MQ7045X, Romanya) kullanılarak kabukları ile birlikte püre haline getirilmiştir. Püre 40 gramlık porsiyonlar halinde sterilize edilmiş tek kullanımlık plastik torbalarla paketlenmiştir. Bir kısmı dondurularak -18°C'de, diğer kısmı ise işlenene kadar 4°C'de saklanmıştır (Gürdaş Mazlum ve Lodos, 2025).



Çalışmada kullanılan Trabzon hurma püresi, 100 g numune başına 15, 17,5 ve 20 g çözünmüş katı madde içeren üç standart örnek uygun miktarda deiyonize su ile seyreltilmiştir (Nindo ve ark., 2007). Örneklerin pH ayarı için analitik saflıkta sitrik asit (Merck) ve sodyum hidroksit (Merck) kullanılarak pH 4, 5,5 ve 7'ye ayarlanmıştır. %15, %17,5 ve %20 toplam çözünür katı madde içeren hurma püresinin viskozitesi 25, 50 ve 75°C'de ölçülmüştür. Sıcaklık ekipmana bağlı termostatik bir banyo ile  $\pm 0,1^\circ\text{C}$  hassasiyetle kontrol edilmiştir. Yüksek sıcaklıkta buharlaşmayı en aza indirmek için numune kabı cam bir kapakla kapatılmıştır.

#### Deneyel Tasarım

Trabzon hurması püresinin kayma akış davranışı üzerinde bağımsız değişkenlerin etkisini incelemek için Box-Behnken tasarımı kullanılmıştır. Deney tasarımında, pH ( $X_1$ ), konsantrasyon ( $X_2$ ) ve sıcaklık ( $X_3$ ) olmak üzere 3 faktör için sınır değerlere göre merkez nokta hesaplanarak üç seviye belirlenmiştir. Tasarım, her bir faktör, yüksek (1), orta (0) ve düşük (-1) olmak üzere üç seviyeye sahip ve merkez noktanın üç tekrarı içeren 15 deneysel noktadan oluşmaktadır. Üç bağımsız değişkenin kodlanmış ( $x_i$ ) ve bunlara karşılık gelen kodlanmamış ( $X_i$ ) değerleri Çizelge 1'de gösterilmektedir. Deney tasarımını

oluşturmak ve verileri analiz etmek için Minitab Statistical Software Release 13 (Minitab Inc, State College, PA) bilgisayar programı kullanılmıştır.

#### Reolojik Ölçüm

Reolojik özellikler silindirik bir mil ile donatılmış döner bir viskozimetre (DV-II+Pro modeli; Brookfield Engineering Labs. Inc., ABD) kullanılarak kontrollü sıcaklıklarda ölçülmüştür (Brasileiro ve ark., 2022). Sıcaklık, termostatik olarak kontrol edilen bir su banyosu ile sabit tutulmuştur. Trabzon hurması püresinin reolojik ölçümlerinde yapılan ön denemelerde numunenin konsantrasyonuna göre rpm değeri belirlenmiş ve uygun miller (mil no: 5 ve 6) seçilmiştir. Mil seçimi tork değerinin %10-90 arasında olması gerektiği dikkate alınarak yapılmıştır. Ölçümler püre 1 dakika boyunca karıştırıldıktan sonra alınmıştır. Ölçümler 10 saniye aralıklarla kaydedilmiş ve her örnek için sabit kayma hızında 60 veri kaydedilmiştir. Pürenin viskozite ölçümleri, mil hızı 30 rpm'den ( $8,4 \text{ s}^{-1}$ ) kademeli olarak 100 rpm'e ( $28 \text{ s}^{-1}$ ) kadar artırılarak gerçekleştirilmiştir. Tüm ölçümler için 500 ml'lik beher kullanılmıştır. Her deneysel çalışmadan önce, hurması püresi örnekleri her reolojik testten 1 saat önce oda sıcaklığında ( $25^\circ\text{C}$ ) çözülümüştür (Gürdaş Mazlum ve Lodos, 2025).

Çizelge 1. The Box-Behnken tasarım matrisi

Table 1. The Box-Behnken design matrix

Yöntem	Çalışma sırası (Run)	pH $X_1$ ( $x_1$ )	Konsantrasyon (%w/w) $X_2$ ( $x_2$ )	Sıcaklık ( $^\circ\text{C}$ ) $X_3$ ( $x_3$ )
T1	1	5,5 (0)	15 (-1)	25 (-1)
T2	2	5,5 (0)	20 (+1)	75 (+1)
T3	3	7 (+1)	15 (-1)	50 (0)
T4	4	5,5 (0)	17,5 (0)	50 (0)
T5	5	4 (-1)	17,5 (0)	25 (-1)
T6	6	7 (+1)	17,5 (0)	25 (-1)
T7	7	5,5 (0)	17,5 (0)	50 (0)
T8	8	5,5 (0)	20 (+1)	25 (-1)
T9	9	7 (+1)	20 (+1)	50 (0)
T10	10	4 (-1)	15 (-1)	50 (0)
T11	11	4 (-1)	17,5 (0)	75 (+1)
T12	12	5,5 (0)	15 (-1)	75 (+1)
T13	13	4 (-1)	20 (+1)	50 (0)
T14	14	5,5 (0)	17,5 (0)	50 (0)
T15	15	7 (+1)	17,5 (0)	75 (+1)

Çizelge 2. Trabzon hurması püresinin reolojik davranışının modellenmesinde kullanılan reolojik denklemler ve model parametreleri (Milani ve ark., 2019)

Table 2. Rheological equations and model parameters used to model the rheological behaviour of persimmon puree (Milani et al., 2019)

Model numarası	Model adı	Denklem	Model parametreleri
1	Power law (Ostwald-de-Waele)	$\tau = k\gamma^n$	k:kıvam katsayısı ( $\text{Pa}\cdot\text{s}^n$ ) n:akış davranış indeksi (boyutsuz)
2	Herschel-Bulkley	$\tau = \tau_0 + k(\gamma)^n$	k: kıvam katsayısı ( $\text{Pa}\cdot\text{s}^n$ ) $\tau_0$ :akma gerilimi (Pa) n:akış davranış indeksi (boyutsuz)
3	Casson	$\tau^{0,5} = \tau_0^{0,5} + k_c\gamma^{0,5}$	$k_c$ : kıvam katsayısı ( $\text{Pa}\cdot\text{s}^{0,5}$ ) $\tau_0$ :akma gerilimi ( $\text{Pa}^{0,5}$ )
4	Mizhari-Berk	$\tau^{0,5} = \tau_0^{0,5} + k_m\gamma^n$	$k_m$ : kıvam katsayısı ( $\text{Pa}^{0,5}\cdot\text{s}^n$ ) $\tau_0$ :akma gerilimi ( $\text{Pa}^{0,5}$ ) n:akış davranış indeksi (boyutsuz)

$\tau$ :kayma gerilimi (Pa),  $\gamma$ : kayma hızı ( $\text{s}^{-1}$ )

Her ölçüm için yeni bir örnek kullanılmıştır. Trabzon hurması püresinin viskozitesi ve torku, 4,0, 5,5, 7,0 pH aralığında, %15, %17,5, %20 konsantrasyonlarda, 25, 50, 75 °C sıcaklıklarda ve 5 mil hızında (30, 50, 60, 90 ve 100 rpm) ölçülmüştür. Tüm deneyler iki tekrarlı olarak yapılmış ve ortalaması alınmıştır. Ortalama kayma gerilmeleri ve kayma hızları Mitschka (1982) yöntemiyle hesaplanmıştır.

#### Veri analizi ve modelleme

Püreler genellikle Newtonyen olmayan akış davranışı gösterirler. Meyvelerin ve türevlerinin reolojik davranışlarını tanımlamada Güç yasası, Herschel-Bulkley, Casson ve Mizhari-Berk en yaygın kullanılan reolojik modellerdir (Kechinski ve ark., 2011). Bu nedenle Trabzon hurması püresinin akış davranışını tanımlamak için bu dört model seçilmiş ve modellerin denklemleri ve parametreleri Çizelge 2’de verilmiştir.

Deneysel verilere en iyi uyum sağlayan modeli belirlemek için pürenin deneysel kayma gerilimi-kayma hızı verileri Güç yasası, Herschel-Bulkley, Casson ve Mizhari-Berk modellerine uygulanmıştır. Tüm modellerin parametre tahminleri doğrusal ve doğrusal olmayan regresyon analizleri ile  $p < 0,05$  anlamlılık düzeyinde hesaplanmıştır. Doğrusal olmayan en küçük kareler regresyon yöntemi Microsoft Excel 2016 (Microsoft Corporation, ABD) çözücü aracı kullanılarak gerçekleştirilmiştir (Mustapha ve ark., 2023).

Uyumun iyiliğini değerlendirmek için belirleme katsayısı ( $R^2$ ), hata kareleri ortalamasının kare kökü ( $RMSE$ ) ve indirgenmiş ki- kare ( $\chi^2$ ) olmak üzere üç istatistiksel ölçüt kullanılmıştır. Reolojik modellerin akış davranışını açıklamadaki uygunluğunu doğrulamak için indirgenmiş ki-kare ( $\chi^2$ ) ve hata kareleri ortalamasının kare kökü ( $RMSE$ ) gibi hata analizi yöntemleri kullanılmış ve belirleme katsayısı ( $R^2$ ) ile karşılaştırılmıştır (Mustapha ve ark., 2023). Çünkü reoloji denklemlerinin doğrusal biçimleri ile deneysel veriler arasındaki uygunluğu temsil ettiği için,  $R^2$  tek başına en iyi reolojik modelin seçiminde yeterli olmayabilir. Deneysel veriler ile modelden elde edilen veriler arasındaki fark  $\chi^2$  ile ölçülür (Mustapha ve

ark., 2023).  $R^2$ ’nin en yüksek değerleri,  $RMSE$  ve  $\chi^2$ ’nin en düşük değerleri, en iyi uyumu gösteren modeli belirlemek için kullanılmıştır.  $R^2$ ,  $RMSE$  ve  $\chi^2$  sırasıyla 1-3 denklemleri kullanılarak hesaplanmıştır (Milani ve ark., 2019; Tavakolipour ve ark., 2020).

$$R^2 = 1 - \frac{\sum_{i=1}^N (\tau_{exp,i} - \tau_{pre,i})^2}{\sum_{i=1}^N (\tau_{exp,i} - \bar{\tau}_{exp,i})^2} \quad (1)$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^N (\tau_{exp,i} - \tau_{pre,i})^2} \quad (2)$$

$$\chi^2 = \frac{\sum_{i=1}^N (\tau_{exp,i} - \tau_{pre,i})^2}{N-m} \quad (3)$$

burada  $\tau_{exp,i}$  deneysel kayma gerilimi,  $\tau_{pre,i}$  tahmin edilen kayma gerilimi,  $\bar{\tau}_{exp,i}$  deneysel kayma geriliminin ortalaması,  $N$  gözlem sayısı ve  $m$  modelin sabitleri.

## Bulgular ve Tartışma

### Matematiksel Modelleme

Trabzon hurması püresinin reolojik modelinin belirlenmesi amacıyla oluşturulan pH, konsantrasyon ve sıcaklık olmak üzere 3 faktör ve üç seviye içeren Box-Behnken deney tasarımı Tablo 1’de gösterilmektedir. Pürenin viskozite ölçümleri  $8,4 \text{ s}^{-1}$ ’den kademeli olarak  $28 \text{ s}^{-1}$  kadar artırılarak gerçekleştirilmiştir. Trabzon hurması püresi için 4,0, 5,5, 7,0 pH, %15, %17,5, %20 konsantrasyon ve 25, 50, 75°C sıcaklıklarda kayma gerilimi ve kayma hızı verileri toplanmıştır. Ölçülen veriler, çalışılan pH, sıcaklık ve konsantrasyon aralıklarında Trabzon hurması püresinin akış davranışını en iyi tanımlayan modeli belirlemek için Power Law, Herschel-Bulkley, Casson ve Mizhari-Berk olmak üzere dört modele uygulanmıştır (Çizelge 2). Parametre tahmininin sonuçları Power Law ve Herschel-Bulkley modelleri için Çizelge 3’de, Casson ve Mizhari-Berk modelleri için Çizelge 4’de verilmiştir.

Çizelge 3. Power law ve Herschel-Bulkley modelleri için parametre tahmini sonuçları

Table 3. Results for parameters estimation for Power law and Herschel-Bulkley models

Yöntem	Power law					Herschel-Bulkley					
	K Pa s <sup>n</sup>	n	R <sup>2</sup>	RMSE	$\chi^2$	K Pa s <sup>n</sup>	n	$\tau_0$ Pa	R <sup>2</sup>	RMSE	$\chi^2$
T1	3,869	0,723	0,989	0,537	0,481	3,046	0,779	2,224	0,997	0,499	0,624
T2	3,574	0,726	0,937	1,091	1,983	2,939	0,771	1,748	0,984	1,079	2,916
T3	3,794	0,627	0,997	0,189	0,059	2,306	0,741	3,391	0,999	0,129	0,042
T4	3,430	0,642	0,994	0,279	0,129	2,798	0,687	1,398	0,998	0,276	0,189
T5	5,064	0,775	0,988	0,942	1,480	4,416	0,808	1,849	0,997	0,866	1,876
T6	9,579	0,644	0,922	2,741	12,526	8,295	0,675	3,013	0,972	2,673	17,867
T7	3,502	0,566	0,929	0,544	0,494	2,219	0,668	2,592	0,983	0,541	0,732
T8	14,487	0,397	0,903	1,356	3,064	10,834	0,452	5,565	0,971	1,289	4,159
T9	13,574	0,285	0,821	0,875	1,275	8,474	0,365	6,540	0,947	0,872	1,899
T10	6,640	0,281	0,878	0,308	0,159	4,032	0,364	3,337	0,967	0,325	0,265
T11	2,887	0,771	0,997	0,246	0,101	2,186	0,838	2,009	0,999	0,179	0,080
T12	3,125	0,700	0,975	0,574	0,549	2,682	0,736	1,088	0,993	0,573	0,822
T13	7,670	0,586	0,959	1,177	2,308	5,631	0,652	4,436	0,988	1,101	3,029
T14	4,778	0,501	0,726	1,119	2,089	1,374	0,783	6,898	0,935	1,099	3,017
T15	4,313	0,775	0,991	0,684	0,781	3,665	0,815	1,837	0,997	0,659	1,086

Çizelge 4 Casson ve Mizrahi-Berk modelleri için parametre tahmini sonuçları  
Table 4 Results for parameters estimation for Casson and Mizrahi-Berk models

Yöntem	Casson					Mizhari-Berk					
	$k_c Pa s^{0,5}$	$\tau_0 Pa^{0,5}$	$R^2$	RMSE	$\chi^2$	$k_m Pa^{0,5} s^n$	N	$\tau_0 Pa^{0,5}$	$R^2$	RMSE	$\chi^2$
T1	0,964	1,487	0,996	0,044	0,003	1,176	0,458	1,162	0,999	0,044	0,005
T2	0,985	1,239	0,992	0,111	0,021	1,121	0,461	1,132	0,988	0,089	0,020
T3	0,729	1,707	0,999	0,018	0,001	1,070	0,420	1,198	0,999	0,013	0,001
T4	0,700	1,700	0,995	0,361	0,217	1,010	0,431	1,157	0,999	0,028	0,002
T5	1,238	1,617	0,995	0,059	0,006	1,419	0,474	1,99	0,997	0,063	0,009
T6	1,111	3,058	0,963	0,174	0,050	1,812	0,411	1,875	0,972	0,172	0,077
T7	0,621	1,617	0,996	0,078	0,012	0,996	0,391	1,144	0,999	0,058	0,008
T8	0,623	4,066	0,968	0,080	0,011	2,268	0,268	1,831	0,969	0,098	0,024
T9	0,390	3,886	0,914	0,082	0,011	2,094	0,209	1,732	0,946	0,080	0,016
T10	0,286	2,650	0,944	0,056	0,005	1,257	0,228	1,434	0,966	0,044	0,006
T11	0,944	1,157	0,999	0,871	1,265	1,003	0,487	1,062	1	0,015	0,001
T12	0,843	1,288	0,993	0,069	0,008	0,975	0,462	1,136	0,993	0,059	0,008
T13	0,872	2,712	0,984	0,079	0,011	1,688	0,375	1,454	0,987	0,087	0,019
T14	0,571	2,085	0,914	0,129	0,028	1,174	0,350	1,268	0,934	0,125	0,039
T15	1,185	1,332	0,997	0,053	0,005	1,277	0,481	1,215	0,997	0,049	0,006

Çizelge 5 Modellerin istatistiksel parametrelerinin ortalama ve kritik (minimum ve maksimum) değerleri (Çizelge 3 ve Çizelge 4 verilerine dayanmaktadır) (Kechinski ve ark., 2011)

Table 5 Average and critical (minimum and maximum) values of the statistical indicators for the different models (based on data of Table 3 and Table 4)

Modellerin istatistiksel parametrelerinin ortalama ve kritik değerleri		Power law	Casson	Herschel-Bulkley	Mizhari-Berk
$R^2$	Average	0,934	0,977	0,982	0,983
	Minimum	0,726	0,914	0,935	0,934
$\chi^2$	Average	1,832	0,110	2,574	0,016
	Maximum	12,526	1,265	17,867	0,077
RMSE	Average	0,844	0,151	0,811	0,068
	Maximum	2,741	0,871	2,673	0,172

Trabzon hurması püresinin çeşitli pH, konsantrasyon ve sıcaklıklardaki kayma gerilimi ve kayma hızı arasındaki ilişki için her modelde  $R^2$ ,  $RMSE$  ve  $\chi^2$  istatistiksel parametreleri 1-3 denklemleri ile hesaplanmış ve Çizelge 3 ve 4'de verilmiştir. Modellerin yeterliliği değerlendirilirken  $R^2$ 'nin en yüksek değerleri,  $RMSE$  ve  $\chi^2$ 'nin için en düşük değerleri kullanılmıştır. Modellerin yeterliliğini karşılaştırmak amacıyla, her bir model için istatistiksel göstergelerin (belirleme katsayısı, hata kareleri ortalamasının kare kökü ve ki-kare) ortalama ve kritik (minimum veya maksimum) değerleri hesaplanmış ve Çizelge 5'de verilmiştir. Bu göstergelerin ortalaması ve minimum ve maksimum değerleri, incelenen tüm modelin genel uyum yeteneği hakkında önemli bilgiler verir (Kechinski ve ark., 2011). Modeller incelenirken, verilerin tamamını temsil eden istatistiksel parametrelerin ortalaması dikkate alınmıştır.

Power Law ve Casson modelleri incelendiğinde ortalama  $R^2$  değeri en yüksek, ortalama  $RMSE$  ve  $\chi^2$  değerleri en düşük olan Casson modeli deneysel verilere daha iyi uyum göstermiştir (Çizelge 5). Kechinski ve ark., (2011) yaptıkları çalışmada yaban mersini püresi için 2 parametrelili Power Law ve Casson modellerinin kıyaslanmasında Casson modelinin daha iyi uyum sağladığını bildirmişlerdir. Power Law modeli, konsantre meyve ve sebze suları, meyve posaları ve püre tipi gıdaların akma gerilimi olmadan akış davranışlarını karakterize etmek için en yaygın kullanılan modeldir. Power Law ve Casson modelleri, köri yaprağı püresi

(Meher ve ark., 2018), Fars üzüm pekmezi (Tavakolipour ve ark., 2020), üzüm pekmezi Milani ve ark., 2019) ve Hint hurması pulunun (Patel ve ark., 2022) akış davranışını tanımlayan en iyi modeller olarak gösterilmiştir.

Herschel-Bulkley modeli  $R^2 \geq 0,982$  ve  $RMSE \leq 0,811$  ile deneysel verilere iyi uyum göstermiştir. Herschel-Bulkley ve Mizhari-Berk modelleri incelendiğinde ortalama  $R^2$  değeri her iki modelde birbirine yakındı ancak, ortalama  $RMSE$  ve  $\chi^2$  değerleri en düşük olan Mizhari-Berk modeli deneysel verilere daha iyi uyum göstermiştir (Çizelge 5). Çünkü Mizhari-Berk modelinin  $\chi^2$  değeri Herschel-Bulkley modelinin  $\chi^2$  değerinden daha düşük bir değere sahiptir. Bu nedenle Trabzon hurması püresinin deneysel verilerine en iyi uyumu sağlayan Mizhari-Berk modelidir. Mizrahi-Berk tarafından önerilen reolojik model, meyve posaları, meyve suları ve pürelerin akış eğrileriyle iyi bir uyum sağlar. Bu yazarlar, modellerini, konsantre bir portakal suyundaki ve diğer psödoplastik çözücülerdeki parçacıkların etkileşimini temsil etmek için değiştirilmiş bir Casson modeli olarak geliştirmişlerdir (Santos ve ark., 2016).

İki parametrelili Casson modeli ile üç parametrelili Mizhari-Berk modeli karşılaştırıldığında deneysel verilere en iyi uyumu gösteren modelin Mizhari-Berk olduğu görülmüştür. Mizhari-Berk modeli  $R^2 \geq 0,983$ ,  $RMSE \leq 0,068$  ve  $\chi^2 \leq 0,016$  istatistiksel parametreleriyle incelenen reolojik modeller arasında hurma püresinin akış davranışını en iyi tanımlayan model olduğu belirlenmiştir

(Çizelge 5). Bazı araştırmacılar, malay elma suyu (Santos ve ark., 2016) vişne püresi (Lukhmana ve ark., 2018), makaiba pulpu (Brasileiro ve ark., 2022) ve jambolan pulpu (Costa ve ark., 2024) gibi meyve ürünlerinin akış davranışlarının Herschel-Bulkley ve Mizrahi-Berk modelleri tarafından tanımlandığını bildirmişlerdir.

Trabzon hurması püresi, çözünür şekerler, organik asitler, çözünür pektinler, vitaminler, mineraller, karotenoidler ve fenolik bileşikler gibi içerdiği bileşenler arasındaki karmaşık etkileşimler sonucu Newtonyen olmayan bir sıvı gibi davrandığını göstermiş ve aynı zamanda akma geriliminin varlığına işaret etmiştir (Matheus ve ark., 2022). Bu durum Casson, Herschel-Bulkley ve Mizrahi-Berk gibi akma gerilim içeren modellerin istatistiksel parametrelerinden görülmektedir. Casson, Herschel-Bulkley ve Mizrahi-Berk modellerinin ortalama  $R^2$  ve  $RMSE$  değerleri sırasıyla 0,977, 0,982 ve 0,983 olarak ve 0,151, 0,811 ve 0,0683 olarak belirlenmiştir. Deneysel verilere iyi uyum sağlayan modeller, ortalama  $R^2$  değerine göre Mizrahi-Berk > Herschel-Bulkley > Casson şeklinde sıralanabilir. Akma gerilimi, pürenin akışını başlatmak için gereken minimum kayma gerilimidir. Akma geriliminin altındaki gerilimde, malzeme elastik bir şekilde deforme olur ve elastik bir katı gibi davranır; akma geriliminin üstünde ise akmaya başlar ve viskoz bir sıvı gibi davranır (Augusto ve ark., 2012).

Power Law, Herschel-Bulkley ve Mizrahi-Berk modellerinin akış davranışı indeksi ( $n$ )  $0 < n < 1$  aralığında bulunmuştur. Püresinin akış davranışı indeksinin  $n < 1$  olması psödoplastik (kayma inceltme) davranışa işaret eder. Trabzon hurması püresinin kayma hızı ve kayma gerilimi arasındaki ilişkinin de Newtonyen olmadığını gösterir. Benzer bulgular Costa ve ark., (2024) tarafından 10, 20, 30, 50 ve 70°C sıcaklıklardaki jambolan pulpu için bildirilmiştir. Literatürde, yaban mersini püresi (Kechinski ve ark., 2011), siriguella pulpu (Augusto ve ark., 2012), hurma şurubu (Gabsi ve ark., 2013), açai pulpu (Costa ve ark., 2018) gibi diğer meyvelerde de kayma inceltme davranışı tanımlanmıştır. Akış davranış indeksinin ( $n$ ) farklı değerleri akışkan davranışını gösterir. Akışkanlar, akış davranışı indeksi,  $n=1$  ise Newtonyen,  $n > 1$  ise dilatan ve  $n < 1$  ise psödoplastik (kayma inceltme) olarak tanımlanır (Martinez-Padilla, 2024). Çoğu meyve püresi kayma inceltme davranışı gösterir ( $0 < n < 1$ ), kayma sırasında oluşan hidrodinamik kuvvetler nedeniyle bir gıdadaki yapısal birimlerin bozulmasının bir göstergesi olarak kabul edilmektedir (Nindo ve ark., 2007). Mevcut çalışmada Mizrahi-Berk modelinin akış davranış indeksi 0,209-0,487 arasında değerler almıştır. Benzer bulgular malay elma suyu konsantreleri için  $n < 0,5$  olduğu bildirilmiştir (Santos ve ark., 2016).

## Sonuç ve Öneriler

Trabzon hurması püresinin reolojik özellikleri farklı pH, konsantrasyon, sıcaklık ve kayma hızında ( $8,4-28 \text{ s}^{-1}$ ) araştırılmıştır. Trabzon hurması püresinin akış davranışını en iyi tanımlayan reolojik modelin belirlenmesi amacıyla deneysel veriler Power Law, Herschel-Bulkley, Casson ve Mizrahi-Berk modellerine uygulanmıştır. Reolojik modellerin uygunluğunu doğrulamak için belirleme katsayısı ( $R^2$ ), indirgenmiş ki-kare ( $\chi^2$ ) ve hata kareleri ortalamasının kare kökü ( $RMSE$ ) gibi istatistiksel parametreler kullanılmıştır.

Herschel-Bulkley ve Mizrahi-Berk modelleri, Trabzon hurması püresinin tüm işlem koşullarında deneysel verilerine iyi uyumu sağlamıştır. Ancak Mizrahi-Berk modeli  $R^2 \geq 0,983$ ,  $RMSE \leq 0,0683$  ve  $\chi^2 \leq 0,0160$  istatistiksel parametreleriyle incelenen reolojik modeller arasında hurma püresinin akış davranışını en iyi tanımlayan model olmuştur. Power Law, Herschel-Bulkley ve Mizrahi-Berk modellerinin akış davranış indeksi değerlerinin  $n < 1$  olması Trabzon hurması püresinin Newtonyen olmayan davranışa sahip, psödoplastik (kayma inceltme) bir akışkan olduğunu göstermiştir. Bu çalışmanın bulguları, Trabzon hurması püresinin reolojisi hakkında daha fazla araştırma yapılabilmesi için yararlı olabilir. Ayrıca gıda işleme endüstrilerinin hurması püresi ve benzer ürünler üretmesine önemli ölçüde katkı sağlayacaktır.

## Beyanlar

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## Prediction of the Antibacterial Effect of Ozone Against *Listeria* Isolated from Chicken Meat Using a Machine Learning Approach

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 12.11.2024 Accepted : 17.12.2024</p> <p>Keywords: <i>Listeria</i> Ozone Antibacterial Effect XGBoost Machine Learning</p>	<p>In this study, an XGBoost-based prediction model with 99.99% accuracy was developed to predict the antibacterial effects of ozone gas on <i>Listeria</i> spp. isolated from poultry plants and chicken meat. Prior to the machine learning process, various pre-processing procedures were performed on 75 pieces of data obtained from experimental data and 70% of the data were randomly allocated as training set and 30% as test set. In this study, five different machine learning algorithms were tested with default settings and the performance of the models were compared. According to the R<sup>2</sup> score, the XGBoost algorithm was found to be the most successful model. Hyper-parameter optimization was performed to improve the accuracy performance of the XGBoost model. As a result of the study, it was observed that the antibacterial effect on <i>Listeria</i> spp. increased with the increase in the duration of ozone gas application, especially at the end of 20 minutes, <i>Listeria ivanovii</i>, <i>Listeria monocytogenes</i> and <i>Listeria innocua</i> species were completely inhibited. In conclusion, it was determined that the antibacterial effect of ozone on <i>Listeria</i> spp. may vary from species to species and ozone application has potential as an effective antibacterial method in food safety practices. The research findings demonstrate the industrial applicability of predictive models in the field of food safety.</p>

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## Makine Öğrenmesi Yaklaşımı Kullanılarak Tavuk Etinden İzole Edilen *Listeria*'ya Karşı Ozonun Antibakteriyel Etkisinin Tahmin Edilmesi

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 12.11.2024 Kabul : 17.12.2024</p> <p>Anahtar Kelimeler: <i>Listeria</i> Ozon Antibakteriyel Etki XGBoost Makine Öğrenmesi</p>	<p>Bu çalışmada, ozon gazının <i>Listeria</i> spp. (tavuk işletmeleri ve tavuk etlerinden izole edilen) üzerine antibakteriyel etkilerini tahmin etmek amacıyla %99.99 doğruluk oranına sahip bir XGBoost tabanlı tahmin modeli geliştirilmiştir. Makine öğrenimi süreci öncesinde, deneysel verilerden elde edilen 75 adet veri üzerinde çeşitli ön işlemler gerçekleştirilmiş ve verilerin %70'i eğitim, %30'u test seti olarak rastgele ayrılmıştır. Çalışma kapsamında varsayılan ayarlarla beş farklı makine öğrenmesi algoritması denenmiş ve modellerin performansı karşılaştırılmıştır. R<sup>2</sup> skoruna göre en başarılı modelin XGBoost algoritması olduğu tespit edilmiştir. XGBoost modelinin doğruluk performansını artırmak amacıyla hiper-parametre optimizasyonu yapılmıştır. Araştırma sonucunda ozon gazı uygulamasının süresinin artmasıyla birlikte <i>Listeria</i> spp. üzerindeki antibakteriyel etkinin arttığı gözlemlenmiş, özellikle 20 dakika sonunda <i>Listeria ivanovii</i>, <i>Listeria monocytogenes</i> ve <i>Listeria innocua</i> türleri tamamen inhibe edilmiştir. Sonuç olarak, ozonun <i>Listeria</i> spp. üzerindeki antibakteriyel etkisinin türden türe değişebileceği ve ozon uygulamasının gıda güvenliği pratiklerinde etkili bir antibakteriyel yöntem olarak potansiyel taşıdığı tespit edilmiştir. Elde edilen bulgular, gıda güvenliği alanında prediktif modellerin endüstride uygulanabilirliğini göstermektedir.</p>

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## Giriş

Ozon uygulaması; gıda işleme yöntemleri arasında çevre dostu ve ısılmayan bir yöntem olarak ümit verici bir çözüm olarak karşımıza çıkmaktadır. Oksijenin allotropu olan ozon, FDA (Food and Agriculture Organisation) tarafından 2001 yılında hem sulu hem de gaz halinde doğrudan gıdalarda antimikrobiyal olarak (GRAS-Generally Recognized As Safe) kabul edilen oksitleyici bir maddedir (Barthwal ve ark., 2025). Gram pozitif-negatif bakteri sporları ve vejetatif hücreleri, küfler ve virüsler olmak üzere birçok mikroorganizma üzerinde ozonun antimikrobiyal etkisi ortaya koyulmuştur (Giménez ve ark., 2021). Ozonun hücrelere nüfuz etmesi ile hücre zarı bileşenleri, enzimler ve DNA ile RNA gibi genetik materyaller de dahil olmak üzere çeşitli hücresel bileşenler etkilenir. Bu etki hücreler içeriklerin hücre dışına sızmasına ve hücrenin parçalanmasına neden olur (Sarron ve ark., 2021). Ozonlama, gıda sanayiinde sebzelerin yüzey sanitasyonu, içme suyu dezenfeksiyonu, atık su arıtımı ve pestisitlerin giderilmesi gibi çeşitli proseslerde ürünün biyolojik ve kimyasal güvenliği ile raf ömrünü artırmak için uygulanan önemli bir yöntemdir (Öztekin ve ark., 2006; Zorlugenç ve ark., 2008; Pandiselvam ve ark., 2020; Botondi ve ark., 2021; Özen ve ark., 2021).

Listeriosis gıda kaynaklı önemli bir hastalıktır ve ciddi klinik sonuçlara neden olabilmektedir. Listeriosis etmeni *Listeria monocytogenes*'dir. *L. monocytogenes*, *Listeria* cinsteki 6 türden biridir ve önemli bir patojen olarak kabul edilen tek türüdür. Diğer *Listeria* türleri *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii* ve *L. grayi*'dir. 2010-2022 yılları arasında klasik *Listeria* türlerine 22 yeni tür eklenmiştir. Yeni tanımlanan bu 22 tür, *Listeria*'nın genetik olarak tahmin edilenden daha çok çeşitlilik gösterdiğini ortaya koymaktadır (Orsi ve ark., 2024). *Listeria* türleri doğada yaygın olarak bulunmakta olup sağlıklı hayvanlar ile insanların yanı sıra toprak, sebze ve doğal sulardan da izole edilebilmektedirler (Lakićević ve ark., 2010). *Listeria monocytogenes* ile kontamine olmuş gıdaların tüketilmesinden kaynaklanmaktadır. Özellikle işlenmiş et, süt ürünleri, önceden paketlenmiş sandviçler, soğuk tütülenmiş balık, hazırlanmış sebzeler, salatalar ve meyveler gibi kontamine gıda ürünlerinin tüketilmesi yoluyla insana bulaşır.

Listeriosis vakalarını azaltmaya yönelik çalışmalar, gıda güvenliği ile ilgili yapılan yasal düzenlemeler ve gıda güvenliği uygulamalarındaki iyileştirmelere rağmen, sanayileşmiş ülkelerde gıda kaynaklı enfeksiyonlardan kaynaklanan ölümlerin en önemli nedeni invazif listeriosis olarak kabul edilmektedir (Magalhães ve ark., 2016). Günümüz gıda endüstrisinde, ozon gazı güçlü antibakteriyel etkileri nedeniyle birçok uygulamalarda kullanılmaktadır. Meyve ve sebzelerdeki gıda patojenlerinin kesikli tip yıkama ile inaktivasyonu üzerine yapılan bir araştırmada, 30 dakikalık ozonlu su ile yıkama prosesi uygulanmıştır. *Listeria innocua*'nın *E.coli* ve *S. Typhimurium* 'a göre daha yüksek düzeyde (4.7 log kob/g) inaktive olduğu bildirilmiştir (Gibson ve ark., 2019).

Gıda maddeleri kompleks bir yapıya sahiptir ve bu durum gıdaların belirli özellikleri ile mikrobiyal popülasyon dinamikleri arasındaki etkileşimi ölçmeyi

zorlaştırabilir. Bunun başlıca nedeni, çevresel faktörlerin bakteriyel büyüme ve inaktivasyon üzerindeki birleşik etkilerinin yeterince anlaşılammış olmasıdır. Özellikle, bakteri popülasyonlarının davranışı ile çeşitli değişkenler arasındaki ilişkileri tanımlamak zordur. Tahmine dayalı gıda mikrobiyolojisinde, işleme ve depolama koşullarının ürünlerin nihai patojen kontaminasyon seviyeleri üzerindeki etkilerini değerlendirmek amacıyla çeşitli istatistiksel modeller kullanılmaktadır (Hiura ve ark., 2021). Makine öğrenimindeki gelişmeler, gıdalardaki mikrobiyal popülasyon davranışını hassas bir şekilde tahmin etmek için alternatif ve güçlü bir yaklaşımın yanı sıra, genellikle mikrobiyal yanıtla ilişkili olan yüksek miktarda değişkenlik ve belirsizlikle başa çıkma kapasitesi sağlar (Zhen ve ark., 2024).

Hiura ve ark. (2021), mikrobiyolojide çevresel faktörler (sıcaklık, pH, su aktivitesi) kullanarak bakteriyel popülasyon davranışını tahmin etmek için istatistiksel modeller kullanmışlardır. Artan veri miktarı ve karmaşıklığı nedeniyle yüksek boyutlu değişkenlerle tüm verileri işlenmesinin zorluğu nedeniyle, mikrobiyal tepkilerin bir veri tabanını kullanarak bakteriyel davranışları tahmin etmek amacıyla bir veri madenciliği yaklaşımı önermişlerdir. Çalışmalarında, 0-25°C arasında değişen sıcaklıklar altında beş farklı gıda kategorisinde (sığır eti, kültür ortamı, domuz eti, deniz ürünleri ve sebzeler) *Listeria monocytogenes* popülasyonunun büyüme ve inaktivasyon verilerini kullanarak, eXtreme Gradient Boosting Tree (XGBoost) algoritması ile sekiz açıklayıcı değişkene dayalı olarak tahmin modelleri geliştirmişlerdir.

Bu çalışmada, tavuk işletmeleri ve tavuk etlerinden izole edilen *Listeria* spp.'lere karşı ozonun antibakteriyel etkisini belirlemek amacıyla zaman değişkenini açıklayıcı değişken olarak kullanarak XGBoost algoritmasıyla bir tahmin modeli oluşturulmuştur. Çalışmadan elde edilecek sonuçlar, gıda güvenliği alanında prediktif modellerin endüstride uygulanabilirliğini göstermesi açısından değerlidir. Uygulanacak prosedür, tahmin edilen bakteri popülasyonu davranışı ile gıda işleme ve depolama koşullarının belirlenmesine yönelik kılavuz sağlayabilir. Ayrıca, makine öğreniminin sağladığı bu tür çalışmaların, antibakteriyel uygulamaların etkinliğinin artırılmasına ve gıda işleme endüstrisinde risk yönetimi stratejilerinin geliştirilmesine katkı sağlayacağı düşünülmektedir.

## Materyal ve Yöntem

### Materyal

Bu çalışmada kullanılan *Listeria ivanovii*, *Listeria welshimeri*, *Listeria innocua* ve *Listeria welshimeri* türleri, FDA metodu ve Vitec 2 compact (Biomerieux) cihazı kullanılarak tavuk karkaslarından izolasyonu yapılmıştır. Çalışma Çukurova Üniversitesi Gıda Mühendisliği Bölümü Gıda Mikrobiyolojisi laboratuvarında gerçekleştirilmiştir. Kontrol amacıyla, liyofilize *Listeria monocytogenes* (Türkiye-Biomerieux) kullanılmıştır (USDA, 2015).

## Yöntem

### Ozon Gazı Uygulaması Öncesinde *Listeria* spp.'lerin Sıvı Besiyerinde Çoğaltılması

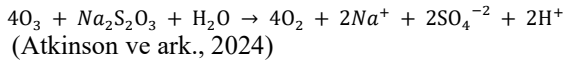
Bu amaçla daha önceden hazırlanan ve sterilize edilen 500 ml hacimli TSB (Tryptic Soy Broth) (Hitchins ve ark., 2022; USDA, 2015) besiyerine *Listeria* spp.'ler aşılansın ve 37°C 48 saat inkübe edildikten sonra ozonlama düzeneğine aktarılmışlardır.

### *Listeria* spp.'lere Ozon gazı Uygulaması

Çalışmada saatte 7 g ozon üretim kapasitesine sahip Ozomax (VTT-1 Model, Kanada) marka ozon jeneratörü kullanılmıştır. Reaksiyon kabı içindeki çözünmüş ozon konsantrasyonunu tespit etmek ve ozon jeneratörü ile oksijen tüpündeki gaz akışının kontrolü amacıyla sisteme PID kontrollü ATI marka (Model: Q45 H/64, ABD) ozon kontrol ve analiz cihazı eklenmiştir. Bu sayede reaksiyon kabı içerisinde 100 ppb çözünmüş ozon seviyesine ulaşıldığında, ozon jeneratörünü ve oksijen gaz akışını sağlayan solenoid valfinin kapatılması sağlanmıştır. Araştırmada kullanılan ozonlama düzeneğinin şeması Şekil 1'de verilmiştir.

Ozonlama işleminin gerçekleştirildiği reaksiyon kabı, ozon gazının yarılanma ömrünün sıcaklığa bağlı olarak değişkenlik göstermesi ve mikroorganizmalarının üreme hızının yavaşlatılabilmesi amacıyla 10°C'ye ayarlı soğutmalı su banyosu içerisine yerleştirilmiştir.

3000 ml hacimli üç boyunlu reaksiyon tankı içerisine 1000 ml steril izotonik çözelti ilave edilmiş hem sistemin dezenfekte edilmesi hem de ozon konsantrasyonunun 100 ppb düzeyinde stabil hale gelmesi için sisteme mikroorganizma aşılama yapılmadan 60 dakika boyunca ozon gazı verilmiştir. Reaksiyon kabı içindeki suda çözünmeyen ozon gazı iş güvenliği açısından tehlike oluşturmaması amacıyla teflon hortum aracılığıyla içerisinde sodyum tiyosülfat çözeltisi bulunan kavanoza aktararak ozon gazı oksijene indirgenmiştir.

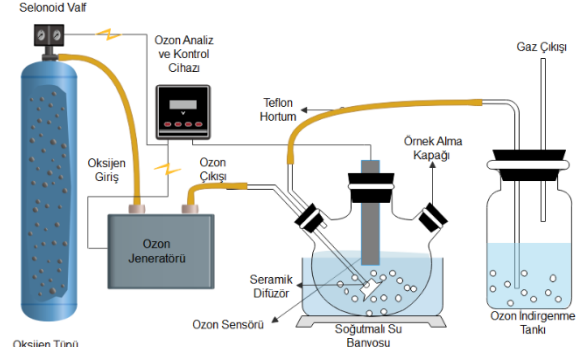


*Listeria* spp. kültürleri, triptic soy broth besi ortamı içerisinde üretilen Şekil 1'de görülen 3 boyunlu reaksiyon kabına aseptik koşullarda (steril kabin içerisinde) bunzen beki yakınında 100 ml olarak ilave edilmiştir. Bu esnada başlangıç mikroorganizma sayısını belirlemek amacıyla yayma ekim yöntemi kullanılarak seri dilüsyonlar hazırlanmış ve PALCAM Agar besiyerine ekim işlemi gerçekleştirilmiştir (Hitchins ve ark., 2022). Sistemden 5, 10, 15 ve 20. dakikalarda örnek alınarak PALCAM Agar besiyerine aynı şekilde ekim yapılmış ve ozon gazının *Listeria* spp. üzerine etkisi belirlenmeye çalışılmıştır.

### Veri Önışleme

Bu çalışmada, deneylerden elde edilen 75 adet veri üzerinde makine öğrenmesi işlemlerine başlamadan önce çeşitli veri ön işleme adımları gerçekleştirilmiştir. İlk olarak, bakteri türleri sayısal olarak kodlanmıştır (örneğin: "*Listeria ivanovii*" 0, "*Listeria monocytogenes*" 1 şeklinde). Daha sonra, verilerin %70'i eğitim, %30'u test verisi olarak rastgele ayrılmıştır. Çalışma kapsamında varsayılan ayarlarla beş farklı makine öğrenmesi algoritması denenmiş ve modellerin performansı karşılaştırılmıştır. R<sup>2</sup> skoruna göre en başarılı modelin XGBoost algoritması olduğu tespit edilmiştir (Çizelge 1).

XGBoost modelinin doğruluk performansını artırmak amacıyla hiper-parametre optimizasyonu yapılmıştır. Bu süreçte, RandomizedSearchCV algoritması kullanılarak belirlenen parametre aralıklarından rastgele kombinasyonlar test edilmiş ve en uygun parametreler belirlenmiştir (Çizelge 2). Optimum parametrelerle yeniden eğitilen model, test verisi üzerinde değerlendirilmiş ve tahmin sonuçları analiz edilmiştir. Çalışmanın tüm veri analizi, model eğitimi, değerlendirme ve grafiksel görselleştirme işlemleri Python programlama dili kullanılarak gerçekleştirilmiştir. Elde edilen sonuçlar, önerilen modelin deneysel veri üzerinde yüksek doğruluk performansı sergilediğini göstermiştir.



Şekil 1. Ozonlama düzeneği  
Figure 1. Ozone application system

### XGBoost Algoritması

XGBoost, Chen ve Guestrin (2016) tarafından geliştirilen bir topluluk (ensemble) öğrenme algoritmasıdır. XGBoost, gradient boosting decision tree (GBDT) algoritmasının geliştirilmiş bir versiyonudur. GBDT, birden fazla karar ağacını içeren yinelemeli bir algoritmadır ve kayıp fonksiyonunun (loss function) gradyanını azaltmaya odaklanan adım adım bir yöntemle çalışır. Bu algoritma, bir karar ağacı modelini temel alarak her adımda hataları düzelten yeni modeller ekleyerek eğitimi sürdürür. Boosting, birden çok zayıf temel modelin birleştirilmesiyle yüksek performanslı bir model oluşturan bir topluluk öğrenme (ensemble learning) yöntemidir. GBDT yöntemleri, makine öğrenimi ve veri madenciliği çalışmalarında yaygın olarak kullanılmaktadır (Chen ve Guestrin, 2016; Hiura ve ark., 2021).

XGBoost algoritmasının temel amacı, aşağıdaki hedef fonksiyonu minimize etmektir; bu fonksiyon, kayıp fonksiyonu ve düzenleme teriminden oluşur:

$$L^{(t)} = \sum_{i=1}^n l(y_i, \hat{y}_i^{(t-1)} + f_t(x_i)) + \Omega(f_t)$$

(Tarwidi ve ark., 2023)

Burada,  $l$  gözlenen veri  $y_i$  ile tahmin edilen veri  $\hat{y}_i$  arasındaki hatayı temsil eden kayıp fonksiyonudur,  $f_t$  t-inci ağacın modelidir ve t optimizasyon sürecindeki iterasyon indeksidir. Düzenleme terimi  $\Omega(f)$  aşağıdaki gibidir:

$$\Omega(f) = \gamma T + \frac{1}{2} \lambda \|w\|^2$$

(Tarwidi ve ark., 2023)

Denklemden T, ağaç yapılarının toplam sayısını,  $\gamma$  ve  $\lambda$  ceza katsayılarını, w ise her bir yaprağın skorunu içeren bir vektörü temsil eder. XGBoost modelleri, XGBoost Python Paketi kullanılarak oluşturulmuştur.



Çizelge 1. Farklı makine öğrenimi algoritmalarının değerlendirme metrikleri ve model sonuçlarının karşılaştırılması  
Table 1. Comparison of evaluation metrics and model results of different machine learning algorithms

Model	R <sup>2</sup>	MSE	RMSE
XGBoost (En iyi Model)	0,9998	0,0009	0,0303
Decision Tree	0,9868	0,0575	0,2397
Gradient Boosting	0,9824	0,0767	0,2770
Random Forest	0,9570	0,1871	0,4326
Support Vector Machine	0,6886	1,3555	1,1643

Çizelge 2. Hiper-parametre optimizasyon işleminin konfigürasyonu ve sonuçları  
Table 2. Configuration and results of the hyper-parameter optimization process

Hiper-parametre	Optimizasyon aralığı	En iyi değer
n_estimators	[100, 200, 300, 400, 500]	300
learning_rate	[0,01, 0,05, 0,1, 0,2, 0,3]	0,3
max_depth	[3, 5, 7, 10]	7
min_child_weight	[1, 3, 5]	3
subsample	[0,6, 0,8, 1,0]	0,6
colsample_bytree	[0,6, 0,8, 1,0]	1,0
gamma	[0, 1, 5]	0

Çizelge 3. Ozon Gazı Uygulamasının *Listeria* spp.'ler Üzerine Etkisi (kob/ml)  
Table 3. Effect of ozone gas application on *Listeria* spp. (cfu/ml)

Türler	Ozon Uygulama Süresi (Dakika)				
	0	5	10	15	20
<i>Listeria ivanovii</i>	1,1×10 <sup>5</sup>	4,2×10 <sup>4</sup>	1,0×10 <sup>3</sup>	8,1×10 <sup>1</sup>	t.e.
<i>Listeria monocytogenes</i>	1,2×10 <sup>5</sup>	9,0×10 <sup>4</sup>	6,0×10 <sup>4</sup>	2,3×10 <sup>3</sup>	t.e.
<i>Listeria innocua</i>	1,1×10 <sup>5</sup>	1,9×10 <sup>4</sup>	2,1×10 <sup>3</sup>	t.e.*	t.e.
<i>Listeria welshimeri</i>	9,8×10 <sup>5</sup>	5,4×10 <sup>4</sup>	2,4×10 <sup>4</sup>	3,4×10 <sup>3</sup>	1,7×10 <sup>2</sup>
<i>Listeria monocytogenes</i> (kontrol)	1,6×10 <sup>6</sup>	6,6×10 <sup>5</sup>	5,0×10 <sup>4</sup>	3,7×10 <sup>3</sup>	7,0×10 <sup>2</sup>

\* t.e.: Tespit edilemedi

## Sonuçlar ve Tartışma

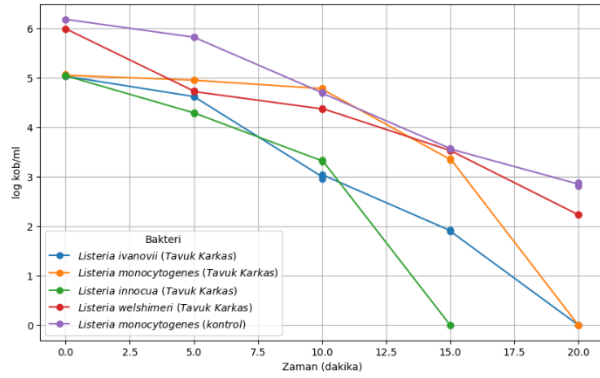
Ozon gazı uygulamasının, tavuk işletmelerinden ve tavuk etlerinden izole edilen *Listeria* spp.'lere karşı etkisi Çizelge 3 ve Şekil 2'de verilmiştir. Uygulama süresi artıkça *Listeria* spp.'lere ozon gazının antibakteriyel etkisi artmıştır. 15. dakikada *Listeria innocua*, 20. dakikada *Listeria ivanovii* ve *Listeria monocytogenes* tamamen inhibe edilmiştir. 20 dakikalık ozonlama sonrasında başlangıç bakterisi yüküne göre *Listeria monocytogenes* (kontrol)'de %80,69 (log kob/ml) ve *Listeria ivanovii* ise %79,71 düzeyinde bir azalma (log kob/ml) meydana gelmiştir. Ozon uygulama süreleri arasında en yüksek antibakteriyel etki 20. dakikada 3,35 logaritmik azalma ile tavuk karkaslarından izole edilmiş olan *Listeria monocytogenes* ile 15. dakikada 3,32 logaritmik azalma ile *Listeria innocua* saptanmıştır.

Şekil 2'de gözlemlenen verilere göre, "*Listeria welshimeri*" ve "*Listeria monocytogenes* (kontrol)" bakterisi türlerinin, diğer türlerin aksine, 20. dakika sonunda tamamen inhibe edilmedikleri görülmektedir. Bu durum, çeşitli makine öğrenmesi modelleri kullanılarak, farklı bakterisi türlerinin gıda üzerindeki azalma eğilimlerini modellemek ve gıda güvenliği açısından kritik zaman noktalarını tahmin etmek için bir fırsat sunmaktadır. Bu amaçla, bu çalışmada Şekil 3'te sonuçları gösterilen lineer regresyon modeli (LR) kullanılmıştır. Şekil 3, "*Listeria welshimeri*" ve "*Listeria monocytogenes* (kontrol)" türlerinin zaman içinde nasıl azaldığını görselleştirmekte ve logaritmik değerlerin (log kob/ml) zamanla nasıl azalan bir eğilim sergilediğini ortaya koymaktadır.

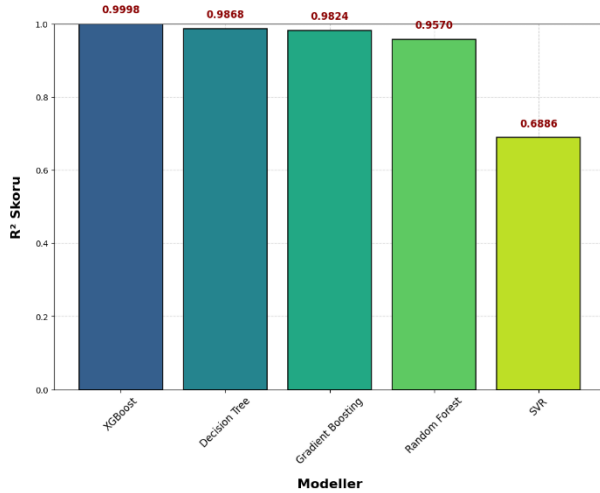
Model, her iki bakterisi türü için de tahmini sifıra ulaşma zamanlarını belirlemiş olup, "*Listeria welshimeri*" için tahmini 33,90 dakika, "*Listeria monocytogenes* (kontrol)" için ise yaklaşık 35,91 dakika olarak öngörülmektedir. Bu tahminler, mevcut verilerde gözlemlenen lineer düşüş eğilimine dayanmakta olup, gerçek deney koşullarında bakterisi sayısının azalmasını bu modelden farklılık gösterip göstermediğini anlamak için daha fazla analiz ve veri gerekmektedir. Şekil 3'ten elde edilen sonuçlar, makine öğrenmesi modellerinin, gıda üzerindeki çeşitli bakterisi türlerinin azalma eğilimlerini başarıyla modelleyebileceğini ve gıda güvenliği açısından kritik zaman noktalarını etkin bir şekilde tahmin edebileceğini göstermesi açısından pratik bir önem taşımaktadır.

Çizelge 3, gerçek deneysel sonuçlardan elde edilen verileri sunmaktadır. Bu veriler ışığında, en dirençli bakterisi türünün *Listeria monocytogenes* (kontrol) olduğu görülmektedir. Bu tür, zamanla işlem sırasında en düşük azalma oranını göstermekte olup, çözülmüş ozona karşı diğer türlere kıyasla daha yüksek direnç sergilemiştir. Bu direnç farklılığının, genetik ve fenotipik çeşitlilikten kaynaklandığı düşünülmektedir (Marino et al., 2018; Orsi et al., 2024)

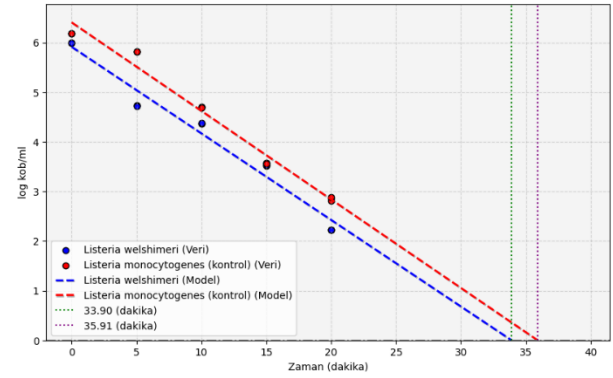
Çizelge 4'teki sonuçlar ise, gerçek deney sonuçlarından elde edilen veriler kullanılarak belirli bir zaman aralığındaki bakterisi yoğunluğundaki değişimi ölçmek için ortalama logaritmik azalmaya göre Python programı kullanılarak hesaplanmıştır.



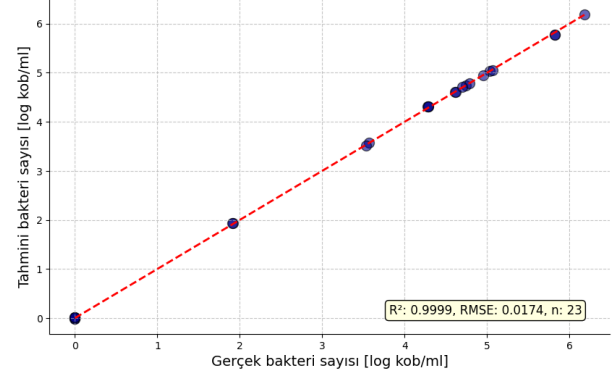
Şekil 2. *Listeria* spp.'ler üzerine ozon gazının etkisi (log kob/ml)  
Figure 2. Effect of ozone gas on *Listeria* spp. (log cfu/ml)



Şekil 4. Model performanslarının R² skorlarına göre karşılaştırması  
Figure 4. Comparison of model performances based on R2 scores



Şekil 3. Bakteri türlerinin log sayımı için LR modeli tahmin sonucu  
Figure 3. LR model estimation result for log count of bacterial species



Şekil 5. Hiper-parametre optimizasyonu sonrası XGBoost model sonucu  
Figure 5. XGBoost model result after hyper-parameter optimization

Çizelge 4. Python Programı Kullanılarak Hesaplanan Ortalama logaritmik Azalmaya Göre Bakteri Direnç Analizi Sonuçları  
Table 4. Bacterial resistance analysis results based on average logarithmic decrease calculated using python program

<i>Listeria</i> Türleri	Azalma Miktarı (log kob/ml)
<i>Listeria monocytogenes</i>	5,06
<i>Listeria innocua</i>	5,04
<i>Listeria ivanovii</i>	5,03
<i>Listeria welshimeri</i>	3,76
<i>Listeria monocytogenes</i> (kontrol)	3,33

Bu yöntemde, her bir bakteri türü için başlangıç zamanı (0 dakika) ve bitiş zamanı (20 dakika) log değerlerinin ortalamaları hesaplanır, ardından bu iki değer arasındaki fark alınarak bakterinin zaman içindeki direnci veya azalma miktarı hesaplanır. Elde edilen sonuçlara göre en dirençli bakteri türü "*Listeria monocytogenes* (kontrol)" olarak belirlenmiştir. Bu bakteri türü, zaman içinde logaritmik olarak en az azalma gösteren türdür. Logaritmik azalma miktarı 3,33 olarak hesaplanmıştır. Elde edilen bu istatistiksel sonuçlar Çizelge 3 sonuçları ile uyum göstermektedir.

Daha önce yapılan bazı çalışmalarda makine öğrenimi algoritmaları bakteriyel davranışları öngörme potansiyelini ortaya koymak amacıyla kullanılmıştır. Hiura ve ark. (2021), bakterilerin gıdalardaki davranışını tahmin etmek için veri madenciliği yaklaşımı bir model geliştirmişlerdir. ComBase veritabanındaki 1.007 farklı çevresel koşul altındaki *Listeria monocytogenes* verilerini kullanarak XGBoost algoritması ile tahminler yapmışlardır. Geliştirdikleri model mikrobiyal bir veri tabanına makine öğrenimi yaklaşımının uygulanabilirliğini göstermesi ve

gıda işleme ve depolama koşullarının belirlenmesi için kılavuzlar sağlama potansiyeli açısından önem arz etmektedir.

Zhen ve ark. (2024), *Listeria monocytogenes* biyofilmlerini paslanmaz çelik yüzeylerde etkisiz hale getirmek için sodyum hipoklorit (NaClO), perasetik asit (PAA), ve klor dioksit (ClO<sub>2</sub>) çözeltilerinin etkinliğini değerlendirmişlerdir. Yapay Sinir Ağları ve Random Forest (RF) modelleri, geleneksel Weibull modeline kıyasla bakteriyel davranış tahmin etmede üstün performans göstermiştir; Özellikle RF modeli eğitim ve doğrulama aşamalarında en başarılı sonuçları vermiştir. Bu sonuçlar, gıda güvenliği ve kaliteyi sağlamak için dezenfeksiyon prosedürlerinin iyileştirilmesine yönelik makine öğrenimi modellerinin potansiyel değerini ortaya koymaktadır.

Bu çalışmada, beş farklı popüler makine öğrenimi algoritmasının performansları karşılaştırılarak değerlendirilmiştir. Algoritmaların başarı düzeyleri, R<sup>2</sup>, MSE ve RMSE metrikleri kullanılarak analiz edilmiştir (Çizelge 1). Varsayılan parametrelerle yapılan değerlendirmede,

XGBoost algoritması  $R^2 = 0,9998$  ve  $RMSE = 0,0303$  değerleriyle en yüksek doğruluk oranını sağlamıştır. Performans sıralamasında XGBoost'u sırasıyla Decision Tree, Gradient Boosting ve Random Forest algoritmaları takip etmiş; SVR algoritması ise  $R^2 = 0,6886$  değeriyle diğer algoritmalara kıyasla daha düşük bir performans sergilemiştir. Bu sonuçlar, XGBoost algoritmasının regresyon problemleri için etkili bir seçenek olduğunu göstermektedir (Şekil 4). Ayrıca, elde edilen bulgular doğrultusunda XGBoost algoritmasına hiper-parametre optimizasyonu uygulanmış (Çizelge 2) ve nihai sonuçlar Şekil 5'te sunulmuştur. Şekil 5, araştırmada kullanılan *Listeria* spp.'lere karşı ozon gazının antibakteriyel etkilerini tahmin etmek amacıyla %99,99 doğruluk oranına ve 0,0174'lük bir RMSE'ye sahip XGBoost tabanlı tahmin modelinin sonucunu göstermektedir.

## Sonuç

Bu çalışmada, tavuk işletmelerinden ve tavuk etlerinden izole edilen *Listeria* spp.'lere karşı ozonun antibakteriyel etkilerini değerlendirmek amacıyla geliştirilen XGBoost tabanlı tahmin modeli, %99,99 gibi yüksek bir doğruluk oranı ile antibakteriyel etkiyi başarıyla tahmin etmiştir. Elde edilen bu sonuç, gıda güvenliği alanında prediktif modellerin endüstride uygulanabilirliğini göstermektedir. Bu prosedür, tahmin edilen bakteri popülasyonu davranışı ile gıda işleme ve depolama koşullarının belirlenmesine yönelik kılavuz sağlayabilir. RandomizedSearchCV kullanılarak yapılan hiper-parametre optimizasyonu, modelin performansını artırmış ve böylece deneysel süreçlerin maliyet ve süresini azaltma potansiyeli sunmuştur. Ayrıca, makine öğreniminin sağladığı bu tür çalışmalar, antibakteriyel uygulamaların etkinliğinin artırılmasında ve gıda işleme endüstrisinde risk yönetimi stratejilerinin geliştirilmesine katkı sağlayabilir. Bu çalışma makine öğrenmesi modellerinin, çeşitli bakteri türlerinin gıda üzerindeki azalma eğilimlerini modelleyerek, gıda güvenliği açısından kritik zaman noktalarını tahmin edebileceğini göstermesi açısından da pratik bir önem arz etmektedir.

Sulu ortamda ozon gazı uygulamasının süresi arttıkça, özellikle *Listeria ivanovii*, *Listeria monocytogenes* ve *Listeria innocua* türlerinde gözlemlenen bakteriyel inhibisyon, ozonun güçlü antibakteriyel kapasitesini göstermektedir. Zaman içinde bakteriyel yüklerdeki azalma eğilimi, ozonun etkinliğini net bir şekilde ortaya koymaktadır; özellikle *Listeria innocua* türü belirgin bir hızla azalmıştır. Ancak, *Listeria monocytogenes* (kontrol) en dirençli tür olarak öne çıkmış, başlangıçta yüksek olan log kob/ml değerleri diğer türlere kıyasla daha yavaş azalmıştır. Bu sonuçlar, *Listeria* spp.'ler üzerindeki suda çözülmüş ozonun etkisinin *listeria* türleri arasındaki genetik çeşitlilikten kaynaklanabileceğini ve bazı durumlarda oldukça etkili olabileceğini göstermektedir. Dolayısıyla, ozonlama gıda güvenliği uygulamalarında önemli bir rol oynayabilecek potansiyel bir antibakteriyel yöntem olarak değerlendirilebilir.

## Beyanlar

### Etik Onay Belgesi

-

## Yazar Katkı Beyanı

B.Z.: mikrobiyolojik analizler, yazım - gözden geçirme ve düzenleme; S.A.: yazım - gözden geçirme ve düzenleme, deneysel verilerin işlenmesi; F.K.Z.: Ozon düzeneğinin kurulması, mikrobiyolojik analizlere yardım, yazım - gözden geçirme ve düzenleme.

## Fon Beyanı

Araştırmada herhangi kurum ya da kuruluştan fon desteği alınmamıştır.

## Çıkar çatışması

Yazarlar bu makalede, diğer kişiler ve kurumlar arasında herhangi bir çıkar çatışmasının olmadığını beyan eder

## Teşekkür

Araştırmada kullanılan bakteri kültürlerinin temininden dolayı Gıda Müh. Dr. S.S.'a teşekkür ederiz.

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## Synthesis of Modified Poly (glycidyl methacrylate) (PGMA) Hydrogels, and Investigation of Their Potential in Dye Removal

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### ABSTRACT

Anthropogenic activities with increasing population lead the pollution of ecosystems. Over one-third of the world's water resources are utilized for agricultural, domestic, and industrial activities, resulting in contamination by synthetic, and geogenic compounds such as dyes, fertilizers, pesticides, and heavy metals. Among these pollutants, dyes are particularly noteworthy due to their extensive use across various sectors, making them one of the leading contributors to water pollution. For this reason, dyes are one of the most important pollutants that cause water pollution. Therefore, the adsorption of Bromophenol blue (BPB) was studied in this study. Firstly, PGMA gels were produced by polymerizing of glycidyl methacrylate (GMA) monomer. Secondly, the PGMA gels were modified to prepare the new adsorbents for the adsorption of BPB dye. Thirdly, the adsorption of BPB dye was carried out. The batch adsorption method was used. The optimum adsorbent amount, initial BPB concentration, pH, and temperature parameters for PGMA gels were determined. The adsorption mechanism between modified PGMA gels, and BPB dye was elucidated by Langmuir, Freundlich, Dubinin-Radushkevich, and Temkin isotherm models. As a result, it was seen that modified PGMA gels showed good performance in the adsorption of BPB.

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## Introduction

Organic dyes are one of the most commonly used and therefore most common hazardous organic pollutants in the environment. The main sources of dyes are primarily leather, textile, plastic, paper, cosmetic, and food industries (Namasivayam et al., 1994). The wastewaters discharged as a result of dyeing processes contain generally high levels of colored components, and this not only causes aesthetic discomfort but also negatively affects biological processes in water bodies. It is well known that azo dyes (e.g. aromatic amines and azo groups) are carcinogenic (Malik, 2004).

The estimates reveal that each year, approximately 12% of synthetic textile dyes are directly discharged during production processes without treatment (Morais et al., 1999). Dyes such as bromophenol blue are frequently preferred compounds for coloring purposes. However, the discharge of these dyes into water resources without treatment poses significant environmental threats by creating toxic effects on both human health, and natural life (Yao et al., 2020). For example, dyes can cause allergic reactions and skin irritation in humans; many of them are also mutagenic and/or carcinogenic (Ghaedi et al., 2013).

In addition, dyes cause water to become colored even in very low concentrations (<1 mg/L). This reduces the transparency of the water, negatively affecting photosynthesis and leading to a decrease in the level of dissolved oxygen (Moradihamedani, 2022).

The removal of dyes from wastewaters is still continued a significant environmental problem and technical challenge. In an effective dye treatment method, large amounts of dyes should be rapidly removed while not causing secondary pollution (Katheresan et al., 2018). To date, various chemical, biological, and physical methods were developed for the dye removal (Mashkoo & Nasar, 2020). Although there are many methods in the literature, most of them are not used due to their lack of sustainability and high costs. For example, biological methods can be effective in removing industrial dyes via biodegradation. However, for biological methods, relatively large areas are required. In addition, these methods are easily affected by environmental conditions. Therefore, the use of these methods is limited. In chemical methods, such as coagulation, ion exchange resins, and advanced oxidation are used; however, these methods are generally high-cost,

and can lead to the formation of undesirable by-products. In other hand, although physical methods such as filtration, and adsorption also have some limitations (Raman & Kanmani, 2016, Mezohegyi et al., 2012), the adsorption method is the most common method for the removal of dyes from industrial wastewater due to its high efficiency, ease of use and low cost. Adsorption stands out as an attractive alternative because it can effectively remove various types of dyes without by-products in wastewater (Mokif, 2019).

In light of the above information, this study aimed to efficiently remove BPB dye, which is widely used in the industry via adsorption method (El-Zahhar et al., 2014; Akpomie et al., 2024). In this context, PGMA gels were synthesized. Then, these gels were modified and characterized. Lastly, the performance of these gels in the adsorption of BPB dye was examined in detail.

## Materials and Methods

### Materials

N,N'-Methylenebisacrylamide (MBA, 99%), Glycidyl methacrylate (GMA, 97%), N,N,N',N'-Tetramethylethylenediamine (TEMED, 99%), and Ethylenediamine (EDA) were purchased from Merck, Ammonium peroxodisulphate (APS) from Sigma Aldrich, and Bromophenol blue (BPB, Mn: 669.96 g mol<sup>-1</sup>) was purchased from AFG Bioscience.

### Synthesis of PGMA gels

PGMA gels were prepared using MBA as a crosslinker. For this, 65 mg (0.75% mol of PGMA) of MBA, and 6 mL of distilled water (DW) were added to 8.0 g of GMA. The mixture was stirred at room temperature for one hour using a magnetic stirrer. Subsequently, 400 µL of TEMED was added to the solution and vortexed for 1 minute, followed by the addition of 1 mol% (140 mg) of APS. The mixture was vortexed again for 2 minutes. Once the milky solution obtained, it was transferred into pipettes. These pipettes were placed on a tube shaker set at 70 rpm and left to react at room temperature for 24 hours. After the reaction was complete, the pipettes were carefully opened and the gels obtained. Then gels washed with DW for two days, and dried at 70°C. Finally, the dried gels were ground for subsequent characterization, and modification.

### Modification of PGMA gels

PGMA gels were modified according to Gokkus et al. (2023). 4 g of PGMA gel, 16.9 g (0.28 mol, 10-fold of PGMA mole) EDA and 25 mL toluene were introduced to the round-bottomed reaction flask. The reaction was conducted 24 h at room temperature and then continued to the reaction at 80 °C for another 8 h. During this period, the solution was continuously stirred at low speed on a magnetic stirrer. End of the reaction, the modified PGMA gels were washed with ethanol, ethanol/distilled water (DW) mixture, and DW, respectively. Thus, PGMA-EDA gels were obtained (Figure 2).

The chemical structures of PGMA-EDA gels were revealed by FT-IR (Bruker, Alpha II), the structures, and gels' surface morphology were investigated by SEM (FEI Quanta FEG250), and their thermal stabilities were analyzed by TGA-DTA analysis (Hitachi STA7300).

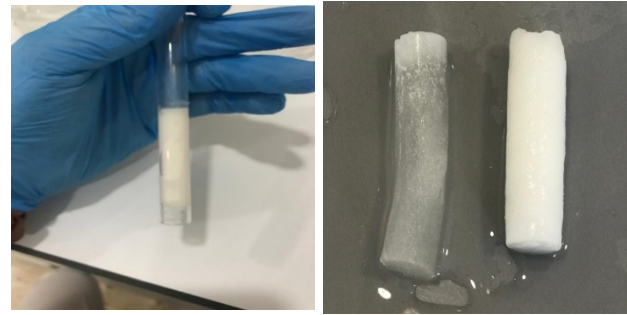


Figure 1. PGMA gels.

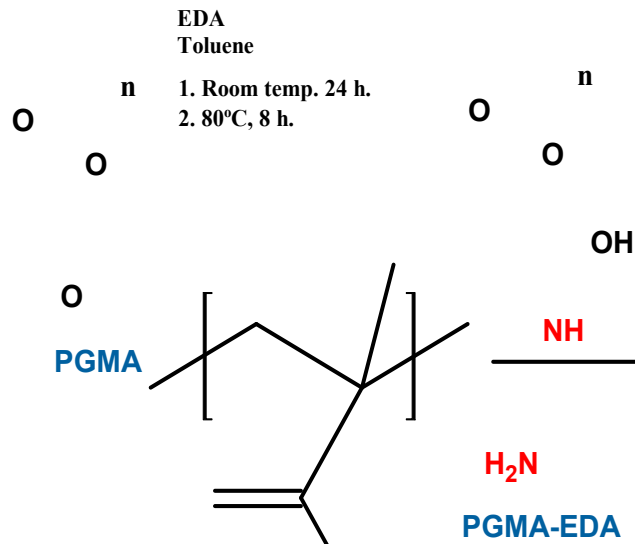


Figure 2. Modification of PGMA gels

### Adsorption Experiments

Adsorption experiments were carried out in three replicates with batch adsorption method. In these studies, the effects of variables such as temperature (15 - 50 °C), pH (2 - 10), contact time (60 - 360 min), initial dye concentration (20 - 500 ppm), and PGMA-EDA amount (5 - 50 mg) on PGMA-EDA gels' adsorption capacity were investigated. The solution volume was 10 mL, and the shaking speed was kept constant at 300 rpm in all adsorption experiments. The absorbance of BPB solutions was measured at 664 nm wavelength with Hach Lange DR9000 UV-VIS spectrophotometer. The adsorption capacity and efficiency (%) of removal calculations of PGMA-EDA gels were carried out using Equation (1), and Equation (2), respectively.

$$q_e = \frac{(C_o - C_e)}{w} V \quad (1)$$

$$\text{Dye removal (\%)} = \frac{(C_o - C_e)}{C_o} * 100 \% \quad (2)$$

The solution volume is V (L), the initial, and final solution concentrations of BPB dye are C<sub>o</sub>, and C<sub>e</sub> (mg L<sup>-1</sup> or ppm), respectively and the adsorbed dry mass is W (g).

### Adsorption Kinetics

The adsorption kinetics were examined to determine the equilibrium time of the adsorbent and the mechanism of BPB removal from wastewater. The resulting data were analyzed using pseudo-first-order (PFO; Lagergren, 1898), pseudo-second-order (PSO; Ho & McKay, 1999), Weber-Morris

intraparticle diffusion (ID; Weber & Morris, 1963), and Elovich (Benjelloun et al., 2021) kinetic models. The equations for the kinetic models are given below. (Eqs 3-6):

$$\text{Pseudo-first order: } \ln(q_e - q_t) = \ln q_e - k_1 \cdot t \quad (3)$$

$$\text{Pseudo-second order: } t/q_t = 1/k_2 \cdot q_e^2 + t/q_e \quad (4)$$

$$\text{Intra particle diffusion: } q_t = k_{id} \cdot t^{1/2} + I \quad (5)$$

$$\text{Elovich: } q_t = \beta \ln(\alpha\beta) - \ln(t) \quad (6)$$

Where the dye amounts adsorbed, and at time t are  $q_e$ , and  $q_t$ , respectively. The equilibrium rate constants of PFO (1/min), PSO (g/mg.min), and ID model (mg/g.min<sup>1/2</sup>) are  $k_1$ ,  $k_2$ , and  $k_{id}$ , respectively.

**Adsorption Isotherms**

Experimental data were analyzed using Langmuir (Langmuir, 1916), Freundlich (Freundlich, 1906), Dubinin-Radushkevich (Radushkevich, 1947), and Temkin (Temkin & Pyzhev 1940) isotherm models to assess the adsorption equilibrium. A detailed explanation of each isotherm model is provided below (Eq 7-13):

**Langmuir**

$$C_e/q_e = \frac{1}{q_{max} b} + \frac{1}{q_{max}} C_e \quad (7)$$

$$R_L = \frac{1}{1 + bC_0} \quad (8)$$

**Freundlich**

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (9)$$

**Temkin**

$$q_e = K_t \cdot \ln(at) + K_t \cdot \ln C_e \quad (10)$$

**D-R**

$$\ln q_e = \ln q_m - \beta \varepsilon^2 \quad (11)$$

$$\varepsilon = RT \ln[1 + (1/C_e)] \quad (12)$$

$$E = \frac{1}{\sqrt{2\beta}} \quad (13)$$

$K_f$ ,  $R_L$ , and  $K_s$  are the constants for the Langmuir, Freundlich and Temkin models, respectively.  $C_e$  is the dye concentration after adsorption,  $q_e$ , and  $q_{max}$  are capacity of adsorption at time t, and maximum adsorption, respectively. n means strength of the adsorption process, and if it is between 1, and 10, it means that the adsorption process is favorable. R is the gas constant (8.314 J K<sup>-1</sup>mol<sup>-1</sup>), T is the absolute temperature in Kelvin,  $\beta$  indicates the mean free energy of sorption per molecule of sorbate.

**Results and Discussion**

**Characterization of Polymer Particles**

FT-IR analyses were performed to evaluate that the synthesis and modifications of PGMA gels were successful. FT-IR spectra of PGMA, and PGMA-EDA are given in Figure 3. When Figure 3A was examined for PGMA; it was determined that the peaks observed at 3000 and 2934 cm<sup>-1</sup> were the stretching belonging to aliphatic C-H bonds in the polymer structure, the peak at 1721 cm<sup>-1</sup> was the stretching belonging to the C=O group of the ester structure, the peak at 1250 cm<sup>-1</sup> was the stretching belonging to both the C-O-C groups in the ester structure, and the C-O-C groups in the epoxy ring, the peak at 1133 cm<sup>-1</sup> was the bending of the C-O-C group of the ester, and the peaks at 905, 842, and 754 cm<sup>-1</sup> were typical stretching, and bending peaks belonging to the C-O-C groups in the epoxy ring.

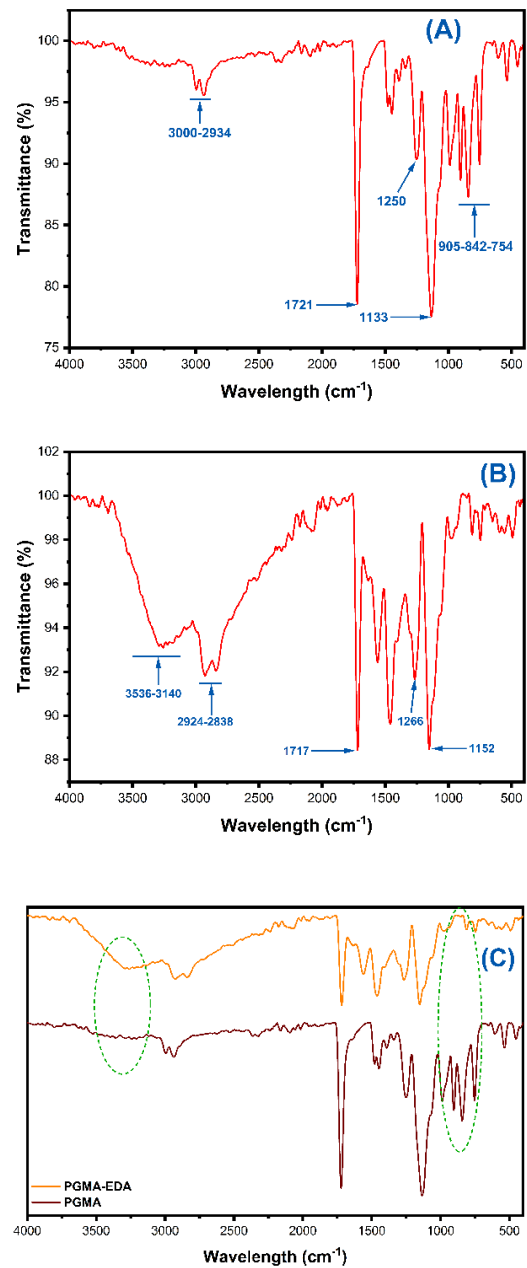


Figure 3. FT-IR spectra. PGMA (A), PGMA-EDA (B), and merged graphs (C).

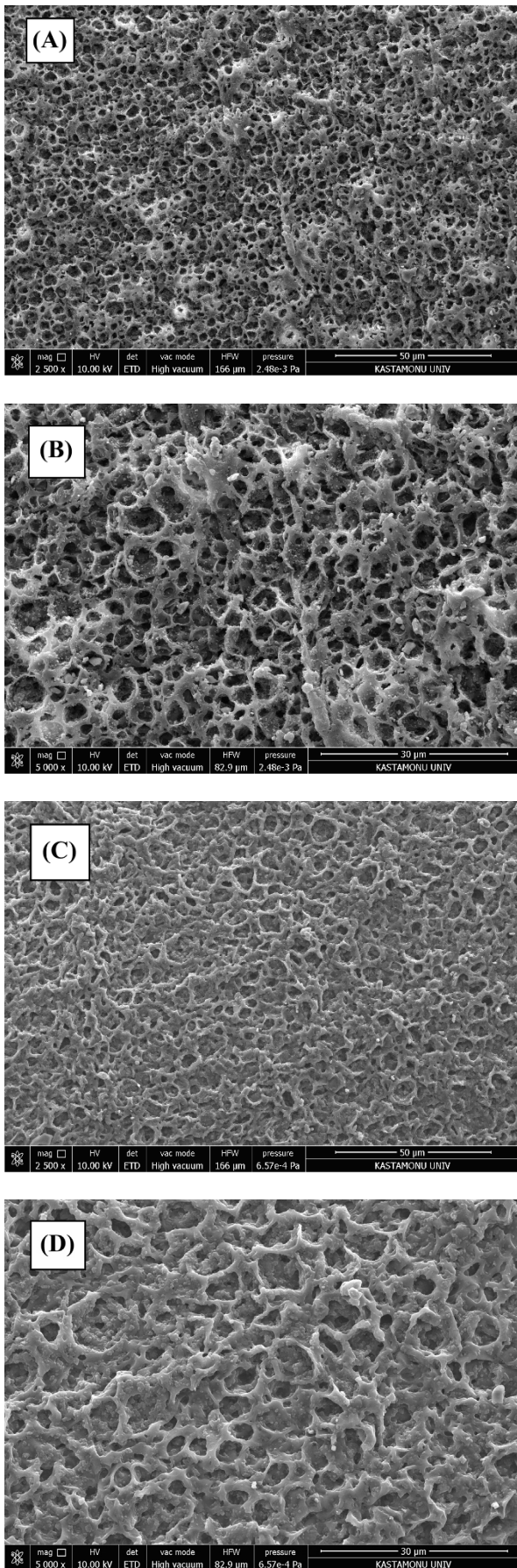


Figure 4. SEM images of PGMA (A) from 50 µm, (B) from 30 µm, and SEM images of PGMA-EDA (C) from 50 µm, (D) from 30 µm.

In PGMA-EDA gels, unlike PGMA, new peaks belonging to N-H, and  $\text{NH}_2$  groups formed as a result of the reaction, and new peaks belonging to -OH group formed by the opening of the epoxy ring should be seen in the FT-IR spectrum. At the same time, the peaks belonging to this ring should disappear due to the opening of the epoxy ring. When the graph belonging to PGMA-EDA is examined in Figure 3, it was seen that the typical stretching vibrations belonging to N-H, and  $\text{NH}_2$  groups were in the range of  $3536\text{-}3140\text{ cm}^{-1}$ , and the peaks belonging to -OH group are widely located from  $3700$  to  $2500\text{ cm}^{-1}$ . At the same time, it was understood that the peaks at  $905$ ,  $842$ , and  $754\text{ cm}^{-1}$  belonging to the epoxy ring of PGMA were not seen in the structure. These results were revealed that the PGMA gels were successfully modified.

The surface morphologies of PGMA gel, and PGMA-EDA were determined by SEM. The images obtained are presented in Figure 4. As it is known, polymeric gels have a weblike structure depending on the type, and size of the crosslinker used. Weblike structures in SEM images showed that polymeric gels were successfully obtained from GMA monomer (Figure 4A). However, this weblike structure was somewhat closed or filled after the modification of PGMA gels with EDA (Figure 4B). The main reason for this was the addition of a bulky molecule (EDA) to the structure of the polymer. This addition naturally led to a closure of the pores. However, the weblike structure still remained as it is.

The thermal stabilities of the synthesized PGMA gels, and their modified forms (PGMA-EDA) within the scope of the study were revealed by TGA analyses. The obtained graphics are presented in Figure 5. Accordingly, the first mass loss for PGMA was observed at  $133\text{ }^\circ\text{C}$ . The mass loss at this temperature was 2.32%. The next mass loss started at  $265\text{ }^\circ\text{C}$ , and after this temperature, PGMA rapidly underwent thermal degradation. The mass loss was 3.03% up to  $265\text{ }^\circ\text{C}$ . This mass loss showed that the PGMA gel had a quite stable structure up to  $265\text{ }^\circ\text{C}$ . On the other hand, the situation was slightly different in PGMA-EDA gels. The first mass losses started to be seen immediately after the polymer was heated and ended at  $148\text{ }^\circ\text{C}$ . It was calculated that PGMA-EDA lost 14.03% mass during this time. From  $148\text{ }^\circ\text{C}$  to  $217\text{ }^\circ\text{C}$ , the mass loss (4.17%) was not too much. However, immediately after this temperature, PGMA-EDA rapidly lost mass and degraded. The main reason for these rapid mass losses observed in PGMA-EDA was thought to be due to the difference in hydrophilicity between PGMA and PGMA-EDA because PGMA is hydrophobic and does not retain water. However, after being modified with EDA, it has N-H, and O-H groups that can make strong hydrogen bonds. It was thought that these mass losses may be due to the removal of water in the structures of PGMA-EDA after washing. However, from the second mass loss, it was seen that the modification by amine groups seriously reduces the thermal stability of PGMA gels.

### Adsorption Studies

#### Determination of contact time

Adsorption is a process in which the interactions between the adsorbent and adsorbate are balanced. Properly finding the contact time between the adsorbate and adsorbent is crucial for maximizing the efficiency of



the adsorbent. Insufficient contact time can hinder the effectiveness of the adsorption process. Otherwise, time and energy will be lost. For this reason, the equilibrium times for BPB were first determined in this study. The experiments were carried out for BPB between 5-360 minutes (100 mg L<sup>-1</sup> BPB concentration, 25 °C, and 30 mg PGMA-EDA gels, pH 4.3, and 300 rpm shaking speed) (Figure 6). According to the Figure 6, BPB was rapidly adsorbed onto PGMA-EDA gels (38.11 mg g<sup>-1</sup>) until the 240th minute. After this, the adsorption increase stopped and did not change. For this reason, the adsorption equilibrium time for BPB was assumed to be 240 min.

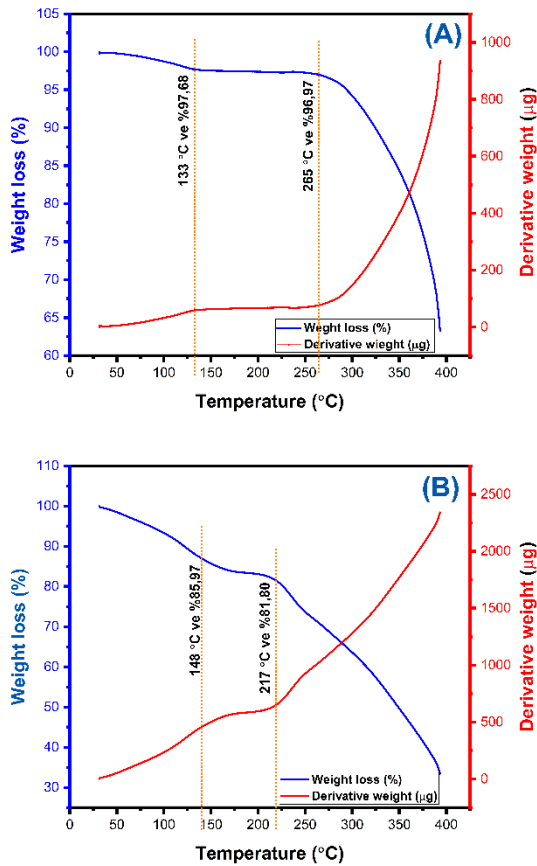


Figure 5. Thermogravimetric analyses of PGMA (A), and PGMA-EDA (B).

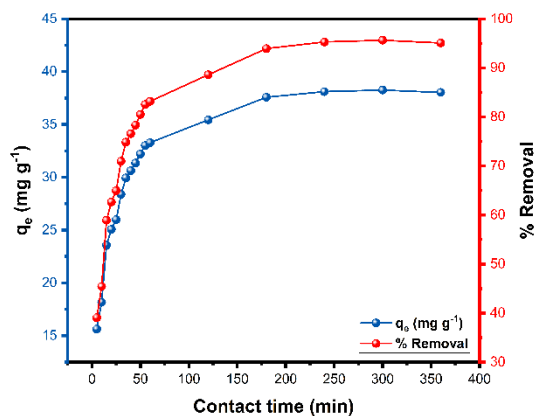


Figure 6. Adsorption equilibrium time graph obtained for PGMA-EDA gels.

### Adsorption Kinetics

The adsorption rate is a critical factor to reveal the mechanism of the adsorption when selecting an effective adsorbent (Repo et al., 2011). Therefore, the mechanism between PGMA-EDA gels, and BPB were investigated via various kinetic models (PFO, PSO, ID, and the Elovich). The calculated results for the kinetic models were summarized in Table 1 and graphs were presented in Figure 7, respectively. The data obtained from contact time experiments were used to calculate the kinetic models.

The PFO model suggests that the occupancy adsorption rate sites are proportional to the occupied areas, while the PSO model argues that this ratio is related to the square of the occupied areas (Chien, 1963, Altaher et al., 2014). When PFO, and PSO correlation coefficients for BPB (0.734, and 0.999, respectively) were compared, it was seen that the PSO model had a higher R<sup>2</sup> value (0.999) (Figure 7A and B, respectively). When the q<sub>e</sub> values for both models (17.71, and 39.53 mg g<sup>-1</sup>, respectively) were compared with the experimental q<sub>exp</sub> values (38.12 mg g<sup>-1</sup>), it was seen that the values obtained with PSO were quite close to each other. These two situations showed that the BPB adsorption was more suitable for the PSO kinetic model. It appears that the rate-determining step could involve chemisorption, characterized by valence forces through electron sharing or exchange between PGMA-EDA gels and BPB molecules (Oter et al., 2024). Similar results were obtained in the literature (El-Zahhar et al., 2014; Gokkus et al., 2023; Gokkus et al., 2024; Oter et al., 2024)

The adsorption process typically occurs in three distinct stages over time. Firstly, dye molecules are transported from the liquid phase to the adsorbent surface through external diffusion. Secondly, the dye molecules diffuse internally into the pores of the adsorbent. Thirdly, chemical and/or physical bonds are formed between the active sites and the adsorbate within the adsorbent pores (Ahmed & Abou-Gamra, 2016). The non-linearity observed in the kinetic studies' graphs indicates that the adsorption process involves sequential steps and displays multiple linear phases. When the adsorption between PGMA-EDA gels, and BPB was evaluated using the ID model, the adsorption occurred in two stages (Figure 7). In the first stage, the adsorption capacity rapidly reached 33.27 mg g<sup>-1</sup>, and this rate decreased in the second stage. At the beginning of the process (first stage), since the surface-active sites of PGMA-EDA gels were empty, BPB molecules quickly adsorbed on these sites. Therefore, adsorption occurred rapidly in the first stage. In the second stage, it was observed that BPB molecules diffused into the macropores of PGMA-EDA gels, which decreased the adsorption rate.

### Determination of adsorbent amount

Determination of the optimum adsorbent amount is extremely critical in order to prevent an increase in cost without a decrease in adsorption efficiency. If the amount of adsorbent is low, sufficient efficiency cannot be obtained from the adsorbent due to insufficient active site. Otherwise, adsorption will not be economical due to the use of excess adsorbent. For this reason, experiments were conducted to find the optimal amount of adsorbent (5, 15, 25, 35, and 50 mg). During the experiments, 25 °C, 100 mg L<sup>-1</sup> BPB concentration, pH 4.3, 10 mL volume, 300 rpm shaking speed, and 240 minutes were kept constant. The

maximum capacity of adsorption was determined as 5 mg adsorbent amount ( $68.74 \text{ mg g}^{-1}$ ), and the minimum capacity of adsorption was found as 50 mg adsorbent amount ( $19.33 \text{ mg g}^{-1}$ ) (Figure 8). When the results were evaluated, it was seen that the  $q_e$  decreased depending on the increase in the adsorbent amount. The adsorption capacity increased rapidly up to 25 mg PGMA-EDA amount ( $38.12 \text{ mg g}^{-1}$  and  $95.03\%$ ) and then the increase rate decreased obviously. Therefore, it was decided that the optimum amount of PGMA-EDA was 25 mg. The subsequent experiments were continued with 25 mg of PGMA-EDA. The most probable reason for this situation was thought to be the agglomeration of PGMA-EDA due

to the low solution volume (10 mL). The aggregation of the gels prevented the distribution of BPB molecules on the adsorbent surface, and this effect became more pronounced when 50 mg of adsorbent was used. Alver & Metin (2012) reported in their studies that the adsorbent amount positively affected the adsorption process up to a certain extent, and then the efficiency decreased because of the agglomeration of the adsorbent. Bulut & Aydın (2006) reported similar results, and implied that this was due to some regions not reaching full saturation during adsorption. These results were also obtained in the other studies (Jirekar et al., 2014; Lapwanit et al., 2018).

Table 1. BPB adsorption kinetic parameters on PGMA-EDA gels.

Kinetic models	Parameters	BPB
PFO	$q_e \text{ (mg.g}^{-1}\text{)}$	38.12
	$k_1 \text{ (min}^{-1}\text{)}$	0.003
	$R^2$	0.734
	$q_{exp} \text{ (mg.g}^{-1}\text{)}$	17.71
PSO	$q_e \text{ (mg.g}^{-1}\text{)}$	38.12
	$k_2 \text{ (g.mg}^{-1}\text{.min}^{-1}\text{)}$	$2.28 \times 10^{-3}$
	$R^2$	0.999
	$q_{exp} \text{ (mg.g}^{-1}\text{)}$	39.53
ID	$k_i \text{ (mg.g}^{-1}\text{min}^{-1/2}\text{)}$	0.867
	$I \text{ (mg.g}^{-1}\text{)}$	20.66
	$R^2$	0.737
Elovich	$\alpha \text{ (mg.g}^{-1}\text{min}^{-1}\text{)}$	0.183
	$\beta \text{ (g.mg}^{-1}\text{)}$	27.91
	$R^2$	0.93

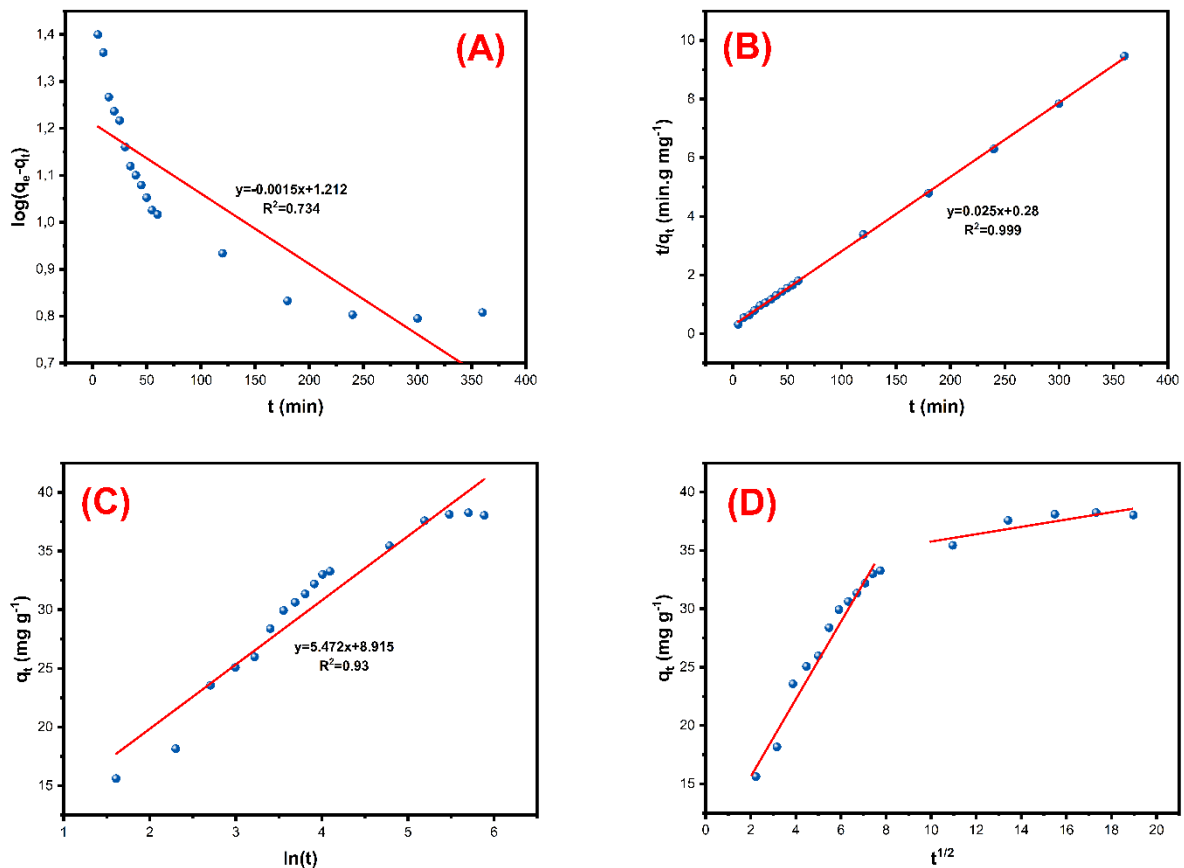


Figure 7. PFO (A), PSO (B), Elovich (C), and ID (D) kinetic models for the adsorption of BPB onto PGMA-EDA.

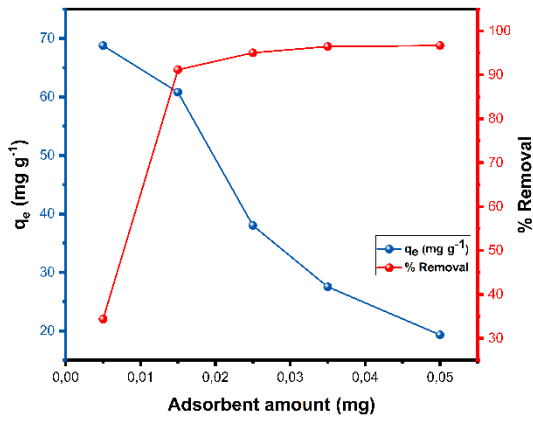


Figure 8. Effect of PGMA-EDA gel amounts on the adsorption of BPB dye.

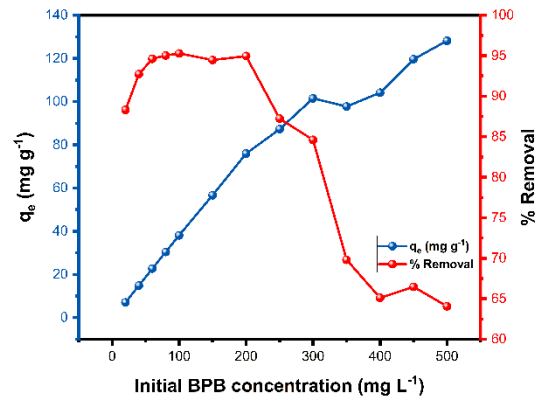


Figure 9. Effect of initial BPB dye concentration on adsorption.

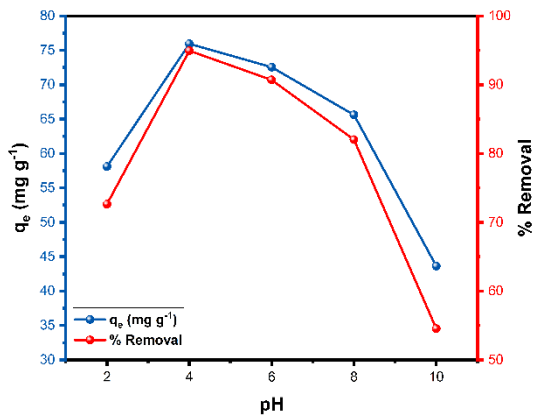


Figure 10. Effect of pH.

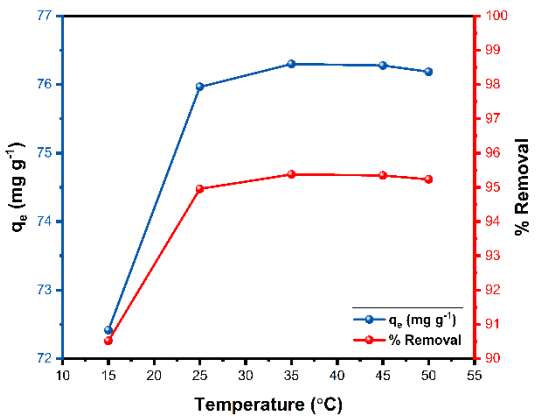


Figure 11. Effect of temperature on the adsorption of BPB.

*Determination of initial dye concentration*

To evaluate potential or capacity of an adsorbent, determination of the optimum dye concentration is an important parameter. Therefore, the optimum BPB concentration was determined for PGMA-EDA gels with 20 - 500 mg L<sup>-1</sup> of concentrations (Figure 9). During the experiments, 25 °C, 25 mg PGMA-EDA gels, pH 4.3, 10 mL solution volume, 300 rpm shaking speed were kept constant. The findings are presented in Figure 9. The adsorption capacities of PGMA-EDA gels showed a continuous increase from 20 mg L<sup>-1</sup> to 500 mg L<sup>-1</sup> BPB concentration. Meanwhile, the adsorption capacity raised from 7.06 mg g<sup>-1</sup> to 128.15 mg g<sup>-1</sup>. The highest  $q_e$  value was obtained with 128.15 mg g<sup>-1</sup> for 500 mg L<sup>-1</sup> BPB concentration. However, at this concentration, % removal efficiency decreased below 65%. At 200 mg L<sup>-1</sup> BPB concentration,  $q_e$  value was 75.96 mg g<sup>-1</sup> corresponding to removal efficiency of 94.96%, and at 250 mg L<sup>-1</sup> concentration,  $q_e$  was calculated 87.24 mg g<sup>-1</sup> corresponding to removal efficiency of 87.24%. Since the efficiency decreased below 90% at 250 mg L<sup>-1</sup> concentration, it was decided that the optimum BPB concentration for PGMA-EDA gels was 200 mg L<sup>-1</sup>. Subsequent experiments were continued with this concentration. The increasing of the adsorption capacity ( $q_e$ ) with the increasing BPB concentration could be revealed by the repulsion of the BPB molecules. With the effect of these repulsion forces, BPB quickly adhered to the surface of PGMA-EDA gels. This repulsion also increased

the adsorption rate and diffusion of BPB into the pores of PGMA-EDA gels. To increase the concentration also caused a raised in the amount of dye adsorbed. This observation was consistent with literature (Gürses et al., 2006; Yagub et al., 2014). If the dye concentration was increased, the available adsorption sites' number decreased and therefore the effect of BPB removal varied depending on the initial BPB concentration and the dyes were physically adsorbed only to the adsorbent's outer surface with a single layer (Mahmoodi et al., 2011).

*Effect of pH*

pH significantly affects the adsorption process by changing the protonation degree and surface charge of functional groups on adsorbents and dyes. Therefore, determining the optimum pH for adsorption is of critical importance. Therefore, in this study, adsorption of BPB dye by PGMA-EDA gels was carried out with 2, 4, 6, 8, and 10 pHs at 25 °C, 25 mg PGMA-EDA gels, 200 mg L<sup>-1</sup> BPB concentration, 10 mL sample volume, 300 rpm shaking speed. The minimum adsorption of BPB was determined at pH 10 (43.64 mg g<sup>-1</sup>), and the highest adsorption was obtained at pH 4 (75.96 mg g<sup>-1</sup>) (Figure 10). PGMA-EDA contains abundant amine groups in its structure. Since these groups are protonated in acidic environments, the surface charges of PGMA-EDA gels increase. In contrast, BPB dye is anionic. Therefore, PGMA-EDA being more cationic at pH 4 led to an increase in the electrostatic attraction force between it and BPB. Thus, the adsorption capacity of PGMA-EDA was the

highest at pH 4. The number of protons increased considerably at pH 2. It is thought that this caused the protons to adsorb on the surface of BPB. Therefore, the adsorption efficiency of PGMA-EDA decreased at pH 2. Similar results were reported in other studies (Liu et al., 2014; Dhananasekaran et al., 2016; Gokkus et al., 2024).

*Adsorption thermodynamics and effect of temperature*

The experiments were conducted at varying temperatures (15–50 °C) keeping the conditions of 25 mg PGMA-EDA gel, pH 4, 10 mL solution volume, 300 rpm shaking speed, 200 mg L<sup>-1</sup> BPB concentration, and 240 minutes constant. Therefore, the effect of temperature on the adsorption process was evaluated. The lowest adsorption capacity was observed as 72.41 mg g<sup>-1</sup> at 15 °C, while the highest was recorded as 76.3 mg g<sup>-1</sup> at 30 °C. The findings indicated a slight increase in the capacity of adsorption when the temperature rose from 25 °C to 50 °C (Figure 11). The increasing of adsorption efficiency with the increasing of temperature was revealed the process was endothermic. This meant that higher temperatures facilitate greater adsorption of BPB molecules onto the PGMA-EDA gel (Figure 11). Similar trends were obtained by Al-Ghouti et al. (2005) and Gürses et al. (2006).

Thermodynamic evaluation of adsorption processes is of great importance in terms of determining whether these processes are spontaneous or not (Sun & Wang, 2010). Therefore, Gibbs free energy ( $\Delta G^\circ$ ), enthalpy ( $\Delta H^\circ$ ), and entropy ( $\Delta S^\circ$ ) changes were calculated from the temperature results. According to the data in Table 2,  $\Delta G^\circ$  was calculated as 4.19 kJ mol<sup>-1</sup>. A positive  $\Delta G^\circ$  value indicates that the adsorption of BPB occurred spontaneously.  $\Delta H^\circ$  represents the enthalpy change during the reaction; a negative value signifies an exothermic reaction, while a positive value indicates an endothermic reaction. As the calculated  $\Delta H^\circ$  value was determined to be 14.39 kJ mol<sup>-1</sup>, it was concluded that the adsorption between PGMA-EDA gels, and BPB was endothermic. A negative  $\Delta S^\circ$  value indicates that the randomness decreased during adsorption and the reaction became less disordered. According to the experimental results, the  $\Delta S^\circ$  value for PGMA-EDA gels was found to be -64.46 J mol<sup>-1</sup>. This highlighted the decreasing irregularity of the adsorption process between PGMA-EDA gels and BPB. The optimum adsorption conditions of PGMA-EDA on the adsorption of BPB dye were summarized in the Table 3.

Table 2. Thermodynamic parameters of BPB adsorption on PGMA-EDA gels.

Temperature (°C)	$\Delta H^\circ$ (kJ mol <sup>-1</sup> )	$\Delta S^\circ$ (J mol <sup>-1</sup> K <sup>-1</sup> )	T $\Delta S^\circ$ (kJ mol <sup>-1</sup> )	$\Delta G^\circ$ (kJ mol <sup>-1</sup> )
15	14.39	-64.46	-18.58	4.19
25				
35				
45				
50				

Table 3. Optimum conditions obtained for the adsorption of BPB dye for PGMA-EDA gels.

Parameters	BPB
pH	4
Temperature (°C)	25
Initial BPB conc. (mg L <sup>-1</sup> )	200
PGMA-EDA amount (mg)	25
Equilibrium time (min)	240

Table 4. Isotherm parameters of BPB adsorption on PGMA-EDA gels

Isotherms	Parameters	BPB
Langmuir	q <sub>max</sub> (mg.g <sup>-1</sup> )	128.15
	q <sub>e,exp</sub> (mg.g <sup>-1</sup> )	125.0
	K <sub>L</sub> (L.mg <sup>-1</sup> )	0.057
	R <sub>L</sub>	0.23
	R <sup>2</sup>	0.97
Freundlich	K <sub>f</sub> [(mg.g <sup>-1</sup> )(L.mg <sup>-1</sup> ) <sup>1/n</sup> ]	12.88
	n	2.11
	R <sup>2</sup>	0.77
Temkin	β <sub>T</sub>	24.19
	KT (L.g <sup>-1</sup> )	1.04
	R <sup>2</sup>	0.94
Dubinin-Radushkevich	q <sub>max</sub> (mg.g <sup>-1</sup> )	128.15
	q <sub>e,exp</sub> (mg.g <sup>-1</sup> )	98.4
	β (mol <sup>-2</sup> .J <sup>-2</sup> )	3.49
	E (kJ.mol <sup>-1</sup> )	0.38
	R <sup>2</sup>	0.97

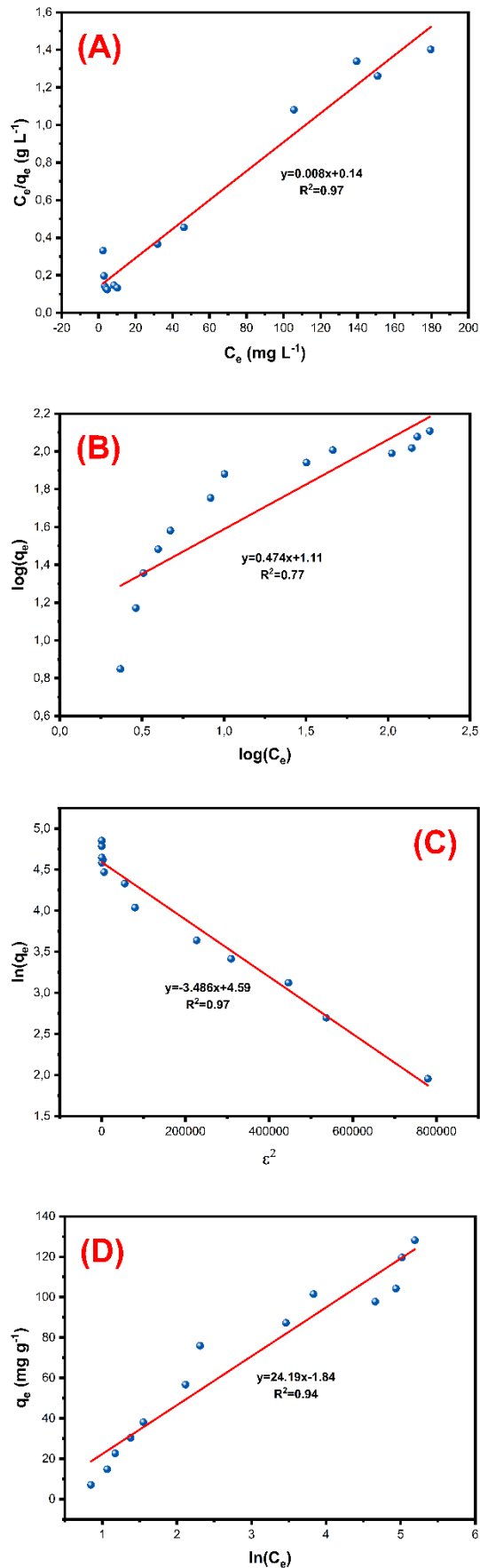


Figure 12. Langmuir (A), Freundlich (B) Dubinin-Radushkevich (C), and Temkin (D) isotherms of BPB on the synthesized PGMA-EDA gels.

### Adsorption isotherms

The adsorption between BPB and PGMA-EDA gels was analyzed using experimental data based on the Freundlich, Langmuir, Dubinin-Radushkevich (DR), and Temkin isotherm models. The isotherm parameters for BPB adsorption on PGMA-EDA gels are summarized in Table 4, while the corresponding isotherm graphs are presented in Figure 12. Among the models, Langmuir isotherm value (the highest; 0.97), indicating that the adsorption occurs on a monolayer and homogeneous surface. This conclusion was further supported by the calculated ( $q_m$ ; 128.15  $\text{mg g}^{-1}$ ), and experimental ( $q_e$ ; 125.0  $\text{mg g}^{-1}$ ) values. Additionally, the correlation values for the Temkin, and D-R isotherms were calculated as 0.94, and 0.97, respectively, as shown in Table 4, highlighting the compatibility of these models with the adsorption process on PGMA-EDA gels. In a similar study, the removal capacity of poly(quaternary ammonium salt) in the removal of Acid Red 18 (AR18), and Acid Blue 25 (AB25) dyes was investigated under both single, and binary systems. It was reported that Langmuir isotherm demonstrated the best fit to the equilibrium data, indicating maximum monolayer adsorption (Mahmoodi et al., 2011).

### Conclusion

This study focused on the efficient removal of BPB dye, which is used intensively in the industry. For this purpose, PGMA gels were modified to produce PGMA-EDA gels and used in the adsorption of BPB dye. Adsorption was performed according to the ‘batch adsorption’ method in three replicates. In the study, the optimum pH, temperature, contact time, shaking speed, dye concentration, and adsorbent amount for PGMA-EDA gels were determined. pH 4, 25 °C, 240 mins, 200  $\text{mg L}^{-1}$  BPB concentration, and 25 mg PGMA-EDA amount were determined the optimum adsorption conditions for PGMA-EDA gels.

In order to elucidate the adsorption mechanism, Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherms were performed with the data obtained from the initial dye concentration experiments. On the other hand, calculations of PFO, and PSO kinetic models were performed with the contact time data. As a result of these calculations, the highest  $R^2$  value among the isotherm models for BPB was obtained for the Langmuir isotherm. This result showed that the adsorption occurred in a monolayer and homogeneous structure and when the correlation coefficients calculated with PFO, and PSO models for BPB were compared, it was seen that the PSO model had a higher  $R^2$  value. This showed that the BPB adsorption was more suitable for the PFO kinetic model.

### Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Synthesis of Quantum Dots Using Biomaterials Derived from Blue Crab and Their Potential Applications

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### ABSTRACT

The blue crab (*Callinectes sapidus*, Rathbun 1896) has become a significant source of raw materials in biotechnology and nanotechnology due to the biomaterials present in its shell. Natural polymers such as chitin and chitosan, derived from the crab's shell, are particularly noteworthy for their environmentally friendly and biologically compatible properties. These biopolymers provide an innovative alternative in the synthesis of quantum dots (QDs). Quantum dots are favored in various applications, including biomedical imaging, environmental sensors, and energy storage, due to their superior optoelectronic properties. Chitosan obtained from blue crab shells acts as both a stabilizer and a coating agent in the green synthesis of quantum dots. This process minimizes the use of toxic chemicals, thus promoting environmental sustainability. Moreover, the antimicrobial and biodegradable properties of chitosan enhance its usability in biomedical applications. For instance, biocompatible carbon-based quantum dots have shown promising results in cancer diagnostics and drug delivery systems. The synthesis of quantum dots using biomaterials is more cost-effective and environmentally friendly compared to traditional methods. Furthermore, utilizing blue crab shells as a waste material contributes to both marine ecosystem preservation and the circular economy. These synthesis methods are reported to create a significant paradigm shift in the field of sustainable technology development. In conclusion, the synthesis of quantum dots using biomaterials derived from blue crabs has the potential to reduce environmental impacts while serving advanced technological applications. This approach significantly contributes to the development of biotechnological innovations and sustainable development goals.

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## Introduction

Marine organisms such as blue crabs are emerging as a promising source for the green synthesis of QDs. Chitosan and other derivatives from the exoskeletons of crustaceans provide a renewable biomass that reduces dependency on hazardous precursors (Ayodele et al., 2018). Recent studies have further highlighted the potential of marine-derived biomaterials for QD synthesis. For example, Pathak et al. (2023) demonstrated the enhanced fluorescence properties of QDs synthesized using crustacean biomaterials, emphasizing their potential in advanced bioimaging applications. Additionally, Kang et al. (2021) explored the role of marine-sourced biopolymers in producing eco-friendly QDs, which showed promising results in applications such as photocatalysis and biosensing.

Carbon-based QDs synthesized from biomaterials are known for their low toxicity and high biocompatibility, making them suitable for applications in biomedical imaging, drug delivery, and biosensing (Anpalagan et al., 2023). For instance, QDs derived from plant-based sources have shown potential in imaging cancer cells, demonstrating their efficacy in bioimaging while ensuring safety (Anpalagan et al., 2023).

A recent study by Torres et al. (2023) explored carbon-based QDs synthesized from marine-derived polysaccharides, reporting superior stability and bioavailability in drug delivery systems. Similarly, chitosan-derived QDs can serve as versatile platforms for monitoring environmental pollutants, offering applications beyond healthcare (Kang et al., 2021).

Blue crab-derived biomaterials enable the production of QDs with unique optical properties, making them applicable for real-time tracking in bioimaging and for use in energy storage devices and environmental monitoring (Iravani & Varma, 2020; Ayodele et al., 2018). The optical tunability of QDs synthesized from marine sources further enhances their value in research and industrial applications. Furthermore, a study by Jing et al. (2023) highlighted the potential of QDs synthesized from crab shells in improving the performance of supercapacitors, showcasing their relevance in energy-related applications. This demonstrates how marine biomaterials can bridge the gap between sustainable practices and high-performance technologies.



In conclusion, this study aims to explore the innovative and sustainable synthesis of quantum dots (QDs) using biomaterials derived from blue crab shells, particularly focusing on chitin and chitosan. By leveraging the natural, renewable, and biodegradable properties of these biopolymers, the research seeks to address the challenges posed by traditional QD synthesis methods, such as environmental toxicity and limited biocompatibility. The study emphasizes the development of eco-friendly and cost-effective green synthesis techniques, showcasing the potential of blue crab-derived QDs in diverse applications, including biomedical imaging, drug delivery, environmental monitoring, and energy storage. Ultimately, the research aims to contribute to sustainable development and circular economy principles while advancing biotechnological innovations.

## Material and Methods

### Materials

Blue crabs (*Callinectes sapidus*, Rathbun 1896) were obtained from Şahin Balıkçılık Buca, İzmir and used as the primary biomaterial for quantum dot (QD) synthesis. Their exoskeletons, rich in chitin and calcium carbonate, were processed to extract chitosan, a key precursor in the synthesis of carbon quantum dots (CQDs). Reagents used in the study included hydrochloric acid (HCl), sodium hydroxide (NaOH), acetic acid, citric acid, and ethylenediamine, all of analytical grade, purchased from Sigma-Aldrich. Deionized water was used for all preparation and purification steps. The methodology for chitosan extraction and QD synthesis was adapted from Iravani and Varma (2020) and Ayodele et al. (2018).

### Extraction of Chitosan

The extraction process involved three critical steps: demineralization, deproteinization, and deacetylation, based on previously established protocols (Ayodele et al., 2018; Croisier & Jerome, 2013).

**Demineralization:** The cleaned and dried crab shells were immersed in 1 M hydrochloric acid (HCl) at room temperature for 24 hours to dissolve calcium carbonate, forming a chitin-rich substrate. The sample was then filtered and washed with deionized water until neutral pH was achieved.

**Deproteinization:** The acid-treated shells were subjected to boiling in a 1 M NaOH solution for 2 hours at 80°C to remove residual proteins and lipids. The resulting material was thoroughly rinsed with deionized water.

**Deacetylation:** To obtain chitosan, the chitin-rich substrate was treated with 50% NaOH at 100°C for 3 hours, converting acetyl groups into amine groups. The product was washed repeatedly with deionized water and dried at 60°C, yielding pure chitosan powder.

### Synthesis of Quantum Dots

Blue crabs (*Callinectes sapidus*) served as the primary source of biomaterials due to their high chitin content in the exoskeletons. Chemicals used for chitosan extraction included hydrochloric acid (HCl), sodium hydroxide (NaOH), and acetic acid. For QD synthesis, citric acid and ethylenediamine were chosen as precursors, while other optional dopants, such as urea and polyethylene glycol (PEG), were used to modify the surface properties of the

QDs. All chemicals were analytical grade and supplied by Sigma-Aldrich. The methods were adapted from green synthesis protocols outlined in Iravani and Varma (2020). The carbon quantum dots (CQDs) were synthesized using a hydrothermal method, a widely accepted green synthesis route for CQDs (Iravani & Varma, 2020; Anpalagan et al., 2023).

**Preparation of Precursor Solution:** Extracted chitosan (0.5 g) was dissolved in 50 mL of 1% acetic acid solution. Citric acid (1.5 g) and ethylenediamine (2 mL) were added to the solution under constant stirring, ensuring homogeneity.

**Hydrothermal Reaction:** The precursor solution was transferred to a 100 mL Teflon-lined stainless-steel autoclave, sealed, and heated to 180°C for 6 hours. The reaction facilitated carbonization and surface functionalization of chitosan, producing CQDs.

**Post-Reaction Processing:** The product was cooled to room temperature, resulting in a brownish colloidal suspension. Centrifugation at 10,000 rpm for 15 minutes was performed to remove larger particles. The supernatant was filtered through a 0.22 µm membrane and stored at 4°C.

The QDs were synthesized via a hydrothermal method, which offers an eco-friendly and efficient route for QD preparation:

**Preparation of Precursor Solution:** Chitosan powder (0.5 g) was dissolved in 1% acetic acid. Citric acid (2 g) and ethylenediamine (2 mL) were added, forming a homogeneous solution. Additional nitrogen (N) doping was achieved by incorporating urea into the mixture.

**Hydrothermal Treatment:** The solution was transferred into a Teflon-lined stainless-steel autoclave and heated at 200°C for 6 hours. The high temperature and pressure facilitated carbonization and surface passivation.

**Purification:** The product was filtered through a 0.22 µm membrane and subjected to dialysis (1 kDa cutoff) for 48 hours to remove impurities and small molecules.

**Drying:** The purified QDs were lyophilized to obtain a powder form for long-term storage.

**Modification of QDs:** To enhance their functional properties, synthesized QDs were surface-modified with polyethylene glycol (PEG). PEGylation improved their dispersion in aqueous media and enhanced biocompatibility (Ayodele et al., 2018).

**PEGylation:** QDs (10 mg) were dispersed in 10 mL of water, followed by the addition of PEG-NH<sub>2</sub> (1 g). The mixture was stirred at room temperature for 4 hours to achieve uniform coating.

**Characterization of Modified QDs:** Modified QDs were characterized using FTIR to confirm PEG attachment.

### Characterization Techniques

The synthesized CQDs were characterized to determine their size, morphology, optical properties, and surface functionalities.

**Transmission Electron Microscopy (TEM):** TEM was used to visualize the morphology and size distribution of the CQDs (Ayodele et al., 2018).

**UV-Vis Spectroscopy:** Absorption spectra were recorded using a UV-Vis spectrophotometer to confirm the characteristic optical properties of CQDs (Iravani & Varma, 2020).

**Photoluminescence (PL) Spectroscopy:** The fluorescence behavior of CQDs was examined by measuring their emission spectra under UV excitation.

**Fourier-Transform Infrared Spectroscopy (FTIR):** FTIR was performed to identify functional groups on the CQD surface, confirming the presence of amine and hydroxyl groups (Anpalagan et al., 2023).

**X-ray Diffraction (XRD):** XRD analysis provided insights into the crystalline structure of the CQDs.

### Applications Testing

**Bioimaging:** HeLa cells were cultured in a standard medium and incubated with varying concentrations of CQDs. The fluorescence imaging was performed using a confocal microscope to evaluate biocompatibility and imaging potential (Anpalagan et al., 2023).

**Environmental Monitoring:** The fluorescence quenching of CQDs in the presence of heavy metal ions (e.g.,  $Pb^{2+}$ ,  $Hg^{2+}$ ) was investigated to evaluate their potential as environmental sensors. Calibration curves were generated to assess sensitivity and detection limits (Ayodele et al., 2018).

**Drug Delivery:** QDs were conjugated with doxorubicin (DOX) via amide bond formation. The release profile of DOX was studied in pH 5.0 and 7.4 buffers, simulating tumor and normal physiological conditions, respectively.

**Photocatalysis:** QDs were tested for photocatalytic degradation of methylene blue under visible light to explore their utility in wastewater treatment.

### Statistical Analysis

The data were analyzed using the MEAN Procedure; the significance level in the tests was accepted as  $\alpha = 0.05$ . Data are given as mean  $\pm$  standard deviation. Data were analyzed using IBM SPSS Statistics version 25.0 for Windows package software (IBM Corp., Armonk, NY, USA).

## Results

The hydrothermal synthesis of carbon quantum dots (CQDs) using blue crab-derived chitosan as the precursor was successfully performed, yielding a stable, brownish colloidal solution. The synthesis process demonstrated an average yield of 85%, based on the weight of the chitosan precursor. The synthesized CQDs exhibited excellent solubility in water due to the inherent functional groups present on their surfaces, such as hydroxyl (-OH) and amino (-NH<sub>2</sub>) groups.

### Morphological and Structural Properties

**Transmission Electron Microscopy (TEM):** The TEM analysis revealed that the synthesized CQDs were spherical and had a uniform size distribution, with diameters ranging from 2 to 6 nm. High-resolution TEM images confirmed the presence of lattice fringes with an interplanar spacing of approximately 0.21 nm, indicative of graphitic carbon. This demonstrates successful carbonization of the chitosan precursor.

**Atomic Force Microscopy (AFM):** AFM measurements further validated the nanoscale dimensions of the CQDs, showing an average height of 3–5 nm and confirming their spherical morphology.

**X-ray Diffraction (XRD):** XRD analysis showed a broad peak centered at 24°, corresponding to the (002) planes of graphitic carbon. The amorphous nature of the CQDs, with minor crystalline regions, is characteristic of carbon-based nanoparticles synthesized from biological materials.

**UV-Vis Spectroscopy:** The UV-Vis spectrum of the CQDs showed a prominent absorption peak at 270 nm, attributed to  $\pi$ - $\pi^*$  transitions of aromatic C=C bonds, and a shoulder around 320 nm, corresponding to n- $\pi^*$  transitions of carbonyl groups. These peaks confirm the formation of conjugated carbon structures within the CQDs.

**Photoluminescence (PL) Spectroscopy:** The CQDs exhibited strong excitation-dependent photoluminescence, with the maximum emission at 450 nm under 360 nm excitation. The quantum yield (QY) was measured to be 18.5%, indicating efficient fluorescence properties. The tunable photoluminescence suggests that the CQDs have potential applications in bioimaging and optoelectronic devices.

**Fourier-Transform Infrared Spectroscopy (FTIR):** FTIR spectra revealed the presence of multiple functional groups essential for biocompatibility and solubility:

Broad peak at 3430  $cm^{-1}$ : O-H and N-H stretching.

Peak at 1650  $cm^{-1}$ : C=O stretching, associated with amide and carboxyl groups.

Peak at 1380  $cm^{-1}$ : C-N stretching vibrations. These findings confirm the successful incorporation of oxygen- and nitrogen-containing groups, originating from the chitosan precursor, onto the CQD surface.

**Zeta Potential:** The zeta potential of the CQDs was measured as -35 mV, indicating high colloidal stability due to electrostatic repulsion. Stability tests over 30 days showed no significant aggregation or fluorescence degradation, making the CQDs ideal for long-term storage and use.

HeLa cells treated with CQDs exhibited bright blue fluorescence, confirming effective cellular uptake and cytoplasmic localization of the QDs. The fluorescence intensity remained stable during prolonged imaging, with no observable photobleaching. The biocompatibility of the CQDs was confirmed through an MTT assay, which showed no significant cytotoxicity at concentrations up to 200  $\mu g/mL$ .

The fluorescence of the CQDs was selectively quenched in the presence of lead ( $Pb^{2+}$ ) and mercury ( $Hg^{2+}$ ) ions. Stern-Volmer plots demonstrated a linear relationship between fluorescence quenching and metal ion concentration. The detection limits for  $Pb^{2+}$  and  $Hg^{2+}$  were determined to be 0.12  $\mu M$  and 0.09  $\mu M$ , respectively, highlighting the sensitivity of CQDs as environmental sensors. These values are below the permissible limits set by the World Health Organization (WHO) for heavy metals in water, indicating practical applicability in real-world environmental monitoring. Doxorubicin (DOX) was successfully conjugated onto the CQD surface, and drug release studies were conducted at physiological (pH 7.4) and acidic (pH 5.0) conditions. At pH 7.4, a controlled release profile was observed, with only 25% of the drug released over 24 hours. At pH 5.0, mimicking the acidic microenvironment of tumor cells, 75% of the drug was released within 12 hours. This pH-dependent release

behavior underscores the potential of CQDs as drug delivery carriers for targeted cancer therapy.

Under visible light irradiation, the CQDs demonstrated effective photocatalytic degradation of methylene blue dye, achieving 85% degradation within 3 hours. The high photocatalytic activity was attributed to the presence of surface functional groups that facilitate reactive oxygen species (ROS) generation and the efficient light absorption properties of CQDs.

The results confirmed the successful synthesis of highly fluorescent and biocompatible CQDs from blue crab-derived chitosan. These CQDs exhibited exceptional optical, structural, and functional properties, making them suitable for diverse applications:

In bioimaging, they enabled high-resolution fluorescence imaging with low cytotoxicity.

In environmental monitoring, they demonstrated sensitivity and selectivity for detecting toxic heavy metals.

In drug delivery, they showcased pH-responsive release profiles for targeted therapies.

In photocatalysis, they efficiently degraded organic pollutants under visible light.

The green synthesis method and multifunctional applications highlight the potential of CQDs as sustainable and versatile nanomaterials for future technological and biomedical advancements.

## Discussion

The synthesis of carbon quantum dots (CQDs) from blue crab-derived chitosan demonstrates an innovative and sustainable approach to nanomaterial production. The hydrothermal process employed in this study effectively converted chitosan, a renewable biomaterial, into highly fluorescent CQDs with excellent physicochemical properties. This method aligns with the principles of green chemistry by utilizing marine waste, reducing environmental impact, and avoiding the use of toxic precursors commonly employed in traditional quantum dot synthesis (Iravani & Varma, 2020). The high yield, stable colloidal dispersion, and uniform size distribution observed in this study underscore the efficiency of the synthesis method and its potential for scalability.

The optical properties of the synthesized CQDs, including strong photoluminescence and excitation-dependent emission, are indicative of their quantum confinement effects and the heterogeneity of their surface states. These characteristics make them suitable for a wide range of applications, particularly in bioimaging, environmental sensing, and drug delivery (Ayodele et al., 2018). The UV-Vis and PL spectral results confirmed the formation of conjugated carbon structures and functionalized surfaces, key factors in determining the CQDs' performance in diverse environments. The quantum yield of 18.5% achieved in this study is comparable to or better than CQDs derived from other biomass sources, suggesting that blue crab-derived chitosan is a competitive precursor for quantum dot synthesis (Anpalagan et al., 2023).

The presence of surface functional groups such as hydroxyl, amine, and carboxyl groups, as confirmed by FTIR, enhances the solubility, biocompatibility, and chemical reactivity of the CQDs. These properties were

crucial for their application in biomedical imaging, where the CQDs exhibited bright fluorescence and effective cellular uptake without significant cytotoxicity. Compared to traditional quantum dots that often involve heavy metals like cadmium or selenium, the CQDs synthesized in this study provide a safer and more environmentally friendly alternative for bioimaging (Ayodele et al., 2018).

The sensitivity and selectivity of CQDs for detecting heavy metal ions, such as  $Pb^{2+}$  and  $Hg^{2+}$ , further highlight their versatility. The fluorescence quenching observed upon exposure to these ions was linear, with detection limits well below the thresholds set by the World Health Organization for safe drinking water. This demonstrates the potential of CQDs as practical tools for environmental monitoring, enabling real-time detection of toxic pollutants in water (Iravani & Varma, 2020).

The pH-responsive drug release behavior of the CQDs conjugated with doxorubicin illustrates their potential as nanocarriers for targeted cancer therapy. The accelerated drug release under acidic conditions mimicking tumor microenvironments provides a controlled and site-specific therapeutic mechanism, reducing systemic side effects and enhancing treatment efficacy. This behavior is consistent with the functionalized surfaces of the CQDs, which respond to changes in pH by facilitating drug dissociation (Ayodele et al., 2018).

In addition to biomedical and environmental applications, the CQDs demonstrated significant photocatalytic activity under visible light, achieving 85% degradation of methylene blue dye within 3 hours. This photocatalytic efficiency can be attributed to the high surface area, light absorption properties, and reactive oxygen species generation capabilities of the CQDs. Such performance positions them as effective catalysts for wastewater treatment, addressing global challenges in environmental pollution.

While the findings of this study are promising, certain limitations and areas for improvement remain. The quantum yield, though competitive, could be further enhanced through doping or advanced surface passivation strategies. Additionally, scaling up the synthesis process while maintaining consistent properties and high yields will be critical for practical applications. Future studies should explore alternative marine biomass sources and optimize the hydrothermal process to achieve greater control over CQD properties. Furthermore, expanding the scope of applications, such as integrating CQDs into advanced sensing platforms or exploring their role in energy storage devices, could unlock new potential for these nanomaterials (Iravani & Varma, 2020; Anpalagan et al., 2023).

In conclusion, the results of this study establish blue crab-derived chitosan as a viable and sustainable precursor for CQD synthesis. The multifunctionality, biocompatibility, and environmental sustainability of the synthesized CQDs underscore their potential as advanced materials for a wide range of applications. This research contributes to the growing body of knowledge on green nanotechnology, demonstrating how waste materials can be transformed into high-value products that address critical challenges in health, environment, and industry. By bridging the gap between sustainability and functionality, this approach offers a pathway toward more responsible and innovative material science solutions.

## Conclusion

This study successfully synthesized carbon quantum dots (CQDs) from blue crab-derived chitosan using a hydrothermal method, demonstrating a sustainable and environmentally friendly approach to nanomaterial production. The CQDs exhibited excellent optical properties, biocompatibility, and stability, with versatile applications in bioimaging, environmental sensing, drug delivery, and photocatalysis. Their strong photoluminescence, functionalized surface groups, and pH-responsive behavior enabled effective cellular imaging, heavy metal detection, targeted drug delivery, and wastewater treatment, positioning them as multifunctional nanomaterials for diverse applications (Iravani & Varma, 2020; Ayodele et al., 2018). The use of marine biomass as a precursor supports green chemistry principles by transforming waste into high-value products, reducing reliance on toxic precursors, and addressing sustainability goals. Future work should focus on enhancing quantum yield, scaling up production, and expanding their application scope in advanced technologies, emphasizing the potential of CQDs as eco-friendly alternatives in nanotechnology (Anpalagan et al., 2023).

## Declarations

### Fund Statement

This study was conducted without any financial support.

### Conflict of Interest

The author declares that there are no financial interests or personal relationships that may have influenced this work.

### Author Contribution Statement

Study design: OG; Literature review: OG; Methodology: OG; Conducting the experiment: OG; Data analysis: OG; Manuscript writing: OG; Editing: OG. All authors approved the final draft.

### Ethical Approval Certificate

Local Ethics Committee Approval was not sought, as the study did not involve the utilization of experimental animals.

### Data Availability Statement

Research data is not shared.

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## Effects of Different Flour Types, Protein Sources and Transglutaminase Enzymes on Gluten-Free Şekerpare Production

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 07.01.2025 Accepted : 04.02.2025</p> <p><b>Keywords:</b> Gluten-free şekerpare Celiac disease Soy protein Pea protein Transglutaminase enzyme</p>	<p>Şekerpare is a well-known dessert in Turkish and Middle Eastern cuisine, traditionally prepared using semolina. However, the presence of gluten in semolina prevents individuals with celiac disease from consuming this dessert. In this study, gluten-free şekerpare formulations were developed using combinations of corn flour, rice flour, potato flour, corn starch, and tapioca starch. Additionally, soy protein, pea protein, and transglutaminase (TG) enzymes were incorporated into these formulations to investigate their effects on dough and final product properties. The study evaluated the properties of the dough, such as pH, hardness, stickiness, work of adhesion, dough strength/cohesiveness, and the color, hardness, fracturability, and sensory properties of the final products. The results demonstrated that the proteins and TG enzyme had varying effects on dough properties depending on the flour combination used. Dough prepared with corn and potato flours exhibited the highest hardness values, while the dough of control group consistently had the lowest hardness. Soy and pea proteins reduced the stickiness of the dough, whereas the use of TG enzyme in combination with pea protein significantly increased the stickiness levels. In the sensory analysis, panelists rated the şekerpare samples prepared using the MuPr formulation, consisting of 62,5% corn flour and 37,5% rice flour, as the most preferred product in terms of texture and taste. However, it was observed that the type of protein used and the addition of TG enzyme did not have a significant overall impact on sensory attributes.</p>

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## Glutensiz Şekerpare Üretiminde Farklı Un Çeşitleri, Protein Kaynakları ve Transglutaminaz Enziminin Etkileri

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 07.01.2025 Kabul : 04.02.2025</p> <p><b>Anahtar Kelimeler:</b> Glutensiz şekerpare Çölyak hastalığı Soya proteini Bezelye proteini Transglutaminaz enzimi</p>	<p>Şekerpare, Türkiye ve Orta Doğu mutfağının önemli tatlılarından biri olup, genellikle irmik kullanılarak hazırlanmaktadır. Ancak irmiğin gluten içermesi, çölyak hastalarının bu tatlıyı tüketmesini engellemektedir. Bu çalışmada, gluten içermeyen mısır unu, pirinç unu, patates unu, mısır nişastası ve tapyoka nişastası kombinasyonları kullanılarak glutensiz şekerpare formülasyonları geliştirilmiştir. Ayrıca, bu formülasyonlara eklenen soya proteini, bezelye proteini ve transglutaminaz (TG) enziminin şekerpare hamuru ve son ürün özellikleri üzerindeki etkileri incelenmiştir. Çalışma kapsamında, hamurların pH, sertlik, yapışkanlık, adhezyon işi ve hamur kuvveti gibi özellikleri değerlendirilirken son ürünlerde ise renk, sertlik, kırılabilirlik ve duyu analizler gerçekleştirilmiştir. Elde edilen sonuçlar, kullanılan protein ve TG enziminin hamur özellikleri üzerindeki etkisinin kullanılan kombinasyonuna bağlı olarak değiştiğini göstermiştir. Mısır ve patates unu ile hazırlanan hamurlar en yüksek sertlik değerine sahipken, tüm örneklerde kontrol grubu hamurları en düşük sertlikte olmuştur. Soya ve bezelye proteini, hamurun yapışkanlığını azaltırken, TG enziminin bezelye proteiniyle birlikte kullanımı yapışkanlık düzeyini önemli ölçüde artırmıştır. Duyusal analizde panelistler, %62,5 mısır unu ve %37,5 pirinç unundan oluşan MuPr reçetesiyle hazırlanan şekerpare örneklerini, hem yapı hem de lezzet açısından en beğenilen ürün olarak değerlendirmiştir. Bununla birlikte, kullanılan protein türü ve TG enzimi duyu özellikleri üzerinde genel anlamda önemli bir fark yaratmamıştır.</p>

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## Giriş

Çölyak hastalığı, genetik olarak yatkın bireylerde gluten alımıyla tetiklenen en yaygın oto-immün bozukluklardan biridir (Caio ve ark., 2019). Öte yandan, çölyak dışı gluten hassasiyeti, çok çeşitli bağırsak ve bağırsak dışı semptomlarla karakterize edilen glutenle ilişkili bozuklukların bir parçası olarak tanımlanmıştır ve çölyak hastalığı olmayan deneklerde gluten içeren gıdaların alınmasından sonra ortaya çıkmaktadır (Ebling ve ark., 2022). Çölyak hastalığının görülme sıklığı Avrupa ülkelerinde ve Amerika Birleşik Devletleri'nde %0,3 ile %1 arasında olduğu bildirilmiştir (Harmancı, 2008), Türkiye'deki sıklığın da benzer düzeylerde olduğu ortaya konmuştur (Dalgic ve ark., 2011). Çölyak hastalığının moleküler mekanizmalarının daha iyi anlaşılmasına ve tedavi yöntemlerinde ilerlemeler kaydedilmesine rağmen, şu anda bilinen tek etkin tedavi yaşam boyu glutensiz diyetdir (Gobbetti ve ark., 2007). Glutensiz diyet, buğday, yulaf, arpa, tritikale, kamut ve çavdar unu ile yapılan gıda ürünlerini içermemelidir (Muthukumar ve ark., 2020).

Gluten, tahılların temel depo proteinlerinden biri olup, buğday bazı hamurlarda reolojik özelliklerin ve yapının oluşumunda kritik bir rol oynar. Gluten, çözünürlüklerine göre prolamin ve glutenin olmak üzere iki alt fraksiyona ayrılır. Prolaminler hamurun viskozitesini ve uzayabilirliğini sağlarken, gluteninler elastikiyet ve kohezyon özelliklerinden sorumludur (Carmen & Popping, 2012; Gujral & Rosell, 2004). Glutensiz hamurlar ise daha az yapışkan, elastik olmayan ve işlenmesi zor yapılarıyla buğday içerikli hamurlara göre dezavantajlıdır (Matos & Rosell, 2015). Glutensiz ürünlerin temel zorluklarından biri, glutenin sağladığı işlevleri yerine getirebilecek alternatif bileşenlere ihtiyaç duyulmasıdır. Bu amaçla hidrokoloidler, protein izolatları, enzimler ve farklı un çeşitleri kullanılmıştır (Xu ve ark., 2020). Glutenin uzaklaştırılmasının bir sonucu olarak, glutensiz ürünler genellikle düşük kalitede, zayıf dokuya sahip ve zayıf kırıntı ve kabuk özellikleri ve ağız hissi sergilemektedir (Gallagher ve ark., 2004). Glutensiz tatlı unlu mamullerin geliştirilmesine yönelik çok sayıda çalışma, nihai ürünlerin yapısını, ağız hissini, kabul edilebilirliğini, raf ömrünü ve besin kalitesini iyileştirmeyi amaçlamıştır (Gularte ve ark., 2012; Matos ve ark., 2014; Park ve ark., 2012). Glutensiz kurabiyeler üzerine yapılan çalışmalarda tahıllar (pirinç, mısır, sorgum), baklagiller, yalancı tahıllar ve bunların kombinasyonlarından elde edilen unlar sıklıkla kullanılmıştır. Pirinç unu, mısır nişastası ve bezelye proteini (Mancebo ve ark., 2016) veya pirinç unu ve patates nişastası (Šarić ve ark., 2019) gibi bileşenlerle reçeteler geliştirilmiştir. Protein izolatları da glutensiz kurabiye reçetelerinde kullanılmış ve örneklerin su hidrasyonunu arttırmada, kurabiyelerin sertlik ve yayılma oranını azaltmada etkili bulunmuşlardır (Xu ve ark., 2020). Glutensiz unların reolojik ve pişirme özelliklerini iyileştirmek amacıyla reçetelerde enzimler de kullanılmıştır. Transglutaminazlar (TG), peptide bağlı glutaminin  $\gamma$ -karboksiamid grubu ile bir lizin kalıntısının  $\epsilon$ -amino grubu arasında açıl transfer reaksiyonlarını, deamidasyonu ve çapraz bağlanmayı katalize eden enzim ailesinden (EC 2.3.2.13), protein-glutamin gama-glutamilttransferazdır (Babiker, 2000; Gaspar & Góes-Favoni, 2015). TG enzimi, proteinlerin çapraz bağlanmasını teşvik ederek gıda

sistemlerinde yapısal iyileştirmeler sağlamaktadır. Özellikle glutensiz sistemlerde TG uygulamaları, hamurun viskoelastik özelliklerini iyileştirmiş ve protein ağını destekleyerek ekmek ve kurabiye gibi ürünlerin kalitesini artırmıştır (Altındağ ve ark., 2015; Ebling ve ark., 2022; Gujral & Rosell, 2004).

Türkiye ve Orta Doğu mutfağında önemli bir yer tutan şerbetli tatlılardan biri olan şekerpare, geleneksel olarak irmikle yapılan ve şeker şurubuyla ıslatılan bir tatlıdır. Ancak, irmiğin gluten içermesi nedeniyle çölyak hastaları bu tatlıyı tüketmemektedir. Bu çalışmanın amacı, mısır unu, pirinç unu, patates unu, mısır nişastası ve tapyoka nişastası kullanılarak glutensiz bir şekerpare reçetesi geliştirmek ve soya proteini, bezelye proteini ve transglutaminaz enzimi takviyesiyle bu reçeteyi optimize etmektir.

## Materyal ve Yöntem

Şekerpare örneklerinde pirinç unu (Dr.Oetker, Almanya), mısır unu, patates unu (Satüdas A.Ş., İstanbul, Türkiye), mısır nişastası (Dr.Oetker, Almanya), tapyoka nişastası (Tito, İstanbul) kullanılmıştır. Bunun yanında soya proteini ve bezelye proteini Tunçkaya firmasından temin edilmiştir. Transglutaminaz (100 IU/g) enzimi BDF Ingredients (Probind TX, BDF ingredients, İspanya) tarafından sağlanmıştır. Yumurta, şeker, tuz, su ve sodyum bikarbonat - yerel marketlerden (İstanbul, Türkiye) temin edilmiştir.

### Glutensiz Şekerpare Üretimi

Şekerpare üretiminde kullanılan glutensiz un kombinasyonları Çizelge 1'de sunulmuştur. Un karışımı oranı ön denemelere göre belirlenmiştir. Un kombinasyonları mısır unu (Mu), pirinç unu (Pr), patates unu (Pt), mısır nişastası (Mn), tapyoka nişastası (Tn) ile beraber 4 farklı şekilde yapılmıştır. Bu 4 grubun her birinde una ikame olarak kütlece %5 soya proteini (SP), %5 bezelye proteini (BP), ve protein eklenen örneklerde de protein başına 0.2 IU olacak şekilde TG enzimi eklenmiştir (SP+TG ve BP+TG). Kontrol örneği ile beraber 5 farklı ürün üretilmiştir. Glutensiz un karışımına ek olarak tüm şekerpare örneklerinde 24,80 g pudra şekeri, 19,85 g toz fındık, 49,93 g hidrojene bitkisel yağ, 29,78 g yumurta, 4,96 sodyum bikarbonat kullanılmıştır.

Pudra şekeri ve hidrojene bitkisel yağ hamur yoğurma makinesinde (Kitchen aid, ABD) hız 2' de 1 dakika karıştırılmış, yumurta eklendikten sonra 3 dakika hız 3' te karıştırılmıştır. Diğer ingrediyeentler eklenerek 6 dakika boyunca hız 4'e çıkarılarak hamur yoğrulmuştur. Hamur 15 dakika dinlendirildikten sonra elle şekil verilerek tepsiye dizilmiş ve 160 °C'de 20 dakika konvansiyonel fırında (Fimak, Türkiye) pişirilmiştir. Şurup için 200 g şeker ve 200 mL su 105 °C'ye getirilmiş ve 1 dakika boyunca kaynatılmıştır. Şekerpare pişirildikten sonra fırın çıkışında şurup üzerine dökülmüştür (Şekil 1).

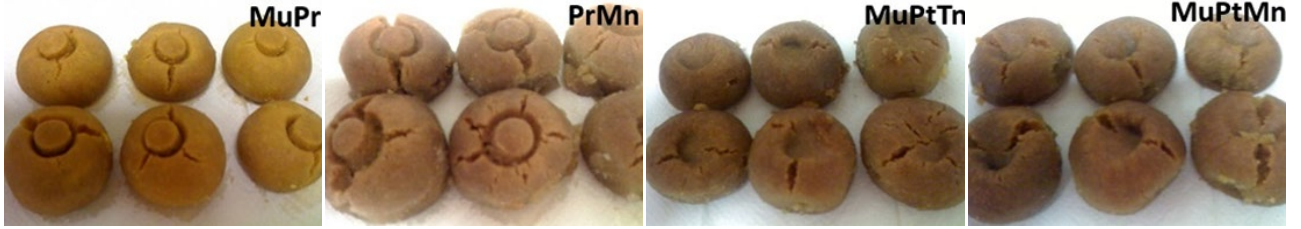
### Örneklerin pH, Nem, Protein, Kül İçeriği ve Renk Özelliklerinin Belirlenmesi

Hamurun pH değeri pH metre (H35010, HANNA Instruments, İtalya) ile ölçülmüştür. Örneklerin renk değerleri 2 saatlik pişirme işleminden sonra oda sıcaklığında  $L^*$ ,  $a^*$  ve  $b^*$  değerlerinin Chroma Meter (Conica, CR-400, Japonya) ölçülmesiyle belirlenmiştir.

Çizelge 1. Glutensiz un karışımları

Table 1. Gluten-free flour blends

	Un Karışımları			
	MuPr	PrMn	MuPtTn	PuPtMn
Mısır Unu (Mu)	62,5		37,5	37,5
Pirinç Unu (Pr)	37,5	62,5		
Patates Unu (Pt)			31,25	31,25
Mısır Nişastası (Mn)		37,5		31,25
Tapyoka Nişastası (Tn)			31,25	



Şekil 1. Farklı un kombinasyonlarıyla hazırlanan şekerpare örnekleri  
Figure 1. Şekerpare samples prepared with different flour combinations

### Tekstür Analizi

Glutensiz un kombinasyonlarının şekerpare hamurunun yapışkanlık, adhezyon işi ve hamur kuvveti/kohesiflik özelliklerine etkisi Encina-Zelada ve ark. (2019) tarafından belirtilen yöntemde bazı modifikasyonlar yapılarak SMS/Chen-Hoseney hamur yapışkanlık düzeneği (A/DSC) ve 25 mm Perspex silindirik prob (P/25P) ile donatılmış TA-XT Tekstür Analiz Cihazıyla (Stable Micro System, İngiltere) ölçülmüştür. Cihaz parametreleri: ön test hızı 0,5 mm/s, test hızı 0,5 mm/s ve test sonrası hız 10 mm/s, tetikleme kuvveti 5 g, uygulanan kuvvet 40 g, temas süresi 0,1 s ve geri dönüş 4 mm olarak ayarlanmıştır. Hamur Hoseney düzeneğinin içine yerleştirilmiş ve iç vida döndürülerek hamurun küçük bir kısmı ekstrüzyon deliklerinden ekstrüde edilmiştir. İlk ekstrüde atıldıktan sonra, vida tekrar döndürülerek hamur yaklaşık 1 mm yüksekliğe kadar ekstrüde edilmiştir. Basıncı azaltmak ve ölçüm sırasında hamurun daha fazla ekstrüde edilmemesini sağlamak için vida hafifçe geriye doğru döndürülmüştür. Her ölçüm 5 kez tekrarlanmıştır. Hamur sertliği ise aynı cihaz üzerinde P/5 probu kullanılarak sıkıştırma modu ile belirlenmiştir. Prob hamurun içine 3 mm/s hızla 20 mm derinliğe kadar nüfuz etmiştir. Penetrasyon sırasında kuvvet, maksimum penetrasyon derinliği noktasına kadar artırılmış ve bu kuvvet değeri, belirtilen bu derinlikteki sertlik olarak ifade edilmiştir. Şekerpare örneklerindeki sertlik (g) ve kırılmalık (mm) değerleri ise pişirmeden 2 saat sonra ölçülmüştür. Örnekler TA-XT Tekstür Analiz Cihazı (Stable Micro System, İngiltere) ile HDP/3PB probu kullanılarak kırılmıştır. Analiz parametreleri olarak 5 g tetikleme kuvveti, 3 mm/s analiz hızı, 5 mm dalma derinliği kullanılmıştır (Mancebo ve ark., 2016).

### Duyusal Değerlendirme

Glutensiz şekerpare örneklerinin duyusal analizleri yaşları 29 ile 40 arası değişen ürünler hakkında bilgi verilen 10 panelist tarafından yapılmıştır. Ürünler pişirilip şerbeti verildikten sonra 5 saat dinlendirilmiş, numaralandırılarak ışığın yeterli olduğu ortamda ayrı ayrı tatlandırılmıştır. Duyusal değerlendirme formunda her bir özelliğin yanına 10 cm'lik skala konulmuş, panelistlerden

kötü, orta ve iyi değerlendirmelerine göre skalada yakın buldukları noktayı işaretlemeleri istenmiştir. Şekerpare için panelistlerden ürünün rengini, görünüşünü, yapışkanlığını, dağılan yapı düzeyini, pütürlü taneli yapısını ve genel beğeni düzeyini değerlendirmeleri istenmiştir.

### İstatistiksel Analizler

Elde edilen sonuçlar JMP 6 programından yararlanılarak analiz edilmiştir, sonuçlar aritmetik ortalama ve standart sapma şeklinde verilmiştir. Örnekler arasındaki istatistiksel farklılık  $p < 0,05$  anlamlılık düzeyinde tek yönlü ANOVA ile analiz edilmiş, çoklu karşılaştırmalar ise TUKEY testi ile değerlendirilmiştir. Örnekler ve analizler üç tekrarlı yapılmıştır.

### Bulgular ve Tartışma

#### Örnek Hamurlarının pH ve Yapısal Özellikleri

Hamur özellikleri nişasta, protein gibi ingredientlerin varlığına ve miktarına bağlı olarak değişmekte ve dolayısıyla hamurun işlenmesini etkilemektedir. Hamurun çok sert ya da kuru oluşu, işlenmesini zorlaştırmaktadır. Bu sebeple hamur, yeterli düzeyde sertliğe ve farklı proseslerde işlenebilecek düzeyde bir arada duracak bir kohesifliğe sahip olmalıdır (Gujral ve ark., 2003). Çalışmada kullanılan şekerpare hamurlarının pH, sertlik, yapışkanlık, adhezyon işi ve hamur kuvveti değerleri Çizelge 2'de verilmiştir. Çizelgeden de görüleceği üzere hamurların pH sonuçları 5,8 ile 6,08 arasında değişkenlik göstermiştir. Kullanılan un kombinasyonları hamurların pH değerini önemli derecede etkilememiştir ( $p > 0,05$ ). Benzer şekilde, bezelye veya soya proteini ve TG enzimi kullanımı da hamurun pH değerini önemli ölçüde etkilememiştir ( $p > 0,05$ ). Hamurların sertliği 248,47 - 463,61 g arasında değişmiştir. MuPr ve PrMn'nin kontrol hamurunun sertlikleri sırasıyla  $248,47 \pm 34,22$  g ve  $280,91 \pm 30,27$  g iken, MuPtTn ve MuPtMn hamurlarının ise sırasıyla  $366,14 \pm 15,84$  g ve  $343,00 \pm 13,49$  g olarak bulunmuştur. En yüksek hamur sertliği mısır unu ve patates ununun birlikte kullanıldığı hamurlarda görülmüştür.

Çizelge 2. Şekerpare hamurlarına ait pH, hamur sertliği, yapışkanlık, adhezyon işi ve hamur kuvveti değerleri  
Table 2. pH, dough hardness, stickiness, work of adhesion and dough strength/cohesiveness values of şekerpare dough

		pH	Hamur Sertliği (g)	Yapışkanlık (g)	Adhezyon işi (g.sec)	Hamur Kuvveti/ Kohesiflik (mm)
MuPr	K	6,04	248,47±34,22 <sup>Bc</sup>	9,28±0,98 <sup>Aa</sup>	0,29±0,03 <sup>Abc</sup>	0,54±0,05 <sup>Abc</sup>
	SP	6,10	375,10±22,10 <sup>Ab</sup>	4,85±0,29 <sup>Cb</sup>	0,14±0,02 <sup>Cc</sup>	0,48±0,07 <sup>Aa</sup>
	BP	5,91	399,60±20,23 <sup>Ac</sup>	6,32±0,44 <sup>Ba</sup>	0,19±0,02 <sup>Ba</sup>	0,50±0,02 <sup>Aa</sup>
	SP+ TG	6,04	395,67±33,35 <sup>Aa</sup>	5,47±0,30 <sup>BCc</sup>	0,16±0,01 <sup>BCb</sup>	0,46±0,02 <sup>Aa</sup>
	BP+ TG	6,02	355,14±20,44 <sup>Ab</sup>	8,25±0,33 <sup>Aa</sup>	0,25±0,02 <sup>Aa</sup>	0,55±0,02 <sup>Aa</sup>
PrMn	K	5,96	280,91±30,27 <sup>Bbc</sup>	9,66±0,24 <sup>Aa</sup>	0,42±0,03 <sup>Aa</sup>	0,66±0,04 <sup>Aa</sup>
	SP	6,08	460,40±26,70 <sup>Aa</sup>	4,12±0,24 <sup>Db</sup>	0,11±0,01 <sup>Dc</sup>	0,40±0,01 <sup>Ca</sup>
	BP	6,01	446,54±18,45 <sup>Aab</sup>	6,25±0,28 <sup>Ca</sup>	0,18±0,02 <sup>Ca</sup>	0,46±0,02 <sup>BCbc</sup>
	SP+ TG	6,04	321,77±15,50 <sup>Bb</sup>	6,64±0,31 <sup>Cb</sup>	0,20±0,02 <sup>Ca</sup>	0,49±0,04 <sup>Ba</sup>
	BP+ TG	6,05	294,40±21,06 <sup>Bc</sup>	8,08±0,21 <sup>Ba</sup>	0,25±0,01 <sup>Ba</sup>	0,52±0,03 <sup>Bab</sup>
MuPtTn	K	5,98	366,14±15,84 <sup>Ba</sup>	7,22±0,20 <sup>Ab</sup>	0,23±0,01 <sup>Ac</sup>	0,49±0,01 <sup>Abc</sup>
	SP	6,05	454,60±19,06 <sup>Aa</sup>	6,53±0,21 <sup>Ba</sup>	0,18±0,01 <sup>Bb</sup>	0,45±0,03 <sup>Ba</sup>
	BP	5,87	463,61±17,35 <sup>Aa</sup>	5,97±0,24 <sup>Ca</sup>	0,17±0,01 <sup>Ba</sup>	0,45±0,01 <sup>Bc</sup>
	SP+ TG	5,86	383,32±8,16 <sup>Ba</sup>	7,06±0,22 <sup>ABab</sup>	0,22±0,01 <sup>Aa</sup>	0,48±0,03 <sup>ABa</sup>
	BP+ TG	5,85	382,89±10,63 <sup>Bb</sup>	7,21±0,17 <sup>Ab</sup>	0,23±0,01 <sup>Aa</sup>	0,51±0,01 <sup>Aab</sup>
MuPtMn	K	5,80	343,00±13,49 <sup>Bab</sup>	8,89±0,16 <sup>Aa</sup>	0,31±0,03 <sup>Ab</sup>	0,60±0,02 <sup>Aab</sup>
	SP	5,84	418,19±14,16 <sup>Aab</sup>	7,15±0,29 <sup>BCa</sup>	0,22±0,02 <sup>Ba</sup>	0,50±0,02 <sup>Ba</sup>
	BP	5,78	409,56±14,74 <sup>Abc</sup>	6,54±0,17 <sup>Da</sup>	0,19±0,01 <sup>Ba</sup>	0,49±0,01 <sup>Bab</sup>
	SP+ TG	5,80	423,00±12,62 <sup>Aa</sup>	7,46±0,20 <sup>Ba</sup>	0,23±0,01 <sup>Ba</sup>	0,50±0,02 <sup>Ba</sup>
	BP+ TG	5,80	444,36±17,56 <sup>Aa</sup>	6,88±0,13 <sup>Cdb</sup>	0,19±0,01 <sup>Bb</sup>	0,48±0,03 <sup>Bb</sup>

\*Farklı büyük harfle işaretlenmiş ortalamalar aynı hamur karışımı içerisinde farklı uygulamaların istatistiki olarak birbirinden farklı olduğunu gösterir (p<0,05). Farklı küçük harfle işaretlenmiş ortalamalar aynı uygulamada hamur karışımları arasındaki farklılığı gösterir (p<0,05). Mu: Mısır Unu, Pr: Pirinç Unu, Mn: Mısır Nişastası, Pt: Patates Unu, Tn: Tapyoka Nişastası, K: Kontrol, SP: Soya Proteinini, BP: Bezelye Proteinini, TG: Transglutaminaz Enzimi

Tüm gruplarda kontrol hamuru en düşük sertliğe sahiptir. Hamur karışımlarına hem SP hem de BP eklenmesi hamur sertliğini arttırmıştır. Buna karşın TG enziminin her iki proteine ek olarak kullanılması PrMn ve MuPtTn hamurlarında hamur sertliğini önemli düzeyde düşürmüştür. Örneğin PrMn reçetesinde kontrol hamurun sertliği 280,91±30,27 g olarak bulunmuşken bu oran SP kullanılması ile 460,40±26,70 g'ye yükselmiş fakat TG enzimi eklenmesiyle bu oranı 321,77±15,50 g'ye düşürmüştür; BP kullanımında ise 446,54±18,45 g olarak ölçülen hamur sertliği TG enzimi kullanımıyla 294,40±21,06 g düzeylerine düşürmüştür. Fakat MuPr ve MuPtMn hamur karışımlarında TG enziminin proteinlere ek olarak kullanılması herhangi bir farklılığa neden olmamıştır.

Hamur yapışkanlığı, adhezyon ve kohezyon arasındaki etkileşimli bir dengeden kaynaklanır. Yapışma, bir malzeme (hamur) ile bir yüzey (prob) arasındaki etkileşimi temsil ederken, kohezyon hamurun içindeki etkileşimleri tanımlar (Hoseney & Smewing, 1999). Şekerpare hamurlarının yapışkanlık oranları 4,12- 9,66 g aralığında değişiklik göstermiştir. Genel olarak tüm hamurlarda soya ve bezelye proteinini eklenmesi hamur yapışkanlıklarının düşmesine neden olmuştur. Tang & Liu (2017) da çalışmalarında buğday unu hamuruna soya proteini ve buğday proteinini eklemiş ve soya proteininin hamurların yapışkanlık düzeylerini düşürdüğünü belirlemişlerdir. MuPr hamurunda protein eklenmesiyle düşen yapışkanlık düzeyi TG enziminin eklenmesiyle artmıştır. Özellikle bezelye proteinine eklenen TG enziminin kullanıldığı hamurdaki yapışkanlık düzeyindeki artış istatistiki anlamda önemli düzeyde bulunmuştur (p<0,05). MuPr hamurunda görülen bu eğilim PrMn ve MuPtTn hamurlarında da görülmüştür. Fakat MuPtMn karışımında TG enzimi eklenmesinin hamurun yapışkanlık düzeyini önemli düzeyde etkilemediği belirlenmiştir. Örnek hamurlarının adhezyon işi değerleri 0,11-0,42 g.sn aralığında değişmiştir.

En yüksek adhezyon işi değerinin en yüksek yapışkanlığa sahip PrMn karışımının hamurunda olduğu görülmüştür. Hamur karışımları baz alınarak sonuçlar incelendiğinde adhezyon işi değerinin yine kontrol örneklerine ait olduğu görülmüştür. Özellikle PrMn ve MuPtTn hamurlarında proteinlere TG enziminin eklenmesi, adhezyon işi değerlerini önemli düzeyde arttırmıştır. Hamur kuvveti ve kohesifliği açısından hamurlar arasında diğer parametrelerde olduğu kadar belirgin ayrımlar görülmemiştir. Yine aynı şekilde kontrol hamurlarının en yüksek hamur kuvvetine sahip olduğu görülmüş, fakat protein türleri arasında ve TG enziminin proteinlere eklenmesinin sadece protein kullanımına göre hamur kuvvetini büyük oranda değiştirmedeği görülmüştür.

#### Glutensiz Şekerpare Örneklerinin Renk Özellikleri ve Tekstür Analizi

Şekerpare örneklerine ait renk ve tekstür analiz sonuçları Çizelge 3'te verilmiştir. Örneklerin renk değerleri incelendiğinde L\* değerinin beklenildiği üzere pirinç unu ve mısır nişastasının kullanıldığı PrMn reçetesinde daha yüksek olduğu görülmüştür. Bu un kombinasyonunda bezelye proteinini kullanıldığı reçetenin diğerlerine göre daha yüksek L\* değeri ve daha düşük a\* ve b\* değerlerine sahip olduğu belirlenmiştir. Bu durum bu şekerpare örneğinin açık renkte görünmesine neden olmuştur. Mancebo ve ark. (2016), pirinç unu, mısır unu ve bezelye proteinini farklı oranlarda kullandığı kurabiyeler üretmiş ve benzer şekilde bunların nişasta oranından ziyade bezelye proteinini kullanmanın L\* değerini düşürdüğünü ve nişasta oranıyla birlikte bu etkinin azaldığını bildirmiştir. Pirinç ununun kullanıldığı MuPr ve PrMn reçetelerinde ortama bezelye proteinini girdiğinde kırmızılık değeri düşmüş, fakat aynı etki patates ununun kullanıldığı MuPtTn ve PuPtMn reçetelerinde



görülmemiştir. Mısır unlarının kullanıldığı reçeteler daha yüksek kırmızılık değerine sahip olmuştur. Daha yüksek protein seviyesi ve dolayısıyla amino asit miktarı Maillard reaksiyonlarını ve dolayısıyla ürünün yüzey rengine katkıda bulunan kahverengi bileşiklerin oluşumunu artırmaktadır (Manley, 2011). Bu sebeple mısır ununun pirinç ununa kıyasla daha yüksek protein içermesi

esmerleşme reaksiyonlarına sebep olmuş ve kırmızılık değerini arttırmıştır. Reçetelere TG ilavesi de genel olarak kırmızılık değerinin artmasına neden olmuştur. Alp & Bilgili (2008) de benzer şekilde buğday unu içeren keklerin kırmızılık değerlerinin tüm protein kaynakları için TG ilavesiyle arttığını bildirmiştir.

Çizelge 3. Şekerpare örneklerine ait renk ve tekstürel özellikler

Table 3. Colour and textural properties of şekerpare samples

		Renk Özellikleri			Tekstürel Özellikler	
		<i>L*</i>	<i>a*</i>	<i>b*</i>	Sertlik (g)	Kırılganlık (mm)
MuPr	K	48,50±1,20 <sup>Cc</sup>	13,30±0,29 <sup>Aa</sup>	17,90±0,51 <sup>Cc</sup>	3185±23,60 <sup>Ba</sup>	49,76±0,74 <sup>Bb</sup>
	SP	53,03±0,02 <sup>Bb</sup>	13,79±0,49 <sup>Aa</sup>	20,06±0,09 <sup>Ba</sup>	3167±186,57 <sup>Ba</sup>	50,31±0,41 <sup>ABb</sup>
	BP	56,05±1,25 <sup>Ab</sup>	11,55±0,62 <sup>Ba</sup>	21,01±0,36 <sup>Aa</sup>	2531±148,94 <sup>Cab</sup>	50,59±0,47 <sup>Ab</sup>
	SP+TG	53,01±0,16 <sup>Bb</sup>	13,45±0,81 <sup>Aa</sup>	20,07±0,47 <sup>Bab</sup>	3791±286,07 <sup>Aa</sup>	49,56±0,18 <sup>Bc</sup>
	BP+TG	52,51±0,54 <sup>Bb</sup>	11,75±0,74 <sup>Bab</sup>	19,83±0,18 <sup>Bb</sup>	3080±266,02 <sup>Ba</sup>	49,97±0,05 <sup>ABbc</sup>
PrMn	K	65,27±0,22 <sup>Ba</sup>	7,69±0,35 <sup>Bc</sup>	18,44±0,32 <sup>Cbc</sup>	1404±7,41 <sup>BCd</sup>	50,61±0,38 <sup>Bb</sup>
	SP	64,74±0,54 <sup>Ba</sup>	8,83±1,05 <sup>Bb</sup>	19,42±1,00 <sup>Ba</sup>	1443±156,13 <sup>Bd</sup>	50,75±0,07 <sup>ABb</sup>
	BP	69,60±1,06 <sup>Aa</sup>	6,92±0,32 <sup>Cb</sup>	17,58±0,51 <sup>Cc</sup>	1177±21,39 <sup>Cc</sup>	50,75±0,37 <sup>ABb</sup>
	SP+TG	62,46±1,01 <sup>Ca</sup>	11,58±0,05 <sup>Ab</sup>	20,85±0,27 <sup>Aa</sup>	1317±234,41 <sup>BCc</sup>	50,12±0,99 <sup>Bbc</sup>
	BP+TG	63,95±2,15 <sup>BCa</sup>	10,75±1,03 <sup>Ab</sup>	20,59±0,09 <sup>Aa</sup>	2341±38,12 <sup>Aba</sup>	51,67±0,28 <sup>Aa</sup>
MuPtTn	K	56,18±1,66 <sup>Ab</sup>	10,67±0,50 <sup>Cb</sup>	21,34±0,25 <sup>Aa</sup>	1726±177,95 <sup>Cc</sup>	52,26±0,54 <sup>Aa</sup>
	SP	48,75±1,85 <sup>Cc</sup>	13,12±0,22 <sup>Aa</sup>	18,17±0,79 <sup>Cb</sup>	2486±10,93 <sup>Ab</sup>	51,99±0,41 <sup>Aa</sup>
	BP	51,83±1,42 <sup>Bc</sup>	11,71±0,21 <sup>BCa</sup>	19,07±0,58 <sup>Bb</sup>	2595±206,78 <sup>Aa</sup>	51,83±0,03 <sup>ABa</sup>
	SP+TG	51,66±1,90 <sup>Bb</sup>	12,20±1,48 <sup>ABab</sup>	19,01±0,42 <sup>BCc</sup>	2533±13,67 <sup>Ab</sup>	51,25±0,30 <sup>Ba</sup>
	BP+TG	50,98±0,17 <sup>BCb</sup>	12,29±0,15 <sup>ABa</sup>	19,24±0,03 <sup>Bbc</sup>	2192±97,63 <sup>Bb</sup>	50,39±0,34 <sup>Cb</sup>
MuPtMn	K	49,19±0,41 <sup>Cc</sup>	13,18±0,40 <sup>Aa</sup>	18,54±0,09 <sup>Bb</sup>	2136±68,19 <sup>Ab</sup>	49,83±0,04 <sup>ABb</sup>
	SP	53,60±0,34 <sup>Ab</sup>	13,99±3,27 <sup>Aa</sup>	20,18±0,35 <sup>Aa</sup>	2168±122,85 <sup>Ac</sup>	50,94±0,98 <sup>Aab</sup>
	BP	52,54±1,69 <sup>ABc</sup>	11,95±0,91 <sup>Aa</sup>	19,66±0,60 <sup>Ab</sup>	2131±372,57 <sup>Ab</sup>	50,86±0,91 <sup>ABab</sup>
	SP+TG	53,06±1,61 <sup>Ab</sup>	11,40±0,50 <sup>Ab</sup>	19,25±0,66 <sup>ABbc</sup>	2137±215,78 <sup>Ab</sup>	50,64±0,19 <sup>ABab</sup>
	BP+TG	50,34±1,69 <sup>BCb</sup>	12,85±0,12 <sup>Aa</sup>	18,56±0,70 <sup>Bc</sup>	2162±51,84 <sup>Ab</sup>	49,72±0,54 <sup>Bc</sup>

\*Farklı büyük harfle işaretlenmiş ortalamalar aynı hamur karışımı içerisinde farklı uygulamaların istatistiki olarak birbirinden farklı olduğunu gösterir (p<0.05). Farklı küçük harfle işaretlenmiş ortalamalar aynı uygulamada hamur karışımları arasındaki farklılığı gösterir (p<0.05). Mu: Mısır Unu, Pr: Pirinç Unu, Mn: Mısır Nişastası, Pt: Patates Unu, Tn: Tapyoka Nişastası, K: Kontrol, SP: Soya Proteini, BP: Bezelye Proteini, TG: Transglutaminaz Enzimi

Çizelge 4. Şekerpare örneklerinin duyuşal değerlendirme sonuçları

Table 4. Sensory evaluation results of şekerpare samples

		Renk	Görünüş	Yapışkanlık	Dağılan Yapı	Pütürlü Taneli Yapı	Genel Beğeni
MuPr	K	8,13±0,26 <sup>Ca</sup>	8,71±0,19 <sup>ABa</sup>	8,32±0,33 <sup>Aa</sup>	8,33±0,27 <sup>Aa</sup>	7,28±0,22 <sup>Aa</sup>	8,28±0,21 <sup>Aa</sup>
	SP	8,08±0,30 <sup>Ca</sup>	8,63±0,27 <sup>Ba</sup>	8,31±0,17 <sup>Aa</sup>	8,32±0,28 <sup>Aa</sup>	7,30±0,26 <sup>Aa</sup>	8,43±0,13 <sup>Aa</sup>
	BP	8,90±0,22 <sup>Aa</sup>	9,04±0,15 <sup>Aa</sup>	8,65±0,24 <sup>Aa</sup>	8,34±0,26 <sup>Aa</sup>	7,33±0,21 <sup>Aa</sup>	8,45±0,23 <sup>Aa</sup>
	SP+TG	8,63±0,41 <sup>ABa</sup>	8,63±0,30 <sup>Ba</sup>	8,22±0,25 <sup>Aa</sup>	8,24±0,22 <sup>Aa</sup>	7,25±0,30 <sup>Aa</sup>	8,49±0,16 <sup>Aa</sup>
	BP+TG	8,23±0,30 <sup>BCa</sup>	8,59±0,32 <sup>Ba</sup>	8,68±0,15 <sup>Aa</sup>	8,38±0,20 <sup>Aa</sup>	7,35±0,26 <sup>Aa</sup>	8,51±0,29 <sup>Aa</sup>
PrMn	K	4,58±0,29 <sup>Ac</sup>	3,51±0,26 <sup>Ac</sup>	8,04±0,43 <sup>Aa</sup>	4,50±0,36 <sup>Ac</sup>	5,63±0,31 <sup>Ac</sup>	4,55±0,19 <sup>Ac</sup>
	SP	4,75±0,45 <sup>Ac</sup>	3,46±0,29 <sup>Ac</sup>	7,84±0,61 <sup>Aa</sup>	4,53±0,31 <sup>Ac</sup>	5,10±0,22 <sup>Bd</sup>	4,53±0,25 <sup>Ac</sup>
	BP	4,45±0,31 <sup>Ac</sup>	3,32±0,36 <sup>Ad</sup>	7,84±0,71 <sup>Aa</sup>	4,32±0,29 <sup>Ac</sup>	5,15±0,24 <sup>Bc</sup>	4,48±0,28 <sup>Ac</sup>
	SP+TG	4,45±0,41 <sup>Ac</sup>	3,29±0,27 <sup>Ac</sup>	7,87±0,52 <sup>Aa</sup>	4,13±0,23 <sup>ABc</sup>	5,20±0,18 <sup>Bc</sup>	4,50±0,26 <sup>Ac</sup>
	BP+TG	3,65±0,39 <sup>Bc</sup>	3,16±0,19 <sup>Ac</sup>	7,66±0,82 <sup>Aa</sup>	3,75±0,21 <sup>Bc</sup>	5,30±0,26 <sup>ABc</sup>	4,38±0,26 <sup>Ac</sup>
MuPtTn	K	7,73±0,36 <sup>Aab</sup>	7,66±0,31 <sup>Bb</sup>	7,81±0,86 <sup>Aa</sup>	8,60±0,31 <sup>Aa</sup>	6,43±0,25 <sup>Ab</sup>	7,30±0,26 <sup>Ab</sup>
	SP	7,68±0,39 <sup>Aab</sup>	7,50±0,29 <sup>Bb</sup>	7,88±0,77 <sup>Aa</sup>	8,18±1,12 <sup>Aa</sup>	6,53±0,32 <sup>Ab</sup>	7,22±0,21 <sup>Ab</sup>
	BP	7,70±0,39 <sup>Ab</sup>	8,20±0,18 <sup>Ab</sup>	7,94±0,70 <sup>Aa</sup>	8,44±0,29 <sup>Aa</sup>	6,43±0,25 <sup>Ab</sup>	7,38±0,23 <sup>Ab</sup>
	SP+TG	7,45±0,37 <sup>Ab</sup>	8,27±0,28 <sup>ABab</sup>	7,94±0,57 <sup>Aa</sup>	8,53±0,19 <sup>Aa</sup>	6,50±0,24 <sup>Ab</sup>	7,52±0,13 <sup>Ab</sup>
	BP+TG	7,53±0,22 <sup>Ab</sup>	8,08±0,24 <sup>Ab</sup>	7,90±0,58 <sup>Aa</sup>	8,65±0,26 <sup>Aa</sup>	6,45±0,30 <sup>Ab</sup>	7,40±0,22 <sup>Ab</sup>
MuPtMn	K	7,50±0,37 <sup>Ab</sup>	7,47±0,19 <sup>Cb</sup>	7,79±0,84 <sup>Aa</sup>	6,50±0,22 <sup>Bb</sup>	5,50±0,41 <sup>Ac</sup>	7,53±0,27 <sup>Ab</sup>
	SP	7,45±0,31 <sup>Ab</sup>	7,58±0,26 <sup>Cb</sup>	7,85±0,77 <sup>Aa</sup>	6,67±0,29 <sup>Bb</sup>	5,55±0,35 <sup>Ac</sup>	7,44±0,21 <sup>Ab</sup>
	BP	7,60±0,36 <sup>Ab</sup>	7,58±0,30 <sup>Cc</sup>	7,94±0,70 <sup>Aa</sup>	7,21±0,26 <sup>Ab</sup>	5,51±0,27 <sup>Ac</sup>	7,49±0,14 <sup>Ab</sup>
	SP+TG	7,60±0,33 <sup>Ab</sup>	8,05±0,21 <sup>Bb</sup>	7,91±0,80 <sup>Aa</sup>	7,35±0,27 <sup>Ab</sup>	5,42±0,33 <sup>Ac</sup>	7,68±0,15 <sup>Ab</sup>
	BP+TG	7,85±0,31 <sup>Aab</sup>	8,60±0,29 <sup>Aa</sup>	7,94±0,84 <sup>Aa</sup>	7,53±0,26 <sup>Ab</sup>	5,49±0,34 <sup>Ac</sup>	7,50±0,18 <sup>Ab</sup>

\*Farklı büyük harfle işaretlenmiş ortalamalar aynı hamur karışımı içerisinde farklı uygulamaların istatistiki olarak birbirinden farklı olduğunu gösterir (p<0.05). Farklı küçük harfle işaretlenmiş ortalamalar aynı uygulamada hamur karışımları arasındaki farklılığı gösterir (p<0.05). Mu: Mısır Unu, Pr: Pirinç Unu, Mn: Mısır Nişastası, Pt: Patates Unu, Tn: Tapyoka Nişastası, K: Kontrol, SP: Soya Proteini, BP: Bezelye Proteini, TG: Transglutaminaz Enzimi

Şekerpare örneklerinin sertlik ve kırılabilirlik değerleri sırasıyla 1404-3791 g, 49,56-52,26 mm aralığında değişmiştir. Kullanılan un karışımları örneklerin sertlik değerini önemli düzeyde etkilemiştir. Mısır unu ve pirinç ununun kullanıldığı MuPr reçetesinin sertlik değeri en yüksektir. Bunun yanında protein kullanımı hem protein türüne göre hem de un karışımına göre farklılık göstermiştir. Örneğin mısır unu ve pirinç ununun kullanıldığı MuPr'nin kontrol örneğinin sertliği 3185±23,60 g olarak bulunmuşken bu reçetede SP kullanıldığında sertlik değeri benzer olurken (3167±186,57 g), BP kullanıldığı zaman ise azalmıştır (2531±148,94 g). MuPr, PrMn ve MuPtMn reçetelerinde SP eklenmesinin önemli düzeyde etkisi olmamıştır. MuPtTn'de SP ve BP eklenmesi ürünün sertleşmesine neden olmuştur. Bunun yanı sıra BP, MuPr ve PrMn'de ise ürünün sertliğini düşürmüştür. TG enziminin etkisi ise un kombinasyonuna ve protein türüne göre farklılık göstermiştir. MuPr'de SP ile beraber kullanılan TG sertliği artırırken kırılabilirliğini azaltmıştır. Diğer un karışımlarında SP ile birlikte TG enziminin kullanılmasının sertliğe önemli bir etkisi olmamıştır. BP'nin TG ile birlikte kullanımı MuPr, PrMn ve MuPtTn reçetelerinde sertliği düşürmüştür. TG uygulamasının protein ağını teşvik ederek su tutma kapasitesini artırdığı daha önceki çalışmalarda bildirilmiştir (Kuraishi ve ark., 2001; Lorenzen ve ark., 2002). Shen ve ark. (2022) çalışmalarının bir bölümünde BP ve TG enziminin etkileşimini incelemişler ve kontrol BP ile karşılaştırıldığında TG ilavesinin, proteinin çapraz bağlanmasını destekleyerek su tutma kapasitesini önemli ölçüde artırdığını belirtmişlerdir. Bu nedenle, TG ilavesiyle hazırlanan şekerparelerin sertliğindeki azalma, artan nem içeriğinin bir sonucu olarak gerçekleşmiştir. Altındağ ve ark. (2015) karabuğday unu, mısır ve pirinç unları kullanarak kurabiye üretmiş ve TG enziminin bu karışımlara etkisini incelemişlerdir. TG enziminin kurabiyelerin sertliğinin düşmesine ve kırılabilirlik değerlerinin artmasına neden olduğu bildirilmiştir. Örneklerimizde, kırılabilirlik değerleri açısından belirgin bir farklılık gözlenmemiştir.

#### **Şekerpare Örneklerinin Duyusal Analiz Sonuçları**

Şekerpare örneklerine ait duyusal analiz sonuçları Çizelge 4'te verilmiştir. Panelistler şekerpare örneklerine ait renk, görünüş, yapışkanlık, dağılan yapı, pütürlü taneli yapı, genel beğeni parametrelerini değerlendirmiştir. Genel olarak tüm parametrelerde panelistlerin en yüksek puan verdiği örnekler % 62,5 mısır unu ve %37,5 pirinç ununun kullanıldığı MuPr reçetesine ait olan örnekler olmuştur. Bununla birlikte kullanılan protein çeşitleri ve enzimin genel olarak ürün yapısında ve lezzetinde herhangi bir farklılık oluşturmadığı görülmüştür. MuPtTn ve MuPtMn reçetelerine ait olan örnekler tüm parametrelerde genel olarak benzer bulunmuştur. Pirinç unu ve mısır nişastasının kullanıldığı PrMn'ye ait olan örnekler panelistler tarafından en düşük puanı almıştır. Bu örneklerin dağılan yapısı ve pütürlü taneli ağız hissi genel beğeniye de etkilemiş ve genel beğeni açısından en düşük skoru almıştır. Bunun yanında mısır unu ve pirinç ununun birlikte kullanıldığı şekerpare örnekleri genel beğeni açısından en yüksek skoru almıştır.

#### **Sonuç**

Yaptığımız çalışmada farklı un karışımları hazırlanmış ve glutensiz şekerpare üretiminde kullanılmıştır. Mısır unu, pirinç unu, patates unu, mısır nişastası, tapyoka nişastası kullanılarak 4 farklı reçete hazırlanmış bu un karışımlarına soya proteini, bezelye proteini, transglutaminaz enzimi eklenerek farklı uygulamalarla geliştirilmeye çalışılmıştır. Hamur yapısının değişimini incelemek amacıyla hamurlarda pH, sertlik, yapışkanlık, hamur kuvveti/kohesiflik parametreleri çalışılmış son ürünlerin ise renk, tekstür, duyusal analizleri yapılmıştır. Kullanılan protein ve enzimin istatistiki olarak sonuçlar üzerine etkisi kullanılan un karışımına bağlı olarak değişmiştir. Her iki hamurda da pH'nın reçetelere göre etkilenmediği belirlenmiştir. En yüksek hamur sertliği mısır unu ve patates ununun birlikte kullanıldığı hamurlarda görülmüştür. Fakat TG enziminin her iki proteine ek olarak kullanılması PrMn ve MuPtTn hamurlarında hamur sertliğini önemli düzeyde düşürmüştür. Son üründe ise mısır unu ve pirinç ununun kullanıldığı MuPr reçetesinin sertlik değeri en yüksek oranda çıkmıştır. Bu durum duyusal olarak istenen bir sonuç oluşturmuştur. Duyusal olarak şekerpare örneklerinde en beğenilen reçete mısır ununun % 62,5 pirinç ununun % 37,5 oranında kullanıldığı MuPr reçetesi olmuştur. Protein çeşidi ya da transglutaminaz enziminin kullanılması duyusal olarak belirgin farklılıklara neden olmamıştır. Bu çalışmada elde edilen başarılı un kombinasyonlarının, glutensiz kek, kurabiye ve ekmek gibi farklı fırıncılık ürünlerinde de değerlendirilmesi gelecek çalışmalar için önemli bir araştırma alanı oluşturabilir. Ayrıca, sonraki çalışmalarda farklı bitkisel protein kaynakları kullanılarak (mercimek proteini, bakla proteini vb.) hamur reolojisi ve son ürün kalitesi üzerindeki etkileri göz önüne alınabilir.

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## Impact of Ultrasound-assisted Cooking and Endpoint Core Temperature on Physicochemical and Microbiological Properties, and Oxidative Stability of Beef

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### ABSTRACT

This research aimed to investigate the impacts of different cooking methods (B; Boiling, US; Ultrasound-assisted slow boiling, UF; Ultrasound-assisted fast boiling) and endpoint core temperatures (ECT; 68°C, 74°C, and 80°C) on the oxidative stability, physicochemical, and microbiological properties of beef during refrigerated storage. The results demonstrated that UF application resulted in the lowest cooking loss (CL) at 74°C ECT. The US application caused a lower water activity ( $a_w$ ) compared to B. The lowest oxidation-reduction potential (ORP) levels were determined in UF, whereas the US had the highest ORP levels. Ultrasound-assisted cooking did not affect pH, yeast-mold and total mesophilic aerobic bacteria (TMAB) counts. On the other hand, UF and US caused an increase in total coliform counts compared to B. According to the results of lipid hydroperoxide (LPO) and thiobarbituric acid reactive substances (TBARS), UF application was more effective in preventing lipid oxidation compared to US and B. pH, CL, ORP, hue angle ( $h_{ab}$ ) and  $b^*$  values increased as the ECT increased, whereas  $a_w$ ,  $a^*$ , chroma ( $C^*_{ab}$ ) and browning index ( $BI$ ; inner) values decreased. In addition, beef pieces cooked at 74°C or 80°C ECT had lower  $L^*$  values, TMAB, and total coliform counts, and higher TBARS and LPO values than those cooked at 68°C ECT. 74°C was more effective in controlling microbiological changes, whereas 68°C was a better ECT for maintaining oxidative stability. In conclusion, UF has the potential to be an effective processing technology for improving oxidative stability and physicochemical properties of beef.

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## Introduction

Meat is a vital component of the human diet, providing numerous bioactive compounds such as essential amino and fatty acids (Şimşek & Kılıç, 2016). The physicochemical and nutritional quality of meat is influenced by various processing methods such as drying, packaging, smoking, freezing and cooking (Gómez et al., 2020). The cooking of meat is crucial for ensuring both deliciousness and food safety (Dominguez et al., 2015). Cooking is one of the most widely used methods to destroy foodborne pathogens, ensure microbial safety, enhance the taste and flavor of meat, and improve digestibility (Broncano et al., 2009). However, improper cooking techniques or conditions can lead to detrimental impacts such as the formation of chemical browning reactions, diminished nutritional and sensorial quality of muscle foods, aromatic polycyclic hydrocarbons (pyrene, chrysene etc.) generation and accelerated lipid oxidation (Rasinska et al., 2019; Gómez et al., 2020). Many studies on cooking

techniques with high final cooking temperatures and prolonged cooking duration demonstrated increased quality losses and lipid oxidation (Serrano et al., 2007; Domínguez et al., 2015). Among different cooking techniques, the three main factors that create differences in meat quality are the applied surface temperature, core temperature, and heat transfer method (Bejerholm & Aaslyng, 2004). Whereas surface temperature plays a crucial role in determining the aroma, taste, and color of the meat, the core temperature is vital for microbial destruction and affects the textural properties of meat. The heat transfer method influences the textural and physicochemical properties and its nutritional value, which plays an important role in consumer preference. Various conventional cooking techniques such as boiling, oven-roasting and grilling are commonly used to cook meat and meat products (Campo et al., 2013; Kerth et al., 2022).

Boiling is a conventional cooking technique used to tenderize meat and enhance its flavor. Moreover, boiling can effectively inhibit pathogenic microorganisms. However, cooking meat using the boiling technique may cause negative effects such as fat and nutrient losses, discoloration, and toughening due to overboiling. For this reason, studies on innovative food processing techniques such as microwave or ultrasound-assisted cooking have gained importance to minimize the adverse changes caused by boiling on the quality characteristics of meat (Suleman et al., 2020).

Ultrasound is an innovative and environmentally friendly technique applied in meat processing for various purposes, such as cooking, extraction, emulsification, homogenization, tenderization, and preservation (Ashar et al., 2022). Ultrasound has the capability to reduce processing time and ensure food safety without compromising the quality of meat products (Zou et al., 2018). The commonly used ultrasound frequency, which can impact the physicochemical, biological and structural characteristics of meat, is 16-100 kHz with an intensity range of 10-1000 Wcm<sup>-2</sup> (Ashar et al., 2022). Ultrasound are typically applied directly to the food or the cooking medium (such as water or oil) using specialized equipment. When ultrasound is implemented in a liquid medium, it generates cavitation bubbles, which are tiny gas-filled voids that form and collapse rapidly under the influence of ultrasound waves. This cavitation phenomenon produces intense localized heating, pressure changes, and microstreaming within the liquid medium. These phenomena are the causes of the alterations in properties, microstructures and chemical reactions of meat (Zhao et al., 2024). In ultrasound-assisted cooking, ultrasound waves can accelerate heat transfer within the meat, leading to shorter cooking times compared to conventional cooking techniques. Rapid and uniform heating with ultrasound-assisted cooking helps ensure that the meat reaches the required temperature for microbial inactivation, reducing the risk of foodborne illnesses and extending the shelf life of cooked meat products. Furthermore, ultrasound waves can tenderize meat by disrupting muscle fibers and breaking down connective tissue, resulting in more tender and juicy meats (Firouz et al., 2022). Recently, ultrasound technology has been effectively applied in numerous studies aimed at enhancing the quality of various meat products such as spiced beef (Zou et al., 2018), fermented sausages (de Lima Alves et al., 2020), mortadella (Cichoski et al., 2021) and meatballs (Zhao et al., 2024). However, there is no study comparing conventional boiling and ultrasound-assisted fast and slow boiling on the quality characteristics of cooked beef. Therefore, this research aimed to evaluate the effects of boiling, ultrasound-assisted fast and slow boiling, and different endpoint core temperatures on the physicochemical and microbiological properties, and the oxidative stability of beef.

## Materials and Methods

### Materials

The fresh *M. semimembranosus* muscles (24 h post-mortem) obtained from 1.5-2 years old cattle carcasses were used as the meat material. To create controlled conditions among replications, the post-mortem age of beef did not exceed 5 days after receipt. The pH of all muscles was measured using a portable digital pH meter (HI 9024, Hanna Instruments, Germany). The pH range of all muscles was between 5.4 and 5.6.

### Sample preparation

Before experiments, the cattle muscles were trimmed to remove all visible fat and connective tissue. They were then cut into pieces (20 mm × 40 mm × 40 mm) in the direction of muscle fibers. The sliced beef pieces were randomly separated into nine groups (Table 1) for the different cooking methods (Boiling; B, ultrasound-assisted slow boiling; US, and ultrasound-assisted fast boiling; UF) and endpoint core temperatures (68°C, 74°C, and 80°C).

Table 1. Experimental groups and treatment conditions.

Cooking method (CM)	ECT
Conventional boiling; B	68°C
	74°C
	80°C
Ultrasound-assisted slow boiling; US	68°C
	74°C
	80°C
Ultrasound-assisted fast boiling; UF	68°C
	74°C
	80°C

ECT: Endpoint core temperature

### Cooking procedures

A total of thirty beef pieces per replication in each treatment group were randomly divided into five equal portions (approximately 250 g each) for each storage day. Each portion (6 beef pieces per treatment) were put into polyamide/polyethylene (PA/PE; 80 µm) bags prior to cooking. The cooking process was carried out without vacuum application in PA/PE bags. For boiling treatment, PA/PE bags containing raw beef pieces were placed in a water bath (Nüvebath NB20, Türkiye) and cooked at different endpoint core temperatures (68°C, 74°C, and 80°C). The initial water bath temperature was 60°C. Water bath temperature was adjusted to 80°C after placing the PA/PE bags. The endpoint core temperature was monitored using a thermocouple (TK100S, Kimo Instruments, France) inserted into the geometric center of the beef pieces. An ultrasonic water bath (Model Sonorex RK103 H, Bandelin Ultrasonic Electronics, Germany) with a fixed 35 kHz frequency and 140 W intensity was used for the ultrasound treatment (Firouz et al., 2022; Nehring et al., 2023). The ultrasonic water bath was initially set to the temperatures of 60°C (US) for slow boiling and 80°C (UF) for fast boiling. For slow boiling, the ultrasonic water bath temperature was adjusted to 80°C after placing the PA/PE bags. The raw beef pieces were cooked with ultrasound-assisted until they reached the target endpoint core temperatures (68°C, 74°C, and 80°C). After that, the beef pieces were naturally cooled at sterile ambient conditions. Cooled beef pieces were vacuum-packaged using a RAMON VP280 vacuum machine (Barcelona, Spain) at a vacuum pressure of -0.85 bar in PA/PE bags (O<sub>2</sub> permeability rate: 10 cm<sup>3</sup>/m<sup>2</sup>/24 h/1 atm) and stored in a refrigerator (4°C) for 40 days. All analyses were performed two times for each replication. Water activity (a<sub>w</sub>), color, ORP, pH, LPO and TBARS measurements were conducted in certain intervals (0, 10, 20, 30, and 40 d) during storage. Microbiological analyses were implemented on days 0, 20, and 40 throughout the storage. Furthermore, cooking loss values were calculated once for every replication on production day.

**Determination of cooking loss, pH, a<sub>w</sub>, color and ORP**

The weights of beef samples were recorded before and after cooking process. The cooked beef samples were cooled at ambient temperature before being weighed. The cooking loss (CL, equation 1) was calculated as follows (López-Vargas et al., 2014);

$$CL (\%) = \frac{(WUB)-(WCB)}{(WUB)} \times 100 \quad (1)$$

WUB: weight of uncooked beef

WCB: weight of cooked beef

The pH measurement of beef samples was implemented as mentioned by Kılıç et al. (2016). A spear electrode connected to a portable pH meter (HI 9024, Hanna Instruments, Germany) was used to measure the pH. The a<sub>w</sub> values of the cooked beef samples were measured at 25°C using an a<sub>w</sub> device (Novasina LabSwift-aw, Lachen, Switzerland) according to the procedure explained by Tenderis et al. (2021).

Color measurements of cooked beef samples were carried out using a Minolta CR-200 colorimeter (Minolta Corp., Ramsey, NJ, U.S.A.) following the method described by Şimşek and Kılıç (2020). The cooked beef pieces were removed after opening the vacuum-sealed package and allowed to bloom for at least 30 min at ambient temperature before color measurement. Before measuring color values, the colorimeter was calibrated with a white reference plate (D65, L\*=97.79, a\*=-0.11, b\*=2.69). CIE L\*, a\*, and b\* values were obtained by measuring random locations on both the inner and outer surfaces of cooked beef samples at certain intervals throughout the storage period. Chroma (C\*<sub>ab</sub>; equation 2), hue angle (h<sub>ab</sub>; equation 3), and browning index (BI; equation 4) values were computed using CIE L\*, a\* and b\* values based on the following formulas (Uysal et al., 2022);

$$C^*_{ab} = (a^2 + b^2)^{1/2} \quad (2)$$

$$h_{ab} = \tan^{-1} \left( \frac{b^*}{a^*} \right) \times \frac{180^\circ}{\pi} \text{ and} \quad (3)$$

$$BI = \frac{[100 \times (X - 0.31)]}{0.17}; X = \frac{(a + 1.75xL)}{(5.645xL + a - 3.012xb)} \quad (4)$$

A pH meter (WTW pH 3110, Germany) equipped with a redox electrode was used to measure ORP values in cooked beef samples (Tenderis et al., 2021). An ORP electrode was firmly placed into the center of the cooked beef. The smallest possible hole was made by a cutter before placing the electrode to minimize the effect of air. ORP values (mV) were recorded precisely 2 min after the electrode insertion into a sample.

**Determination of TBARS and LPO values**

TBARS of cooked beef samples was evaluated using the extraction procedure as outlined by Kılıç et al. (2014). Beef samples (2 g) were homogenized (15 s, 13500 rpm) in 12 mL of TCA extraction solution (0.1% propylgallate (PG), 0.1% ethylenediaminetetraacetic acid, disodium salt (EDTA), and 7.5% trichloroacetic acid (TCA)). The

homogenates were filtered using filter paper (Whatman no:1). The filtrate (1.0 mL) was mixed with thiobarbituric acid (1.0 mL, 0.02 M) and incubated at 100°C for 40 min. The mixture was cooled (25°C) and centrifuged (10 min, 4,200 rpm, 4°C). The absorbance of the supernatant was determined at 532 nm using a spectrophotometer (T80 UV/VIS, PG Instruments, England). The TBARS results were expressed as µmol MDA/kg of meat. A calibration curve was prepared from 1,1,3,3-tetraethoxypropane (TEP).

The procedure described by Kılıç et al. (2014) was performed to determine the LPO levels. According to this procedure, cooked beef sample (0.5 g) was mixed with 5 mL of chloroform/methanol (1:1) and homogenized for 30 s. NaCl (3.08 mL; 0.5%) solution was added to this mixture and vortexed for 30 s. Then, this mixture was centrifuged at 2000 rpm for 10 min to separate the mixture into two phases. The lower phase (2 mL) was mixed with 1.33 mL of cold chloroform/methanol (1:1), and then vortexed. Ammonium thiocyanate (25 µL; 4.38 M) and iron (II) chloride (25 µL; 18 mM) were added to the mixture to determine lipid hydroperoxides. The mixture was kept at ambient temperature for 20 min, and then the absorbance was measured at 500 nm. A calibration curve was formed by using cumene hydroperoxide. LPO results were expressed as µmol LPO/kg of meat.

**Microbiological analyses**

The microbiological analyses were implemented using the procedures stated by Uysal et al. (2022). Plate Count Agar (PCA, Merck, Germany), Potato Dextrose Agar (PDA, Merck, Germany) and Eosin Methylene-blue Lactose Sucrose Agar (EMB, Merck, Germany) were used as selective agar mediums for total mesophilic aerobic bacteria (TMAB), yeast-mold and total coliform counts, respectively. The plates for TMAB, total coliforms and yeast-mold were counted following an incubation at 30°C for 24-48 h, 37°C for 24-48 h and 25°C for 72-120 h, respectively. The microbiological results were expressed as log cfu/g of meat.

**Statistical analysis**

The experiments were designed as a completely randomized block design as two replications. All analyses were repeated in duplicate. The statistical model to evaluate the collected data of ORP, pH, a<sub>w</sub>, color, TBARS, and LPO was implemented using a 3 × 3 × 5 factorial design, which included three factors: cooking method, endpoint core temperature, and storage time. To evaluate the data from microbiological analyses, 3 × 3 × 3 factorial design was applied. For cooking loss determination, the data were analyzed with a 3×3 factorial design. All collected data were analyzed using analysis of variance (ANOVA) with the generalized linear mixed model (GLMM) of Minitab 19.2.0 (Minitab Statistical Software, USA). The main effects used in the fixed model were cooking methods (B, US and UF), endpoint core temperatures (68°C, 74°C and 80°C) and storage times (0, 10, 20, 30 and 40 days). The replicates were assigned as a random effect. All main effects and responsible interactions were evaluated. Non-significant interactions were not included in the model. For significant interactions (p<0.05), distinctions between means were assessed by Tukey's Multiple Comparison Test. The results are presented as mean and SEM (standard error of the mean).

## Results and Discussions

### Cooking Loss

The cooking method (CM), endpoint core temperature (ECT), and their interaction (CMxECT) had an impact ( $p < 0.05$ ) on the cooking loss (Figure 1). Cooking loss is primarily influenced by moisture evaporation and protein denaturation during thermal processing. Studies have consistently shown that higher cooking temperatures cause greater cooking loss due to increased protein shrinkage and moisture expulsion (Tornberg, 2005; Pang et al., 2021). Moreover, Klinhom et al. (2017) reported that cooking loss depends on mass transfer during thermal processing. Therefore, different cooking methods result in varying losses due to differences in heat transfer rates and exposure times. In the present research, CM x ECT interaction demonstrated that cooking loss was higher in UF compared with both US and B at 68°C ECT, whereas the methods of US or B resulted in a higher cooking loss compared to UF at 74°C ECT ( $p < 0.05$ ). At 68°C ECT, UF exhibited a greater cooking loss than B or US, possibly due to the higher initial temperature applied for fast cooking. Similarly, Aaslyng et al. (2003) observed that at 60°C ECT, the fast-heating rate (190°C oven temperature) resulted in higher cooking loss compared to a slower heating rate (90°C oven temperature). On the other hand, at 74°C ECT, UF displayed a lower cooking loss ( $p < 0.05$ ). This situation may be due to the shorter cooking time associated with ultrasound-assisted fast cooking. Another possible reason is that the cavitation effect produced by ultrasound can change the structure of muscle proteins by disrupting the myofibrils. This process can create small spaces with uniform and ordered network structures among myofibrillar proteins, allowing them to retain more moisture (Aaslyng et al., 2003; Zhao et al., 2024). Likewise, Zhao et al. (2024) demonstrated that ultrasound-assisted cooking improved the cooking yield of pork meatballs. In the present research, at 80°C ECT, there was no difference in cooking loss among cooking methods. Moreover, the cooking loss was significantly increased ( $p < 0.05$ ) with the increasing ECT in all cooking methods. The cooking losses were comparable among methods at 80°C ECT, likely because the protein denaturation and structural changes plateau at high temperatures, limiting further differences

among methods. Aaslyng et al. (2003) observed that the differences in cooking loss became smaller as the endpoint internal temperature increased, and at 80°C, the cooking procedure no longer had an effect. These results align with Tornberg (2005), who noted that protein denaturation begins at 40°C and accelerates with increasing temperature, peaking around 90°C. Similarly, numerous researchers have reported an increase in cooking loss of various meat types with increased endpoint core temperatures (García-Segovia et al., 2007; Smith et al., 2011; Cauble et al., 2021). Furthermore, Adhikari et al. (2004) noted that cooking losses increased linearly with higher time and temperature.

### pH, $a_w$ , ORP and color

Study results (Table 2) indicated that pH values were influenced ( $p < 0.01$ ) by ECT and storage time (ST). In contrast, CM had no significant impact on pH values of beef. Likewise, previous studies conducted on pork meatballs and broiler meat revealed that there were no significant differences in pH values among ultrasound-assisted cooking and water-bath cooking treatments (Ashar et al., 2022; Zhao et al., 2024). According to the present study results, pH values significantly increased with the increasing ECT ( $p < 0.01$ ). Similarly, Huang et al. (2011) observed that pH values in pork consistently increased as internal temperatures increased. Researchers explained that pH changes in meat during heating were likely due to the dynamic balance of acid-base groups at the surface of sarcoplasmic proteins, which degrade as the temperature increases. In addition, Khan et al. (2019) revealed that cooking causes an increase in pH due to protein degradation, resulting from the divergence of bonds involving hydroxyl, sulfhydryl, and imidazole groups. In the present study, pH values of cooked meats gradually decreased during storage ( $p < 0.01$ ). Likewise, Yingyud et al. (2006) observed a gradual decrease in pH values of vacuum-packed grilled pork during storage. The primary cause of pH reduction in vacuum-packed meat products is the growth of lactic acid bacteria, which leads to the production of lactic acid (Fernández-López et al., 2008).

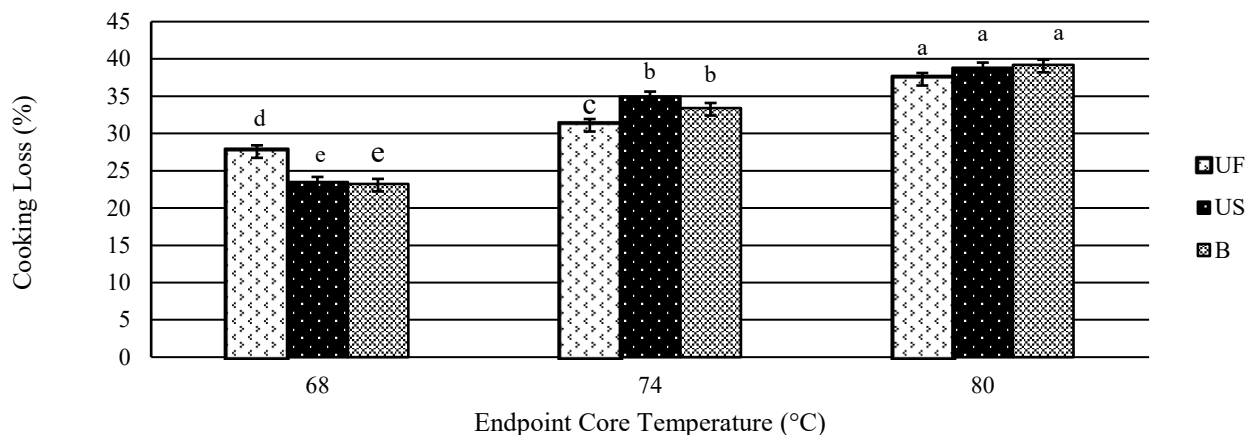


Figure 1. Influences of cooking methods and endpoint core temperatures on cooking loss values of beef. The results were expressed as mean values with standard error bars. <sup>a-c</sup>Means with different superscript letters in the figure indicate statistically significant differences at ( $p < 0.05$ ).

Table 2. pH,  $a_w$  and ORP values of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	pH	ORP	$a_w$
Cooking Method (CM, n=60)			
B	5.58 <sup>a</sup>	-66.88 <sup>b</sup>	0.940 <sup>a</sup>
US	5.59 <sup>a</sup>	-62.63 <sup>a</sup>	0.937 <sup>b</sup>
UF	5.58 <sup>a</sup>	-77.68 <sup>c</sup>	0.938 <sup>ab</sup>
SEM	0.02	1.62	0.001
Significance	NS	**	*
Endpoint Core Temperature (°C; ECT, n=60)			
68	5.52 <sup>c</sup>	-87.89 <sup>c</sup>	0.940 <sup>a</sup>
74	5.59 <sup>b</sup>	-69.19 <sup>b</sup>	0.939 <sup>ab</sup>
80	5.65 <sup>a</sup>	-50.11 <sup>a</sup>	0.937 <sup>b</sup>
SEM	0.02	1.62	0.001
Significance	**	**	*
Storage Time (Day; ST, n=36)			
0	5.66 <sup>a</sup>	-106.25 <sup>c</sup>	0.942 <sup>a</sup>
10	5.63 <sup>b</sup>	-77.29 <sup>d</sup>	0.940 <sup>ab</sup>
20	5.61 <sup>b</sup>	-62.98 <sup>c</sup>	0.936 <sup>c</sup>
30	5.52 <sup>c</sup>	-56.45 <sup>b</sup>	0.938 <sup>bc</sup>
40	5.51 <sup>c</sup>	-42.35 <sup>a</sup>	0.936 <sup>c</sup>
SEM	0.02	1.67	0.001
Significance	**	**	**
CM×ECT	**	**	*
CM×ST	NS	**	*
ECT×ST	**	**	NS
CM×ECT×ST	NS	**	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, SEM: Standard error of the mean, NS: Not significant, <sup>a-c</sup>Means with different superscript letters in the same column indicate statistically significant differences at (\*\* $p < 0.01$ ) and (\* $p < 0.05$ ).

The CM, ECT and ST significantly affected the ORP values of cooked meats ( $p < 0.01$ ). ORP measurements (Table 2) revealed that the lowest ORP levels were determined in UF, whereas US had the highest ORP levels ( $p < 0.01$ ). In addition, increasing ECT caused an increase in ORP levels of cooked beef pieces ( $p < 0.01$ ). Regardless of CM and ECT, ORP levels of cooked beef pieces gradually increased during storage ( $p < 0.01$ ). ORP is a key indicator for tracking chemical reactions and biological processes that lead to oxidation. ORP values indicate the relative tendency of a system to either gain or lose electrons (Latoch & Stasiak, 2015). Ignatova et al. (2010) reported that ORP is affected by environmental factors such as dissolved oxygen, temperature, and pH. Claus and Jeong (2017) indicated that lower ORP values are associated with higher pH. On the other hand, researchers noted that the endpoint internal cooking temperature did not affect ORP values in cooked ground turkey breasts (Claus & Jeong, 2017). Zhang et al. (2022) reported that the ORP values increased steadily in heat-processed beef with increasing storage time. Moreover, Latoch and Stasiak (2015) noted that alterations in ORP levels could result from increased lipid oxidation during storage.

In the present study,  $a_w$  values (Table 2) were influenced by CM, ECT ( $p < 0.05$ ) and ST ( $p < 0.01$ ). Comparisons of CM revealed that US had a lower  $a_w$  value compared to B ( $p < 0.05$ ). There were no  $a_w$  value differences in B versus UF; and US versus UF. Bao et al. (2022) stated that the use of ultrasound resulted in reduced  $a_w$  values. Researchers have attributed this phenomenon to ultrasound generating air turbulence at the air-product interface, which enhances moisture removal from the surface. In addition, Leães et al. (2020) observed that long-

term ultrasound application (20 min) was more effective in reducing  $a_w$  compared to short-term application (10 min). Researchers generally did not find a significant difference in  $a_w$  between the short-term ultrasound application and the untreated group. Regarding ECT application, beef cooked at 80°C ECT presented a lower  $a_w$  value than that cooked at 68°C ECT ( $p < 0.05$ ). Furthermore, although  $a_w$  values gradually decreased during the first 20 days of storage ( $p < 0.01$ ),  $a_w$  values were quite stable during the rest of the storage period. Pandey et al. (2014) noted that shami kebab samples processed at high temperatures exhibited lower  $a_w$ , which was attributed to the effective removal of moisture.

Table 3 and Table 4 illustrated the effects of different cooking methods and endpoint core temperatures on the color properties of cooked beef. Color measurements demonstrated that  $L^*$ ,  $b^*$ ,  $h_{ab}$  and  $BI$  values ( $p < 0.05$ ) obtained from cooked beef's inner surface and  $a^*$ ,  $b^*$ ,  $C^*_{ab}$ ,  $h_{ab}$  and  $BI$  values ( $p < 0.01$ ) obtained from cooked beef's outer surface were affected by CM. Moreover, all color parameters obtained from both the inner and outer surfaces of cooked beef pieces were affected by ECT and ST ( $p < 0.05$ ). On the other hand, ECT did not affect  $BI$  values of cooked beef's outer surface.

As far as cooking methods are considered, color measurements revealed that the lower  $L^*$  value and the higher  $BI$  and  $b^*$  values obtained from cooked beef's inner surface were determined in UF compared to B ( $p < 0.05$ ). Ultrasonic cavitation causes a decrease in  $L^*$  value, and an increase in  $BI$  value by stimulating the formation of free radicals and leading to enzymatic browning (Zhao et al., 2024). In addition, the higher  $a^*$ ,  $b^*$ ,  $C^*_{ab}$  and  $BI$  values obtained from cooked beef's outer surface were determined in UF compared to B ( $p < 0.01$ ).



Table 3. Colour values (inner surface) of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	Inner Surface					
	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i> * <sub>ab</sub>	<i>h</i> <sub>ab</sub>	<i>BI</i>
Cooking Method (CM, n=60)						
B	57.98 <sup>a</sup>	15.18 <sup>a</sup>	2.87 <sup>c</sup>	15.59 <sup>a</sup>	13.03 <sup>b</sup>	23.19 <sup>b</sup>
US	57.31 <sup>ab</sup>	15.02 <sup>a</sup>	3.17 <sup>b</sup>	15.52 <sup>a</sup>	14.33 <sup>a</sup>	23.84 <sup>ab</sup>
UF	57.00 <sup>b</sup>	14.86 <sup>a</sup>	3.47 <sup>a</sup>	15.32 <sup>a</sup>	13.95 <sup>ab</sup>	24.48 <sup>a</sup>
SEM	0.39	0.22	0.07	0.23	0.34	0.34
Significance	*	NS	**	NS	*	*
Endpoint Core Temperature (°C; ECT, n=60)						
68	58.15 <sup>a</sup>	20.44 <sup>a</sup>	1.83 <sup>c</sup>	20.54 <sup>a</sup>	5.23 <sup>c</sup>	27.28 <sup>a</sup>
74	57.05 <sup>b</sup>	13.99 <sup>b</sup>	3.40 <sup>b</sup>	14.42 <sup>b</sup>	13.85 <sup>b</sup>	23.34 <sup>b</sup>
80	57.09 <sup>b</sup>	10.62 <sup>c</sup>	4.27 <sup>a</sup>	11.47 <sup>c</sup>	22.23 <sup>a</sup>	20.90 <sup>c</sup>
SEM	0.39	0.22	0.07	0.23	0.34	0.34
Significance	*	**	**	**	**	**
Storage Time (Day; ST, n=36)						
0	55.51 <sup>c</sup>	16.85 <sup>a</sup>	3.89 <sup>a</sup>	17.46 <sup>a</sup>	15.02 <sup>a</sup>	28.24 <sup>a</sup>
10	55.54 <sup>c</sup>	15.81 <sup>b</sup>	3.44 <sup>b</sup>	16.32 <sup>b</sup>	14.01 <sup>ab</sup>	26.07 <sup>b</sup>
20	57.31 <sup>b</sup>	15.06 <sup>b</sup>	3.07 <sup>c</sup>	15.49 <sup>bc</sup>	13.28 <sup>b</sup>	23.64 <sup>c</sup>
30	58.87 <sup>a</sup>	14.24 <sup>c</sup>	2.86 <sup>cd</sup>	14.63 <sup>c</sup>	13.07 <sup>b</sup>	21.69 <sup>d</sup>
40	59.92 <sup>a</sup>	13.12 <sup>d</sup>	2.58 <sup>d</sup>	13.49 <sup>d</sup>	13.46 <sup>ab</sup>	19.55 <sup>e</sup>
SEM	0.46	0.28	0.08	0.29	0.44	0.43
Significance	**	**	**	**	*	**
CM×ECT	*	**	**	**	**	*
CM×ST	NS	*	*	*	NS	NS
ECT×ST	NS	NS	*	NS	NS	NS
CM×ECT×ST	NS	NS	**	NS	*	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, *C*\*<sub>ab</sub>: Chroma, *h*<sub>ab</sub>: hue angle, *BI*: browning index, SEM: Standard error of the mean, NS: Not significant, <sup>a-c</sup>Means with different superscript letters in the same column indicate statistically significant differences at (\*\*p<0.01) and (\*p<0.05).

Table 4. Colour values (outer surface) of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	Outer Surface					
	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i> * <sub>ab</sub>	<i>h</i> <sub>ab</sub>	<i>BI</i>
Cooking Method (CM, n=60)						
B	44.14 <sup>a</sup>	7.76 <sup>b</sup>	5.89 <sup>c</sup>	9.88 <sup>b</sup>	39.09 <sup>b</sup>	27.11 <sup>b</sup>
US	43.72 <sup>a</sup>	7.29 <sup>c</sup>	6.36 <sup>b</sup>	9.81 <sup>b</sup>	42.00 <sup>a</sup>	28.27 <sup>b</sup>
UF	44.42 <sup>a</sup>	8.74 <sup>a</sup>	7.03 <sup>a</sup>	11.34 <sup>a</sup>	39.20 <sup>b</sup>	31.98 <sup>a</sup>
SEM	0.56	0.18	0.11	0.15	0.87	0.64
Significance	NS	**	**	**	**	**
Endpoint Core Temperature (°C; ECT, n=60)						
68	45.90 <sup>a</sup>	9.99 <sup>a</sup>	5.84 <sup>b</sup>	11.63 <sup>a</sup>	30.65 <sup>c</sup>	29.40 <sup>a</sup>
74	43.46 <sup>b</sup>	7.61 <sup>b</sup>	6.66 <sup>a</sup>	10.18 <sup>b</sup>	41.44 <sup>b</sup>	29.86 <sup>a</sup>
80	42.92 <sup>b</sup>	6.18 <sup>c</sup>	6.78 <sup>a</sup>	9.22 <sup>c</sup>	48.19 <sup>a</sup>	28.10 <sup>a</sup>
SEM	0.56	0.18	0.11	0.15	0.87	0.64
Significance	**	**	**	**	**	NS
Storage Time (Day; ST, n=36)						
0	42.85 <sup>b</sup>	9.90 <sup>a</sup>	7.25 <sup>ab</sup>	12.41 <sup>a</sup>	36.46 <sup>c</sup>	35.79 <sup>a</sup>
10	41.18 <sup>b</sup>	8.51 <sup>b</sup>	7.42 <sup>a</sup>	11.41 <sup>b</sup>	41.76 <sup>ab</sup>	34.86 <sup>a</sup>
20	42.46 <sup>b</sup>	7.81 <sup>bc</sup>	6.83 <sup>b</sup>	10.50 <sup>c</sup>	42.21 <sup>a</sup>	30.82 <sup>b</sup>
30	46.75 <sup>a</sup>	7.15 <sup>c</sup>	5.69 <sup>c</sup>	9.27 <sup>d</sup>	39.93 <sup>b</sup>	23.73 <sup>c</sup>
40	47.21 <sup>a</sup>	6.27 <sup>d</sup>	4.95 <sup>d</sup>	8.13 <sup>c</sup>	40.12 <sup>b</sup>	20.39 <sup>d</sup>
SEM	0.63	0.21	0.15	0.20	0.98	0.83
Significance	**	**	**	**	**	**
CM×ECT	*	NS	**	**	*	**
CM×ST	*	**	*	*	**	NS
ECT×ST	NS	*	NS	NS	**	NS
CM×ECT×ST	NS	**	*	**	NS	**

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, *C*\*<sub>ab</sub>: Chroma, *h*<sub>ab</sub>: hue angle, *BI*: browning index, SEM: Standard error of the mean, NS: Not significant, <sup>a-c</sup>Means with different superscript letters in the same column indicate statistically significant differences at (\*\*p<0.01) and (\*p<0.05).

Table 5. LPO ( $\mu\text{mol LPO/kg}$ ) and TBARS ( $\mu\text{mol MDA/kg}$ ) values of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	LPO	TBARS
Cooking Method (CM, n=60)		
B	55.81 <sup>a</sup>	4.31 <sup>a</sup>
US	53.22 <sup>b</sup>	4.11 <sup>b</sup>
UF	48.99 <sup>c</sup>	3.65 <sup>c</sup>
SEM	0.76	0.05
Significance	**	**
Endpoint Core Temperature ( $^{\circ}\text{C}$ ; ECT, n=60)		
68	48.90 <sup>b</sup>	3.80 <sup>b</sup>
74	55.02 <sup>a</sup>	4.16 <sup>a</sup>
80	54.11 <sup>a</sup>	4.12 <sup>a</sup>
SEM	0.76	0.05
Significance	**	**
Storage Time (Day; ST, n=36)		
0	27.46 <sup>c</sup>	2.03 <sup>c</sup>
10	41.23 <sup>d</sup>	3.08 <sup>d</sup>
20	52.25 <sup>c</sup>	3.86 <sup>c</sup>
30	64.79 <sup>b</sup>	4.95 <sup>b</sup>
40	77.65 <sup>a</sup>	6.22 <sup>a</sup>
SEM	0.83	0.07
Significance	**	**
CMxECT	NS	NS
CMxST	**	**
ECTxST	*	NS
CMxECTxST	NS	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, LPO: Lipid hydroperoxide, TBARS: Thiobarbituric acid reactive substances, SEM: Standard error of the mean, NS: Not significant, <sup>a-c</sup>Means with different superscript letters in the same column indicate statistically significant differences at (\*\* $p < 0.01$ ) and (\* $p < 0.05$ ).

Wang et al. (2019) reported an increase in  $a^*$  and  $b^*$  values in ultrasound-assisted fried meatballs. Furthermore, US had higher  $h_{ab}$  values obtained from both the inner and outer surfaces of cooked beef pieces compared to those cooked with the B method ( $p < 0.05$ ). There were no differences among cooking methods in terms of  $L^*$  values of the outer surface and  $a^*$  and  $C^*_{ab}$  values of the inner surface.

Comparisons of color results concerning different ECT applications indicated that  $a^*$  and  $C^*_{ab}$  values obtained from both the inner and outer surfaces and  $BI$  values of the inner surface significantly decreased with the increasing ECT ( $p < 0.01$ ). Similarly, previous studies revealed that  $a^*$  values of meat products significantly decreased as endpoint core temperature increased (Lien et al., 2002; Sen et al., 2014; Cauble et al., 2021). The reduction in the redness ( $a^*$ ) values of cooked meats is related to the myoglobin denaturation that occurs in fresh meats during cooking (Rincon et al., 2015). On the other hand,  $h_{ab}$  values of both inner and outer surfaces and  $b^*$  values of the inner surface increased with increasing ECT ( $p < 0.01$ ). Likewise, Mancini et al. (2005) and Sen et al. (2014) observed a linear increase in  $h_{ab}$  values of pork and mutton chops with increasing endpoint internal temperatures. Torun et al. (2023) noted that higher cooking temperatures increased  $b^*$  values in beef. Furthermore, beef cooked at  $74^{\circ}\text{C}$  or  $80^{\circ}\text{C}$  ECT had lower  $L^*$  values in both inner and outer surfaces than that cooked at  $68^{\circ}\text{C}$  ECT ( $p < 0.05$ ). Yancey et al. (2011) revealed a slight decline in  $L^*$  values of beef steaks with increasing internal cooked temperatures. Torun et al. (2023) found a similar result for  $L^*$  value changes in beef due to the internal cooked temperatures. There were no  $BI$  value differences in the outer surface among different ECT applications.

Regarding the storage time,  $a^*$ ,  $b^*$ ,  $C^*_{ab}$ , and  $BI$  values obtained in both inner and outer surfaces gradually decreased during the storage period ( $p < 0.01$ ). These results are in line with the reports of Zhang et al. (2021), who found that color values decreased in spiced beef during cold storage. Molins et al. (1987) similarly observed a decline in color values throughout the storage period. Although  $L^*$  values obtained on the outer surface were quite stable during the first 20 days, these values increased on day 30 ( $p < 0.01$ ), and did not change during the rest of the storage. Moreover,  $L^*$  values determined on the inner surface of cooked beef were quite stable during the first 10 days, this value gradually increased on days 20 and 30 ( $p < 0.01$ ), and it did not change during the rest of the storage. In addition,  $h_{ab}$  value determined on the outer surface increased on day 10 ( $p < 0.01$ ), whereas the values of  $h_{ab}$  did not change during the rest of the storage. On the other hand,  $h_{ab}$  values obtained from the inner surface did not generally change during the whole storage period.

#### TBARS and LPO

TBARS and LPO results (Table 5) demonstrated that CM, ECT and ST had an evident influence on TBARS and LPO values of cooked beef pieces ( $p < 0.01$ ). Regarding CM, the lowest TBARS and LPO values were obtained in the samples cooked with UF method, whereas the samples cooked with B method had the highest TBARS and LPO values ( $p < 0.01$ ). Furthermore, TBARS and LPO values obtained in the samples cooked with US method remained between the values obtained in the samples cooked with UF and those cooked with B method ( $p < 0.01$ ). Similarly, Cichoski et al. (2015) reported a reduction in the oxidation

of ultrasound-treated sausages. In addition, Zhang et al. (2021) noted that ultrasound-assisted cooking effectively reduced the degree of lipid oxidation during cold storage, thereby helping to prevent the formation of undesired flavors in spiced beef. da Silva et al. (2020) revealed that the application of ultrasonic-assisted cooking resulted in lower peroxide index, conjugated diene levels, and TBARS levels throughout storage compared to control. Moreover, Ashar et al. (2022) showed that broiler meat cooked at 50°C in an ultrasonic bath had a lower TBARS level compared to broiler meat cooked at 72°C in a water bath and cooked at 60°C, 70°C and 80°C in an ultrasonic bath. Researchers also reported that broiler meat cooked at 72°C in a water bath and 60°C and 70°C in an ultrasonic bath had the same TBARS levels. In contrast, other studies have indicated that ultrasound-assisted cooking increases lipid oxidation levels in meat products (Zou et al., 2018; Zhang et al., 2020). Considering TBARS and LPO values according to ECT, beef cooked at 74°C or 80°C ECT had higher TBARS and LPO values than those cooked at 68°C ECT ( $p < 0.01$ ). On the other hand, there was no difference in terms of TBARS and LPO values between beef cooked at 74°C and 80°C ECT. As far as considering ST, both TBARS and LPO values increased as the storage time increased ( $p < 0.01$ ). Likewise, Spanier and Miller (1996) observed that lipid oxidation increased with higher endpoint cooking temperatures. The cooking temperature, method, and duration influence the production of free radicals, which may result in lipid oxidation in meat (Schwartz et al., 2022). Sen et al. (2014) noted that the endpoint cooking temperatures did not significantly affect lipid oxidation, whereas the storage duration had a significant impact on the degree of lipid oxidation.

### Microbiological properties of cooked beef

Microbiological analysis results (Table 6) showed that TMAB counts were influenced by ECT and ST ( $p < 0.01$ ) but not CM. On the other hand, CM, ECT and ST had no significant impact on yeast-mold counts of cooked beef. Moreover, CM, ST ( $p < 0.01$ ) and ECT ( $p < 0.05$ ) significantly changed the total coliform counts of cooked beef. Following the present study results, there were no TMAB and yeast-mold count differences among different cooking methods. On the other hand, the cooking methods of US or UF had a higher total coliform count in comparison to B method ( $p < 0.01$ ). Additionally, the highest coliform count was detected in the samples cooked with US method. da Silva et al. (2020) reported that the ultrasonic-assisted cooking treatment had similar mesophilic and psychrotrophic bacteria counts compared to control. Previous studies have reported conflicting results regarding the antimicrobial effects of ultrasound application (Piyasena et al., 2003; Cichoski et al., 2015; Piñon et al., 2020). Piñon et al. (2020) observed an increase in the numbers of psychrotrophic, mesophilic, lactic acid bacteria, and *Staphylococcus aureus* in chicken breasts after the ultrasonication process. Sams and Ferial (1991) suggested that the increase in microbial counts was attributed to the release of nutrients from meat undergoing ultrasonication. Conversely, other studies have highlighted a positive effect of ultrasound on microbial reduction (Piyasena et al., 2003; Cichoski et al., 2015). The main theory on how ultrasound affects the viability of microorganisms is based on the phenomenon of cavitation, which can disrupt the system and lead to microbial cell rupture (da Silva et al., 2020).

Table 6. Microbiological analysis results (log cfu/g) of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	TMAB	Yeast and Mold	Total Coliforms
Cooking Method (CM, n=36)			
B	4.85 <sup>a</sup>	< 1	2.61 <sup>c</sup>
US	4.93 <sup>a</sup>	< 1	3.42 <sup>a</sup>
UF	5.00 <sup>a</sup>	< 1	3.00 <sup>b</sup>
SEM	0.49	0.06	0.18
Significance	NS	NS	**
Endpoint Core Temperature (°C; ECT, n=36)			
68	5.27 <sup>a</sup>	< 1	3.25 <sup>a</sup>
74	4.85 <sup>b</sup>	< 1	2.94 <sup>b</sup>
80	4.67 <sup>b</sup>	< 1	2.83 <sup>b</sup>
SEM	0.49	0.06	0.18
Significance	**	NS	*
Storage Time (Day; ST, n=36)			
0	< 1 <sup>c</sup>	< 1	< 1 <sup>c</sup>
20	6.83 <sup>b</sup>	< 1	4.01 <sup>b</sup>
40	7.47 <sup>a</sup>	< 1	4.66 <sup>a</sup>
SEM	0.49	0.06	0.18
Significance	**	NS	**
CMxECT	NS	NS	NS
CMxST	NS	NS	**
ECTxST	NS	NS	NS
CMxECTxST	NS	NS	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, TMAB: Total mesophilic aerobic bacteria, SEM: Standard error of the mean, NS: Not significant, <sup>a-c</sup>Means with different superscript letters in the same column indicate statistically significant differences at (\*\* $p < 0.01$ ) and (\* $p < 0.05$ ).

Considering the microbiological analysis results regarding ECT, beef cooked at 74°C or 80°C ECT had lower TMAB and total coliform counts compared to those cooked at 68°C ECT ( $p < 0.05$ ). Conversely, no significant difference in TMAB and total coliform counts was observed between 74°C and 80°C ECT applications. Regarding ST, TMAB and total coliform counts gradually increased throughout the storage ( $p < 0.01$ ). Yeast-mold count results according to CM, ECT and ST were determined below the detection limits. Sen et al. (2014) stated that initial aerobic plate counts were lower at higher endpoint cooking temperatures. A similar result was demonstrated by Torun et al. (2023) who noted that the lower cooking temperatures resulted in higher microbial counts.

## Conclusion

The study results indicated that ultrasound-assisted cooking positively influenced the oxidative stability of cooked beef. Based on the results of TBARS and LPO assessments, UF demonstrated a greater ability to delay lipid oxidation. The cooking loss values of US and B samples at the endpoint core temperature of 68°C were lower than those of UF samples. On the other hand, B or US at 74°C ECT increased cooking loss values compared to UF. US exhibited lower  $a_w$  values compared to B. Ultrasound-assisted cooking had no significant impact on pH. Regarding the color values of the outer surface, there were no differences in  $L^*$  values among cooking methods. On the other hand, the higher  $a^*$ ,  $b^*$ ,  $C^*_{ab}$  and  $BI$  values were obtained from the outer surfaces of cooked beef were determined in UF compared to B. UF samples exhibited lower  $L^*$  values and higher  $BI$  and  $b^*$  values on the inner surface compared to B. Moreover, US samples displayed higher  $h_{ab}$  values on both the inner and outer surfaces of cooked beef than those of B. No differences in  $a^*$  and  $C^*_{ab}$  values were observed on the inner surfaces of cooked beef among different cooking methods. There were no significant differences in TMAB and yeast-mold counts among the cooking methods, however, coliform counts increased with ultrasound-assisted cooking. pH, CL, ORP, hue angle ( $h_{ab}$ ) and  $b^*$  values increased as the ECT increased, whereas  $a_w$ ,  $a^*$ , chroma ( $C^*_{ab}$ ) and browning index ( $BI$ ; inner) values decreased. In addition, beef cooked at 74°C or 80°C ECT had lower  $L^*$  values, TMAB, and total coliform counts, and higher TBARS and LPO values than those cooked at 68°C ECT. There were no differences in terms of TMAB and total coliform counts, as well as  $L^*$ , TBARS and LPO values between beef pieces cooked at 74°C and 80°C ECT. Therefore, ultrasound-assisted fast boiling (UF) could be an effective processing technology for enhancing the oxidative stability and physicochemical properties of beef.

## Declarations

### Author Contribution Statement

Dilara Aydın: Investigation, Data collection, Formal analysis. Birol Kılıç: Methodology, Conceptualization, Supervision, Review and editing. Azim Şimşek: Methodology, Conceptualization, Data collection, Formal analysis, Writing original draft, Review and editing.

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## Conflict of Interest

The authors do not have any conflict of interest.

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## Gülez Method in Determining Landscape and Recreational Potential: The Case of Gölbaşı Lakes Nature Park (Adıyaman)

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 13.12.2024 Accepted : 30.12.2024</p> <p><b>Keywords:</b> Adıyaman Gölbaşı Lakes Nature Park Gülez Method Recreational Potential Landscape</p>	<p>With the increase in population, rapid and unplanned urbanisation, all other natural areas, especially green areas in the city, have lost their quality or decreased due to construction. For people living in the city, the decrease in these areas and the disappearance of their quality has caused recreational needs to be unmet. This situation has made it possible for nature parks, recreation areas and forest areas in or near the city to become more valuable. The aim of this study is to determine the recreational potential of Gölbaşı Lakes Nature Park within the borders of Gölbaşı district of Adıyaman province according to Gülez method. For this purpose, Gülez method, which is preferred in determining the landscape condition in the forest and determining the recreational potential, was used. n Gülez method, recreational potential is calculated by scoring the landscape value, climate value, accessibility, recreational convenience and negative factors of the recreational area. According to this calculation, the recreational potential of Gölbaşı Lakes Nature Park was evaluated in the 'high' category with a total score of '67'. As a result of the field studies carried out in Gölbaşı Lakes Nature Park, it was determined that some of the elements specified in the method are deficient in quality and the negative factor elements are quite high. The recreational activity potential of Gölbaşı Lakes Nature Park is higher than the determined one. It is predicted that the recreational activity potential of the area can be increased by '19' points by reducing the negative factors in the area, thus the total score of Gölbaşı Lakes Nature Park can reach '86' and its potential can reach the 'very high' class.</p>

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(2): 487-496, 2025

## Peyzaj ve Rekreatiyonel Potansiyelin Belirlenmesinde Gülez Yöntemi: Gölbaşı Gölleri Tabiat Parkı (Adıyaman) Örneği

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 13.12.2024 Kabul : 30.12.2024</p> <p><b>Anahtar Kelimeler:</b> Adıyaman Gölbaşı Gölleri Tabiat Parkı Gülez Yöntemi Rekreatiyonel Potansiyel Peyzaj</p>	<p>Nüfus artışı, hızlı ve plansız kentleşme ile birlikte kent içerisindeki yeşil alanlar başta olmak üzere diğer tüm doğal alanlar yapılaşma nedeniyle niteliğini kaybetmiş veya azalmıştır. Kentte yaşayan insanlar için bu alanların azalması ve niteliğinin ortadan kalkması rekreatif ihtiyaçların karşılanamamasına neden olmuştur. Bu durum kent içinde veya yakın alanlarındaki tabiat parklarının, mesire alanlarının ve ormanlık alanlarının daha kıymetli hale gelmesine imkan tanımıştır. Bu çalışmanın amacını, Adıyaman ili, Gölbaşı ilçesi sınırları içinde kalan Gölbaşı Gölleri Tabiat Parkı'nın Gülez yöntemine göre rekreatiyonel potansiyelinin belirlenmesi oluşturmaktadır. Gülez yönteminde, rekreatiyonel alanın peyzaj değeri, iklim değeri, ulaşılabilirlik durumu, rekreatif kolaylık ve olumsuz etkenlerin puanlandırılmasıyla rekreatiyon potansiyeli hesaplanmaktadır. Bu hesaplama göre, Gölbaşı Gölleri Tabiat Parkı'nın rekreatiyonel potansiyeli toplamda "67" puan olarak "yüksek" kategoride değerlendirilmiştir. Gölbaşı Gölleri Tabiat Parkı'nda gerçekleştirilen arazi çalışmaları sonucunda, yöntemde belirtilen bazı öğelerinin nitelik olarak eksik olduğu ve olumsuz etken öğelerinin oldukça fazla olduğu tespit edilmiştir. Gölbaşı Gölleri Tabiat Parkı'nın rekreatiyonel faaliyet potansiyeli tespit edilenden daha fazladır. Sahadaki olumsuz etkenlerin azaltılmasıyla alanın rekreatiyonel faaliyet potansiyelinin "19" puan daha artırılabilceği, bu sayede Gölbaşı Gölleri Tabiat Parkı'nın toplam puanın "86" olarak potansiyelinin "çok yüksek" sınıfına ulaşabileceği öngörülmektedir.</p>

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## Giriş

Nüfus artışı, kentleşme, sanayileşme ve ulaşım sistemlerindeki gelişmeler, plansız ve çarpık şehirleşmeyi ortaya çıkarmıştır (Akten, 2003). Plansız ve çarpık şehirleşme sonucunda da betonlaşma artarak yeşil alanlar ve peyzaj alanları azalmıştır. Çarpık şehirleşme, peyzaj alanlarının azalması ve çevre kirliliği şehirlerde doğal alanların bozulmasına ve azalmasına yol açmış, insanların rekreasyonel alanlara olan ihtiyacını artırmıştır (Sü Eröz ve Aslan, 2017). Eski çağlardan beri var olan rekreasyon faaliyetleri, şehirleşmenin yoğun ve yıkıcı etkilerinin arttığı 21. Yüzyılda daha da önem kazanmıştır (Büyük ve Kömürcü Sarıbaş, 2021). Rekreasyon kavramı yenilenme, yeniden yapılanma anlamına gelen Latince recreare kelimesinden ortaya çıkarılmıştır (Aylan ve Yetiş, 2021:1281; Yılmaz ve ark., 2009:55). Ortaya çıkan bu kavram, insanların boş vakitlerini eğlenme ve dinlenme amacı ile gönüllü olarak katıldıkları faaliyetlerinde değerlendirmelerini kapsamaktadır (Özçalık ve Kumru, 2019). Açık alan rekreasyon faaliyetleri ve kapalı alan rekreasyon faaliyetleri olmak üzere iki türde de yapılabilmektedir (Göker ve Ünlüönen, 2019). Doğa temelli veya açık alanlar yapılan tüm sportif faaliyetler açık alan rekreasyonel faaliyeti olarak değerlendirilmektedir. Kapalı alan rekreasyonel faaliyetleri ise kapalı ortamlarda yapılan tüm rekreasyonel faaliyetleri kapsamaktadır.

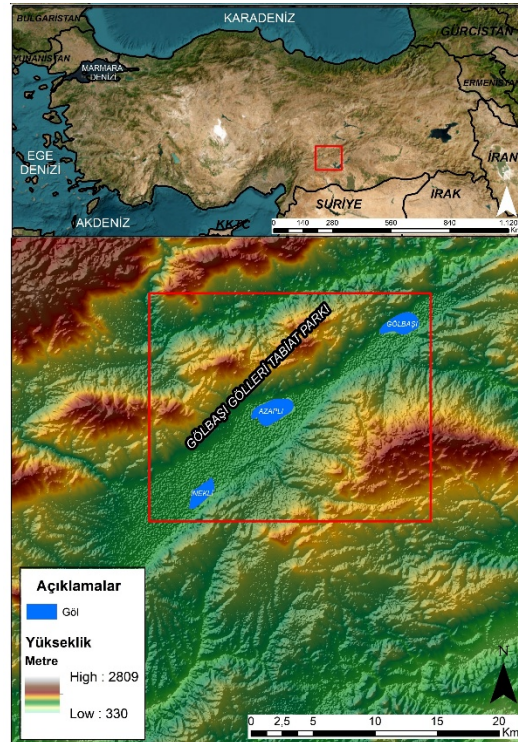
Türkiye’de birçok doğal ve kültürel mekân çeşitli kurum ve kuruluşlar tarafından koruma altına alınmıştır (Bahat, 2011). Tabiat parkları, milli parklar, tabiat anıtları hem doğal alan olmaları hem de insanların rekreasyon ihtiyaçlarını karşılanması için koruma altına alınmıştır (Orman Kanunu, 1956). Ülkemizde korunan alanlar olarak atfedilen; 48 Milli Park, 266 Tabiat Parkı, 31 Tabiat Koruma Alanı, 85 Yaban Hayatı Geliştirme Sahası, 110 Tabiat Anıtı, 47 Mahalli Öne Haiz Sulak Alan, 59 Ulusal Öne Haiz Sulak Alan ve 14 Ramsar Alanı bulunmaktadır. Güneydoğu Anadolu Bölgesi illerinde toplamda 15 Tabiat Parkı bulunmaktadır. Bunların sayısı; Gaziantep’te 5, Adıyaman’da 2, Diyarbakır’da 2, Siirt’te 2, Mardin’de 1, Batman’da 1, Şanlıurfa’da 1, Kilis’te 1 iken Şırnak’ta ise tabiat parkı bulunmamaktadır (Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü, 2023). Tabiat parkları, bitki örtüsü ve yaban hayatı özelliklerine sahip, manzara bütünlüğü olan, halkın dinlenme-eğlenme gibi rekreasyonel faaliyetlerini gerçekleştirebileceği uygun tabiat parçalarıdır (Tarım ve Orman Bakanlığı, 2023). Adıyaman ili Gölbaşı ilçesi sınırları içinde bulunan “Gölbaşı Gölleri Tabiat Parkı” da Adıyaman ilinin iki tabiat parkından biridir. “Gölbaşı Gölleri Tabiat Parkı” birçok beşerî ve doğal unsuru barındırmasının yanı sıra bölgede önemli sayılabilecek Gölbaşı, İneklı ve Azaplı göllerini de kapsamaktadır. “Gölbaşı Gölleri Tabiat Parkı” 2008 yılında tabiat parkı olarak ilan edilmiştir. Bölge tabiat parklarının sayısının az olduğu alanlar arasında yer alması, bölgede önemli doğal ve beşerî çekiciliklerinin bulunması ve bölgenin bu çekiciliklerden dolayı potansiyelinin olması araştırmaya değer bulunmuştur.

## Çalışma Sahasının Yeri ve Sınırları

Çalışma alanını, Güneydoğu Anadolu Bölgesi’nin Orta Fırat Bölümü’nde yer alan Adıyaman ilinin “Gölbaşı Göller Tabiat Parkı” oluşturmaktadır. Tabiat parkı

Güneydoğu Anadolu, Doğu Anadolu ve Akdeniz bölgelerinin birleşim noktasında yer almaktadır. Park 37° 48’ 25” - 37° 41’ 39” Kuzey enlemleri ile 37° 39’ 42” - 37° 29’ 25” Doğu boylamları arasında yer almaktadır. Gölbaşı ilçesinde bulunan tabiat parkı Adıyaman il merkezine yaklaşık olarak 65 km uzaklıktadır. Tabiat parkı 2080 ha. kaplamaktadır. 2008 yılında tabiat parkı ilan edilen “Gölbaşı Gölleri Tabiat Parkı” 3 gölden oluşmaktadır. Bunlar; Gölbaşı, Azaplı ve İneklı (Yeşilova) Gölü’dür. Bu göller kuzeydoğu ve güneybatı yönünde uzanmış ve birbirine bağlıdır. Gölbaşı Gölü’nün deniz seviyesinden yüksekliği 863 m., Azaplı Gölü’nün 840 m ve İneklı (Yeşilova) Gölü’nün ise 820 m.’dir. Gölbaşı Gölleri Tabiat Parkı’nın kuzeyinde Meydan Dağı, doğusunda Körkün Dağı, güneyinde Guz Dağı yer almaktadır. Çalışma sahasında belli başlı tepelere bakıldığında; kuzeyde Adaca tepe, Balçukuru tepe; güneyde deveboynu tepe; doğuda Camuz Alağı tepe, Seske tepe; batıda Değirmen tepe ve Kıracı tepe yer almaktadır (Şekil 1). Tabiat parkında 3 tane orman, 2 tane bataklık ve 2 tane de sucul vejetasyona ait bitki birliği bulunmaktadır (Tel ve Eğilmez, 2015:13).

Çalışma sahasında Akdeniz iklim özellikleri görülürken, yer yer de karasal iklim özellikleri görülmektedir. Sahada ortalama sıcaklık değerleri en düşük -1,2°C ile ocak ayında, en yüksek ise 27°C ile ağustos ayında görülmüştür. Maksimum sıcaklık temmuz ve ağustos aylarında 34 °C olarak ölçülmüşken, minimum sıcaklık ise ocak ayında -5,3°C olarak ölçülmüştür. Sahada yağış durumu bakımından en çok yağış kış mevsiminde (ocak ve kasım) düştüğü görülmektedir. En az yağış ise yaz mevsiminde (temmuz ve ağustos) düşmektedir. Buna paralel olarak yağışlı günler en fazla kış mevsiminde, en az ise yaz mevsimindedir (MGM, 2024), (Şekil 2).



Şekil 1. Çalışma sahasının lokasyonu  
Figure 1. Location of the study area



## Materyal ve Yöntem

Çalışmada kullanılan peyzaj verileri arazi çalışması sonucunda elde edilmiştir. Tarım ve Orman Bakanlığı 3. Bölge Müdürlüğü'ne bağlı olan Gölbaşı Gölleri Tabiat Parkı yetkilileri ile görüşülmüş tabiat parkı içindeki bitki türleri ile ilgili veriler temin edilmiştir. Sahanın uzun yıllara ait iklim verileri sıcaklık, yağış, nem, güneşli günler sayısı ve yağmurlu günler sayısı kullanılmıştır. Alana ilişkin görsel kalitenin değerlendirilmesi amacıyla arazi çalışmaları sırasında fotoğraf çekimleri gerçekleştirilmiştir. Ayrıca TÜİK'ten saha ve yakın çevresinin nüfus verileri alınmıştır. Çalışmada kullanılan haritalar ArcGIS 10. 4 yazılımında görselleştirilmiştir.

Gölbaşı Gölleri Tabiat Parkı'nın rekreasyon potansiyelini belirlemek için, Gülez'in 1990 yılında yapmış olduğu "Gülez Yöntemi" kullanılmıştır. Gülez yönteminde peyzaj değeri (P), iklim değeri (I), Ulaşılabilirlik (U), rekreatif kolaylık (RK) ve olumsuz etkenler (OSE) formüle edilmiş ve her ögeye ağırlık puanı verilmiştir. Bu ağırlık puanına göre de sahanın rekreasyon potansiyeli belirlenmektedir. Bu potansiyelin belirlenmesinde aşağıda yer alan matematiksel formül kullanılmaktadır (Gülez, 1990) (Tablo 1).

$$P + I + U + RK + OSE = \% RP$$

Formüllerin sembolleri ve öge ağırlıkları aşağıda gösterildiği gibidir.

### **Peyzaj Değeri (P)**

Bir sahanın rekreatif faaliyetler amacıyla kullanılmasına imkân tanıyan peyzaj durumunu ve potansiyeli yansıtmaktadır. Bir alanın alacağı maksimum değer peyzaj değeri alanı için toplam 35 puandır. Peyzaj değeri hesaplanırken; alan büyüklüğü 4 puan, bitki örtüsü 8 puan, deniz, göl veya akarsuyun varlığı 8 puan, yüzey durumu 5 puan, görsel kalite 4 puan ve diğer özellikler (doğal anıt, çağlayan, mağara tarihsel ve kültürel değerler, yabani hayvan varlığı, kuşlar vb. 6 puan değerindedir.

### **İklim Değeri (I)**

Rekreasyonel faaliyetlerde hava şartları ve iklim de önemli unsurlar arasında yer almaktadır. Bir alanın iklim değeri kapsamında alacağı maksimum puan değeri 25'dir. Bu bağlamda sahanın sıcaklığı 10 puan, yağış durumu 8 puan, güneşlenme süresi 5 puan, rüzgâr durumu 2 puan şeklinde hesaplanmaktadır.

### **Ulaşılabilirlik (U)**

Rekreasyonel faaliyetlere katılmada ulaşılabilirlik, ulaşım araçları, yol durumu vb. unsurlar büyük önem taşır.

Bu manada bulunduğu bölgede en az 100.000 nüfuslu kent olması 5 puan, ulaşılan zaman süresi (yakınındaki en 5.000 nüfuslu kentten) 4 puan, ulaşım (taksi ve özel araç dışında) 4 puan, ulaşımında diğer kolaylıklar (teleferik, denizden ulaşım olması vb.) 3 puan, bulunduğu bölgenin turistik önemine göre de maksimum 4 puan alınabilmektedir. Bu madde kapsamında da toplam 20 puan alınabilmektedir.

### **Rekreatif Kolaylık (RK)**

Rekreatif kolaylıklar; piknik tesisinin varlığı 4 puan, su durumu 3 puan, geceleme tesisleri 2 puan, WC'ler 2 puan, otopark durumu 2 puan, kır gazinosu-satış büfesi varlığı 2 puan ve bekçi-diğer görevlilerin olması 2 puan ve diğer kolaylıklar (plaj, kabin, duş tesisi, sandal olanakları vb.) 3 puan şeklinde hesaplanmaktadır. Bu madde kapsamında da bir alan maksimum 20 puan puan alabilmektedir.

### **Olumsuz Etkenler (OSE)**

Olumsuz etkenler rekreasyonel faaliyeti olumsuz yönde etkilemekte ve insanların aynı sahayı bir kez daha tercih edip etmeme nedenleri arasında yer almaktadır. Olumsuz etkenler; hava kirliliği -3 puan, güvenli olmaması -2 puan, su kirliliği -1 puan, bakımsızlık -1 puan, gürültü kirliliği -1 puan ve diğer olumsuz etkenler (taş-kum-kireç ocağı varlığı, inşaat ve fabrika kalıntıları vb.) -2 puan olarak hesaplanmaktadır. Olumsuz etkenler toplam puana etkisi negatif yöndedir. Sahada olumsuz etken varsa niteliğine göre toplam puandan maksimum 10 puan düşürülmektedir.

## **Bulgular ve Tartışma**

Bir alanın rekreasyon değeri üzerinde, o alanın sahip olduğu doğal ve beşerî çevre özelliklerinin önemli etkileri vardır (Gül ve Yılmaz, 2019). Doğal ve beşerî çevre çekiciliklerinin çeşitli ve fazla olduğu alanlar insanlar tarafından yoğun bir şekilde tercih edilmektedir. Gölbaşı Gölleri Tabiat Parkı da bu alanlardan biridir.

### **Peyzaj Değeri (P)**

Gülez yönteminde rekreatif faaliyetlerin potansiyelinin belirlenmesi için alanın büyüklüğü, bitki örtüsü, yer şekilleri, görsel kalite ve diğer özellikler ana kriter olarak değerlendirilmektedir. Bu ana kriterler toplam 100 puanın 35 puanını oluşturmaktadır (Tablo 2).

Gölbaşı Gölleri Tabiat Parkı sınırları içinde birbirine bağlı 3 göl bulunmaktadır. Bu göller ile birlikte tabiat parkının toplam alanı 2080 ha.'dır. Gülez yönteminde 10 ha. geçen rekreasyon alanları için 4 puan önerilmiş ve Gölbaşı Gölleri Tabiat Parkı'na "4" puan verilmiştir.

Tablo 1. Gülez yönteminin sembol, anlam ve maksimum puanları  
Table 1. Symbol, meaning and maximum scores of Gülez method

Sembol	Anlamı	Maksimum Puan (Ögenin Ağırlık Puanı)
P	Peyzaj Değeri	35
I	İklim Değeri	25
U	Ulaşılabilirlik	20
RK	Rekreatif Kolaylık	20
OSE	Olumsuz Etkenler	0 (minimum -10)
% RP	Rekreasyon Potansiyeli	100

Kaynak: Gülez, 1990

Tablo 2. Gülez yöntemine göre Gölbaşı Gölleri Tabiat Parkı'nın peyzaj değeri  
 Table 2. Landscape value of Gölbaşı Lakes Nature Park according to Gülez method

Kriter	Alt Kriter	Maksimum Puan	Değerlendirilen Puan
Alanın Büyüklüğü	10 ha.'dan büyük	4	4
	5-10 ha.	3	
	1-5 ha.	2	
	0,5-1 ha.	1	
Bitki Örtüsü	Ağaçlık, Çalılık, Çayırılık	7-8	8
	Yalnız Ağaçlık ve Çalılık	6-7	
	Çalı, Çayır, Seyrek Ağaç	5-6	
	Çayırılık ve Seyrek Ağaçlık	4-5	
	Yalnız Çalılık ve Çayırılık	3-4	
	Çalılık, Seyrek Ağaçlık	2-3	
	Yalnız Çayırılık	1-3	
Konum	Deniz Kıyısı	7-8	7
	Göl Kıyısı	6-7	
	Akarsu Kıyısı	4-5	
	Dere Kıyısı	1-4	
Yüzeysel Durum	Düz Alan	5	3
	Hafif Dalgalı	4	
	Az Meyilli, Yer Yer Düzlük	3	
	Az Engebeli	2	
	Orta Engebeli	1	
Görsel Kalite	Panoramik Görünümler	3-4	4
	Güzel Görüş ve Vistalar	2-3	
	Alanın Genel Görsel, Estetik Değeri	1-3	
Diğer Özellikler	Anıt Ağaç, Çağlayan, Mağara, Tarihsel ve Kültürel Değer, Yaban Hayvanları, Kuşlar vb.	1-6	1
Toplam		35	25

Kaynak: Gülez, 1990



Şekil 2. Gölbaşı Gölleri Tabiat Parkı ağaç türleri  
 Figure 2. Gölbaşı Lakes Nature Park tree species

Tabiat parkında orman, orman-step karışımı ve sucul ortam bitkileri bulunmaktadır (Eğilmez ve Tel, 2016). Sahada *Carpinus betulus L.* (gürgen), *Quercus (meşe)*, *Onobrychis sativa (korunga)*, *Phlomis armeniaca (şavlak)* vb. türler bulunmaktadır. Bunun dışında sahada göl varlığına bağlı olarak turbalık alanlar bulunmaktadır (Sandıkçioğlu ve Uzun, 2023). Bu bağlamda Gülez yönteminde belirtilen ağaçlık, çalılık ve çayırılık alt kriteri uyarınca sahaya “8” puan verilmiştir (Şekil 2).

Gölbaşı Gölleri Tabiat Parkı'nın önemli özelliklerinden biri birbirine bağlı 3 gölün bulunmasıdır. Bu göller Gölbaşı, İnekli (Yeşilova) ve Azaplı Göl'leridir. Göl varlığına bağlı olarak “7” puan verilmiştir.

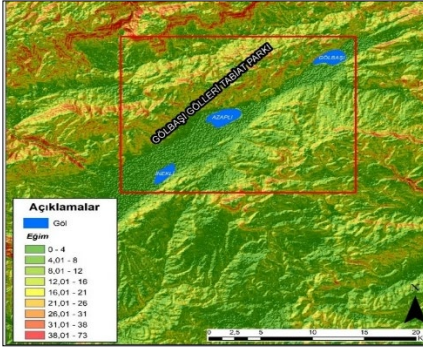
Sahanın eğim değerleri dikkate alındığında; tabiat parkı ve çevresi eğimi 0-73° arasında değişiklik göstermektedir. Tabiat parkının kuzeybatısında ve güneydoğusunda dağlık alan varlığına bağlı olarak eğim derecesi yüksek

seyretmektedir. Bunun dışında tabiat parkının bulunduğu alanın eğim değerleri 0-4° arasında değişiklik göstermekte ve Gülez yöntemine göre az meyilli yer yer düzlük alan olduğu için “3” puan verilmiştir (Şekil 3).

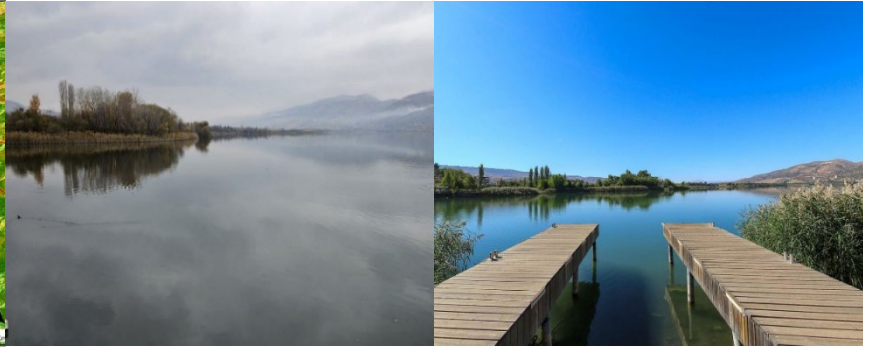
Gülez yönteminde peyzaj değeri kriterinin alt kriteri olan görsel kalite bölümünde sahada panoramik görünüm, güzel görüş ve alanın dağ ve göl varlığından dolayı estetik değeri olduğu düşünüldüğünde “4” puan verilmiştir (Şekil 4).

Tabiat parkı içinde diğer özellikler kapsamında anıt ağaç, çağlayan, mağara, tarihsel ve kültürel değerlerin olmayışı bunun yanı sıra yaban hayvanlarının ve kuşların varlığına bağlı olarak “1” puan verilmiştir.

Gülez yöntemi Peyzaj değeri ve alt kriterleri değerlendirildiğinde Gölbaşı Gölleri Tabiat Parkı'na 35 puandan üzerinden “25” puan verilmiştir.



Şekil 3. Çalışma sahasının eğim haritası  
Figure 3. Slope map of the study area

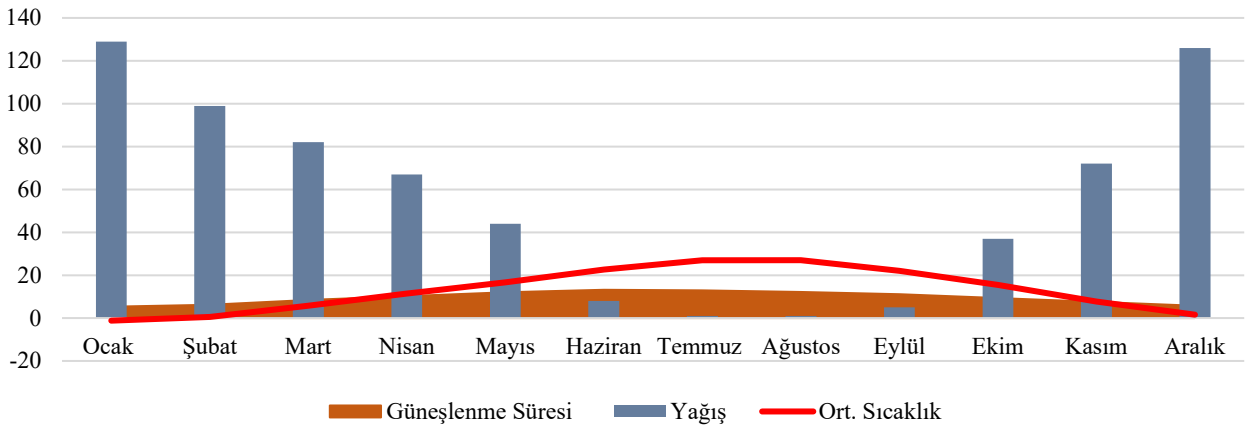


Şekil 4. Gölbaşı Gölüleri Tabiat Parkı'ndan panoramik görüntüler  
Figure 4. Panoramic views from Gölbaşı Lakes Nature Park

Tablo 2. Gülez yöntemine göre Gölbaşı Gölüleri Tabiat Parkı'nın iklim değeri  
Table 2. Climate value of Gölbaşı Lakes Nature Park according to Gülez method

Kriter	Alt Kriter	Maksimum Puan	Değerlendirilen Puan
Sıcaklık (Yaz Ayları Ort.)	25-25,9	10	10
	24-24,9 ve 26-26,9	9	
	23-23,9 ve 27-27,9	8	
	22-22,9 ve 28-28,9	7	
	21-21,9 ve 29-29,9	6	
	20-20,9 ve 30-30,9	5	
	19-19,9 ve 31-31,9	4	
	18-18,9 ve 32-32,9	3	
	17-17,9 ve 33-33,9	2	
	16-16,9 ve 34-34,9	1	
Yağış (Yaz Ayları Ort.)	50 mm'den az	8	8
	50-100 mm.	7	
	100-150 mm.	6	
	150-200 mm.	5	
	200-250 mm.	4	
	250-300 mm.	3	
	300-350 mm.	2	
	350-400 mm.	1	
Güneşlenme (Yaz Ayları Ort.)	0-2	5	5
	2-4	4	
	4-6	3	
	6-8	2	
	8-9	1	
Rüzgâr Durumu (Yaz Ayları Ort.)	1m/sn'den az	2	1
	1-3m/sn arası	1	
<b>Toplam</b>		<b>25</b>	<b>24</b>

Kaynak: Gülez, 1990



Şekil 5. Gölbaşı ilçesinin güneşlenme süresi, yağış ve ortalama sıcaklık diagramı  
Figure 5. Sunshine Duration, Precipitation and Mean Temperature Diagram of Gölbaşı District

Tablo 3. Gülez yöntemine göre Gölbaşı Gölleri Tabiat Parkı'nın ulaşılabilirlik değeri  
 Table 3. Accessibility value of Gölbaşı Lakes Nature Park according to Gülez method

Kriter	Alt Kriter	Maksimum Puan	Değerlendirilen Puan
Bulunduğu Bölgenin Turistik Önemi	Akdeniz, Ege, Marmara Kıyı Bandı	3-4	3
	Karadeniz Kıyı Bandı	2-3	
	Önemli Karayolu Güzergahları	1-3	
	Turizmde Öncelikli Yöreler	1-3	
Bulunduğu Bölgede En Az 100.000 Nüfuslu Kent Olması	20 km'ye Kadar Uzaklık	4-5	3
	20-50 km'ye Kadar Uzaklık	3-4	
	50-100 km'ye Kadar Uzaklık	2-3	
	100-200 km'ye Kadar Uzaklık	1-2	
Ulaşım Zaman Süresi (Yakınındaki En Az 5.000 Nüfuslu Yerden)	Taşıtla 0- 30 dk	4	4
	Taşıtla 30 dk-1 Saat	3	
	Taşıtla 1-2 Saat	2	
	Taşıtla 2-3 Saat	1	
Ulaşım (Taksi ve Özel Araç Dışında)	Yürüyerek Gidebilme veya Her An Taşıt Bulabilme	3-4	3
	Belirli Saatlerde Taşıt Bulabilme	1-3	
	Teleferik Olması, Denizden Ulaşabilme vb.	3	
Ulaşımında Diğer Kolaylıklar	Teleferik Olması, Denizden Ulaşabilme vb.	3	0
Toplam		20	12

Kaynak: Gülez, 1990

### İklim Değeri (I)

Gülez yönteminde rekreatif faaliyet potansiyelinin değerlendirilebilmesi için yaz mevsimine ait iklim verileri kullanılmaktadır. Yaz mevsimine ilişkin sıcaklık, yağış, rüzgâr durumu ve bulutluluk değerleri dikkate alınmaktadır. Kriterlerin öge puanlaması 100 üzerinden 24 puan ile değerlendirilmektedir.

Gölbaşı Meteoroloji İstasyonu, tabiat parkının çok yakınında bulunmaktadır. 1991-2021 yılları arası verilerin ortalamaları değerlendirildiğinde; en soğuk ay ocak -1,2 °C, en sıcak ay ise ağustos 27 °C olarak ölçülmüştür. Sahada yaz mevsimi sıcaklık ortalamaları haziran ayında 22,6 °C, temmuz ayında 26,9 °C ve ağustos ayında ise 27 °C'dir. Sahada yıllık ortalama sıcaklık 13 °C, yaz mevsimi sıcaklık ortalamaları ise 25,5 °C'dir. Bu bağlamda Gülez yöntemine göre değerlendirme puanı "10" olarak verilmiştir (Tablo 2 ve Şekil 5).

Gölbaşı Meteoroloji İstasyonu yağış verileri değerlendirildiğinde, sahanın yıllık yağış ortalaması 671 mm'dir. Yağışın en fazla olduğu ay 129 mm ile ocak, yağışın en az olduğu ay ise 1 mm ile temmuz ve ağustos aylarında görülmektedir. Yaz mevsiminde ise sahaya 10 mm. yağış düşmektedir. Haziran ayında 8 mm., temmuz ayında 1 mm. ve ağustos ayında 1 mm. yağış düşmektedir. Bu bağlamda veriler incelendiğinde, "8" puan verilmiştir (Tablo 2 ve Şekil 5).

Çalışma alanının 1991-2021 bulutluluk değerlerine bakıldığında, bulutluluk ortalaması en fazla ocak ayında, en az ise temmuz ve ağustos aylarında görülmektedir. Bulutluluğun yaz mevsimi ortalaması 2'dir. Haziran ayında 2, temmuz ve ağustos ayında ise 0 bulutluluk görülmektedir. Bu nedenle bulutluluk ortalaması puanlaması "5" olarak verilmiştir (Tablo 2 ve Şekil 5).

Sahanın ortalama rüzgâr hızı 11,03 m/sn'dir. Rüzgâr hızının en fazla olduğu ay temmuz ayında 15.4 m/sn, en az ise kasım ayında 9.9 m/sn'dir. Yaz mevsiminde ise haziran ayında 14.2 m/sn, temmuz ayında 15.4 m/sn ve ağustos ayında ise 14 m/sn olarak ölçülmüştür. Sahanın rüzgâr hızı

değerleri 1 m/sn'den fazla olduğu gerekçesiyle 1 puan verilmiştir (Tablo 2).

Gülez yöntemine göre iklim değerleri kapsamında maksimum alınabilecek puan 25'tir. Gölbaşı Gölleri Tabiat Parkı'nın iklim değeri puanı toplam "24" olarak hesaplanmıştır.

### Ulaşılabilirlik Değeri (U)

Gülez yöntemine göre rekreasyonel alanların potansiyelinin belirlenebilmesi için yerin ulaşılabilirlik değerinin hesaplanması gerekmektedir. Bu hesaplamada bulunduğu turistik önem, bulunduğu bölgede en az 100.000 nüfuslu kent olması, en az 5.000 nüfuslu yerleşmeden rekreasyonel alana ulaşım süresi, ulaşımın erişilebilir olması ve ulaşımında diğer kolaylıklar puanlanarak 100 üzerinden maksimum 20 puan alınabilmektedir (Tablo 3).

Gölbaşı karayolu güzergahı; Kahramanmaraş, Malatya ve Adıyaman'ı birbirine bağlayan önemli karayoluna sahiptir. Bu nedenle Gülez yönteminde çalışma sahasına önemli karayolu güzergâh üzerinde bulunduğu için "3" puan verilmiştir. Yöntemdeki diğer unsurlar Akdeniz, Ege ve Marmara kıyı bandı, Karadeniz kıyı bandı alt kriterleri çalışma alanında yer almadığı için puanlandırılmamıştır.

Yöntemdeki bir diğer alt kriter ise nüfus ve rekreasyonel faaliyetler arasındaki erişilebilirliğin sağlanabilmesi için 100.000 nüfuslu yerleşmeye yakınlık zaman mesafe açısından derecelendirilmesi gerekmektedir. Bu bağlamda Gölbaşı Gölleri Tabiat Parkı; Adıyaman ve Kahramanmaraş illerine 50-100 km'ye kadar uzaklığı olduğu için "3" puan verilmiştir.

Çalışma alanına özel araç ve taksi dışında toplu ulaşım ile ulaşmak mümkündür. Yürüyerek gidebilme veya her an taşıt bulabilme maddesinin kapsamında değerlendirildiği için "4" puan verilmiştir. Yakınındaki en az 5.000 nüfuslu yerden tabiat parkına 0-30 dk arasında ulaşım sağlanabileceği için "4" puan verilmiştir.

Tablo 4. Gülez yöntemine göre Gölbaşı Gölleri Tabiat Parkı'nın rekreatif kolaylık değeri

Table 4. According to Gülez method, recreational facilities of Gölbaşı Lakes Nature Park

Kriter	Alt Kriter	Maksimum Puan	Değerlendirilen Puan
Piknik Tesisleri	Sabit Piknik Masa, Ocak vb.	4	2
Su Durumu	İçme ve Kullanma Suyu Olanakları	3	2
Geceleme Tesisleri	Sabit Geceleme Tesisleri Çadırılı veya Çadırsız Kamp Kurabilme İmkanları	2 1-2	1
WC'ler	WC Varlığı	1-2	1
Otopark	Otopark Varlığı	1-2	1
Kır Gazinosu, Satış Büfesi	Varlığı	1-2	1
Bekçi ve Görevliler	Sürekli Bekçi-Görevli Varlığı Sadece Hafta Sonlarında Bekçi-Görevli Varlığı	2 1	2
Diğer Kolaylıklar	Plaj, Kabin, Duş Tesisleri, Kiralık Sandal İmkanları, Top Oyun Alanları, Diğer Tesisler vb.	3	3
Toplam		20	13

Kaynak: Gülez, 1990



Şekil 6. Gölbaşı Gölleri Tabiat Parkı rekreatif kolaylıklar kapsamında kuş gözlem kulesi, karavan alanı, çocuk oyun parkı ve WC

Figure 6. Bird observation tower, Caravan area, children's playground and WC within the scope of recreational facilities in Gölbaşı Lakes Nature Park

Tablo 5. Gülez yöntemine göre Gölbaşı Gölleri Tabiat Parkı'nın olumsuz etkenler değeri

Table 5. Negative factors value of Gölbaşı Lakes Nature Park according to Gülez method

Kriter	Alt Kriter	Maksimum Puan	Değerlendirilen Puan
Güvenceli Olmaması	Güvence Durumuna Göre	-2	-1
Hava Kirliliği	Kirlilik Derecesine Göre	-3	-2
Su Kirliliği	Deniz, Göl ve Akarsular için	-1	-1
Gürültü Kirliliği	Trafik, Kalabalık vb. Gürültüler	-1	-1
Bakımsızlık	Alanda Yeterli Bakımın Yapılmaması	-1	-1
Diğer Olumsuz Etkenler	Taş, Çakıl, Kireç Ocağı, İnşaat ve Fabrika Kalıntıları vb.	-2	-1
Toplam		-10	-7

Kaynak: Gülez, 1990

Gülez yöntemine göre ulaşılabilirlik değeri kapsamında ulaşımda diğer kolaylıklar alt kriterinde toplu ulaşım dışında başka ulaşım türü olmadığı için ilgili alana "0" puan verilmiştir. Çalışma alanının rekreatif kolaylık değeri yönteme göre incelendiğinde, sahadaki WC'ler, piknik tesisleri, su durumu, konaklama tesisleri, otopark, bekçi ve diğer görevlilerin varlığı, kır gazinosu, büfe ve diğer kolaylıklar dikkate alınmaktadır. Endekste 100 puan üzerinden maksimum 20 puan alınabilmektedir (Tablo 4).

Gölbaşı Gölleri Tabiat Parkı'nda oturma alanları, banklar ve kamelya tarzı oturma bölümleri yer almaktadır. Fakat yöntemde sadece bu alanların varlığı değil aynı zamanda bakım durumu da göz önünde bulundurulmaktadır. Bu nedenle çalışma alanı bu madde kapsamında "2" puan verilmiştir.

Tabiat parkı içinde içme ve kullanma suyu belirli aralıklarla bulunmaktadır. Fakat sayısı yetersizdir. Bu nedenle çalışma alanında bulunan su durumu kriterine

bağlı olarak "2" puan verilmiştir. Çalışma alanı içinde sabit geceleme veya konaklama durumu söz konusu değildir. Fakat kamp alanları ile karavan için alanlar ayrılmış durumdadır. Fakat alt yapı ve alanın genişliği göz önüne alındığında yetersiz bulunmaktadır. Bu nedenle ilgili madde kapsamında "1" puan verilmiştir.

Gölbaşı Gölleri Tabiat Parkı içinde WC'ler bulunmaktadır. WC'ler yanı sıra abdesthane vb. ayrılmış alanlarda yer almaktadır. Fakat bunların nitelikleri ve sayısı yetersizdir. Tüm bu gerekçelerle çalışma alanına "1" puan verilmiştir.

Otopark alanları, rekreasyonel faaliyetlere katılanlar için oldukça önemli bir unsurdur. Otoparkın yakınlığı ve genişliği rekreasyonel faaliyetlere katılmada öncelikli konulardan biridir. Tabiat parkı içinde genişliği çok büyük olmamakla birlikte otopark mevcuttur. Bu nedenle çalışma alanına "1" puan verilmiştir.

Çalışma alanı içinde satış büfesi (yiyecek-içecek satan büfe) bulunmaktadır. Satış büfesi, tabiat parkı içinde 5 farklı noktada bulunmaktadır. Bu kapsamda puanlandırmada “1” puan verilmiştir (Şekil 6).

Tabiat parkı girişinde ve içinde belirli noktalarda güvenlik görevlisi, bekçi ve diğer hizmetler için personeller bulunmaktadır. Alanda sürekli bekçi ve diğer hizmet personelleri bulunmaktadır. Tabiat parkı çevresi tel örgülerle çevrilmiş ve girişler çıkışlar kontrol edilmektedir. Bu nedenle yöntemde de belirttiği üzere maksimum puan olan “2” puan verilmiştir.

Diğer kolaylıklar değerlendirildiğinde, bebek bakım odası, bilgi edinme noktası vb. birimlerin ve noktaların olması tabiat parkı içinde insanların rekreasyonel faaliyetlerini kolaylaştırmaktadır. Ayrıca, top oyun alanları, gölde tekne turu gibi hizmetlerde bulunduğundan kriter için maksimum puan olan “3” puan verilmiştir.

Güleç yöntemine göre “rekreatif kolaylık” kategorisi kapsamında alınabilecek maksimum puan 20’dir. Gölbaşı Gölleri Tabiat Parkı bu kategoriden toplamda “13” puan almıştır.

Güleç yöntemindeki son değerlendirme ise saha rekreasyonel faaliyetlere katılacak kişileri olumsuz yönde etkileyen kriterlerdir. Bu kriterler “olumsuz etkenler” şeklinde sınıflandırılmış ve eğer olumsuz bir durum var ise toplam puandan düşürülmektedir. Olumsuz etkenler güvenceli olmaması, hava kirliliği, su kirliliği, gürültü kirliliği, bakımsızlık ve diğer olumsuz etkenler şeklinde sınıflandırılmaktadır. Bu kategoriden toplam puandan 10 puan çıkarılması önerilmektedir (Tablo 5).

Gölbaşı Gölleri Tabiat Parkı’nda rekreasyonel faaliyetleri etkileyecek olumsuz etkenler bulunmaktadır. Alanda her ne kadar sürekli bekçi ve diğer hizmetler için gerekli personel olsa dahi park çok geniş olması dolayısıyla güvenlik durumu akşam ve gece saatlerinde olumsuzluklar doğurabilmektedir. Bu nedenle -1 puan olarak değerlendirilmiştir.

Hava kirliliği konusunda sahada enkaz kaldırımı ve inşaat faaliyetleri sebebiyle partikül madde ve diğer kirletici unsurların varlığından söz edilebilir. Bu kapsamda tabiat parkı ve çevresinin hava kalitesi endeksi nispeten “orta-kötü” durumdadır (Şekil 7). Bu nedenle alt kritere “-2” puan verilmiştir.

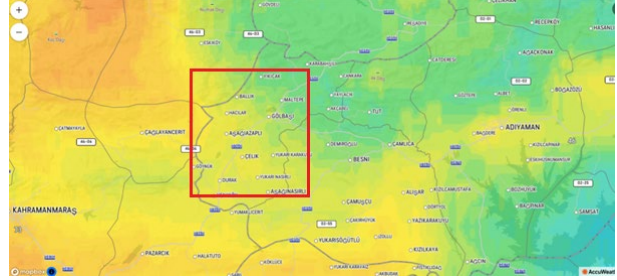
Su kirliliği kapsamında sahada 3 tane göl bulunmaktadır. Göllerin yüzeyleri incelendiğinde kirli olduğu görülmektedir. Genellikle göl yüzeyinde çöp vb. gibi kirletici unsurların olduğu görülmektedir. Bu nedenle “-1” puan verilmiştir.

Gürültü kirliliği kapsamında ise tabiat parkının hemen yanına deprem sonrası enkaz yığın alanı oluşturulmuştur. Bu sebeple sürekli olarak iş makineleri çalıştırılmaktadır. Bu da sahada önemli derecede gürültü kirliliğinin oluşmasına neden olmaktadır. Tüm bu sebeplerde birlikte bu madde kapsamında “-1” puan verilmiştir.

Gölbaşı Gölleri Tabiat Parkı yaşanan Kahramanmaraş depreminde zarar almıştır. Bu zararlar hızlı bir şekilde giderilmeye çalışılmaktadır. Fakat tabiat parkı bu süreçte ziyaretçilere açık durumdadır. İsteyen ziyaretçiler tabiat parkına giriş yapabilmekte ve rekreasyonel faaliyetleri gerçekleştirebilmektedir. Tabiat parkının depremden zarar görmeyen alanlarında bakımsızlık mevcuttur. Bu nedenle olumsuz etkenler kategorisinde “-1” puan olarak değerlendirilmiştir.

Tabiat parkında diğer olumsuz etkenler dahilinde taş, çakıl ocağı bulunmamakta fakat inşaat ve moloz yığın alanları park oldukça yakın konumdadır. Bu nedenle çalışma sahası ilgili madde kapsamında “-1” puan verilerek değerlendirilmiştir.

Gölbaşı Gölleri Tabiat Parkı yukarıdaki nedenlere bağlı olarak “olumsuz etkenler” ana kategorisinden toplamda “-7” puan olarak değerlendirilmiştir. Bu puan kriterlerin toplam puanından düşürülmek suretiyle değerlendirilmeye tabii tutulmuştur (Şekil 8).



Şekil 7. Çalışma sahasının hava kalitesi durumu

Figure 7. Air quality status of the study area

Kaynak: <https://www.accuweather.com/tr/tr/golbasi/1301559/air-quality-index/1301559>



Şekil 8. Gölbaşı Gölleri Tabiat Parkı olumsuz etkenler (Bakımsızlık ve Tabiat Parkı Yakınında Enkaz Yığını)

Figure 8. Gölbaşı Lakes Nature Park negative factors (Poor Maintenance and Debris Pile Near Nature Park)

Çizelge 6. Gülez yöntemine göre Gölbaşı Gölleri Tabiat Parkı'nın rekreasyonel potansiyeli  
Table 6. Recreational Potential of Gölbaşı Lakes Nature Park According to Gülez Method

Özellikler	Maksimum Puan	Değerlendirilen Puan	İyileştirmelerle Alınacak Puan
Peyzaj Değeri (P)	35	25	29
İklim Değeri (I)	25	24	-
Ulaşılabilirlik Değeri (U)	20	12	15
Rekreatif Kolaylık Değeri (RK)	20	13	20
Olumsuz Etkenler (OSE)	-10	-7	-2
Toplam	100	67	86

## Sonuç ve Öneriler

Gölbaşı Gölleri Tabiat Parkı'nın peyzaj değeri, iklim özellikleri, ulaşılabilirliği ve sağladığı rekreatif kolaylıkların toplanması ve belirlenen olumsuz etkenlerin puanlarının toplam puandan çıkarılması ile alanın genel bir değerlendirme skoru hesaplanmıştır. Bu hesaplama göre; Gölbaşı Gölleri Tabiat Parkı'nın rekreasyonel potansiyel değeri "67" puan olarak ölçülmüş; "yüksek" rekreasyon potansiyeline sahip olduğu belirlenmiştir (Çizelge 6).

Gülez yönteminin kullanıldığı çalışmalar incelendiğinde; Çavuş ve Aker (2021), Malatya ilinde bulunan Turgut Özal Tabiat Parkı'nın rekreasyonel potansiyelini incelemiş ve incelemeler sonucunda sahaya 70 puan vermiştir. Beşeri çekiciliklerde yapılacak iyileştirmelerle olumsuzlukların giderilerek rekreasyon potansiyelinin yükseltilebileceği ön görülmüştür. Gül ve Yılmaz (2019) Samsun Şehri kıyı şeridinin rekreasyon potansiyelinin değerlendirildiği çalışmalarında saha toplamda 100 puan üzerinden 78 puan almış ve rekreasyonel potansiyeli "çok yüksek" olarak belirlenmiştir. Ayrıca kıyı şeridinin SWOT analizi de yapılmış ve yöntemin eksik kalan yanları çözülmeye çalışılmıştır. Sü Eröz ve Aslan (2017)'in Istaranca (Yıldız) ormanlarında rekreasyonel potansiyelinin belirlendiği çalışmalarında, ormanlık alan toplamda 77 puan almış ve "çok yüksek" potansiyele sahip olduğu belirlenmiştir. Alt-üst yapı vb. eksikliklerin giderilmesi ile potansiyelinin daha da artırılacağı vurgulanmıştır. Yılmaz ve ark. (2009) Kafkasör ormanının rekreasyonel potansiyelini incelediği çalışmalarında, ormanlık alanın rekreasyonel potansiyelini anket ile desteklenmiş ve 66,9 puan olarak ölçülmüştür. Akten (2003) Isparta ilindeki bazı alanların rekreasyon potansiyellerinin belirlendiği çalışmada, 9 rekreasyonel alan belirlemiş ve değerlendirmiştir. Rekreasyonel alanlar arasında en yüksek potansiyel 72 puan ile Ayazma'da hesaplanmıştır. En düşük ise 33 puan ile Suçıktı orman içi dinlenme alanında tespit edilmiştir. Ayrıca yazar, bu alanların rekreasyonel potansiyelinin hesaplanmasına ek olarak yerli halk ile de anket yaparak yöntemi daha objektif hale getirmiştir. Gülez yöntemi ile ilgili bir çok çalışma bulunmaktadır. Yöntemin avantaj ve dezavantajları vardır fakat en önemli dezavantajı subjektif bir yöntem olmasıdır. Diğer çalışma alanları ile Gölbaşı Gölleri Tabiat Parkı'nın potansiyeli kıyaslandığında, parkın birçok çalışmaya oranla potansiyelinin düşük olduğu görülmüştür. Gölbaşı Gölleri Tabiat Parkı'nın potansiyelinin diğer alanlara göre düşük olmasının nedeni sadece beşeri çekiciliklerin eksikliği değil aynı zamanda depremde aldığı hasar ile de ilgilidir.

Gülez yöntemi; kolay, anlaşılabilir ve basit bir yöntem olmasının yanı sıra alanın güçlü ve zayıf yönleri ile alana ilişkin fırsat ve tehditlerin belirlenebilmesi açısından önemlidir. Fakat yöntem objektif olmaktan ziyade subjektif değerlendirme yapılmasına olanak sağladığı için diğer alanlarla olan mukayeseleri zorlaştırmaktadır. Gölbaşı Gölleri Tabiat Parkı bulunduğu konum sebebiyle oldukça önemlidir. Adıyaman ve Kahramanmaraş illerine yakınlığı göz önüne alındığında park, bölgenin en önemli tabiat parklarından biridir. Tabiat parkı gerek doğa gerekse de rekreasyonel bağlamda potansiyele sahiptir. Tabiat parkı içinde birbiri ile bağlantılı 3 göl (Gölbaşı, İnekli, Azaplı Gölü) bulunmakta ve ziyaretçilere tekne turu, göl bisikleti vb. imkanlar sunabilmektedir. Ayrıca parkın belirli alanlarında piknik, oyun alanı, spor aletleri, yürüyüş yolları gibi donatılar bulunmaktadır. Fakat tabiat parkı dağ, göl ve ağaçlık alan üçlemesi potansiyelinin çok yüksek olmasına rağmen istenilen seviyede kullanılamamaktadır. Bu ve benzeri çalışmalar rekreasyonel kullanımın çeşitlendirilmesine, mevcut durumun iyileştirilmesine ve potansiyelinin artırılmasına imkân tanıyacaktır.

Tabiat parkının potansiyelinin yüksek olmasına rağmen istenilen düzeyde kullanılabilmesi için; deprem sonrası hızlı bir bakımın yapılması gerekmektedir. Geceleme tesislerinin yapılması, çadırı veya çadırsız kamp kurma alanlarının yapılması ve karavan konulabilecek alanların yapılması gerekmektedir. Parkın büyüklüğüne oranlandığında WC'ler nispeten yetersizdir, WC sayısı artırılmalıdır. Gölbaşı Gölleri Tabiat Parkı'nın doğal yapısı korunarak; yöreye uygun mimarisi stiliyle ilgi çekebilecek yapılar oluşturularak 4, ulaşım türünün çeşitlendirilmesini sağlayarak 3, rekreatif kolaylıklar kategorisindeki eksiklikleri tamamlayarak 7, olumsuz etkenleri azaltılmasını sağlayarak 5, toplamda da "19" puan artırılması sağlanabilir. Toplamda da mevcut "67" puan ile potansiyeli "yüksek" kategorisinden iyileştirmelerle birlikte "86" puana "çok yüksek" kategorisine yükseltilebilir.

## Beyanlar

*E.V.:* Sahada gezi gözlem, veri toplanması, verilerin değerlendirilmesi, çalışmanın dizaynı, çalışmanın yazımı ve düzenlenmesi.

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## Comparative Analysis of Machine Learning Algorithms for Irrigation Status Prediction

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 21.01.2025 Accepted : 27.02.2025</p> <p><b>Keywords:</b> Irrigation status estimation Machine learning Agricultural irrigation Artificial intelligence. Spatial Irrigation Estimation</p>	<p>Compared to traditional methods, systems supported by machine learning have been found to make more accurate irrigation decisions. In this study, Kaggle, a data sharing platform frequently used in data science, was utilized. The “Weather Data” dataset was used for irrigation status prediction. Missing and outlier data were corrected and dependent (irrigation status) and independent (air temperature, humidity, soil moisture value, precipitation) parameters were obtained. Focusing on the provinces in the Southeastern Anatolia Region (Adıyaman, Batman, Diyarbakır, Gaziantep, Kilis, Mardin, Siirt, Şanlıurfa and Şırnak), the accuracy of the algorithms was tested under different conditions. In the analyses conducted separately for each province, irrigation status estimation was performed using machine learning algorithms such as Decision Trees, Support Vector Machines, Random Forest, Naive Bayes, Gradient Boosting, Logistic Regression, K-Nearest Neighbor and Artificial Neural Network models. As a result of the predictions made on the dataset, the algorithms were compared using the accuracy metric and the most effective algorithms were Random Forest (95%), Decision Tree (97%), Gradient Boosting (93%) and Artificial Neural Network (98%) models with over 90% accuracy in all cities. Other algorithms also showed remarkable performance with high accuracy rates (above 75%). In the analysis for each city, the algorithm performance ranking was similar. In conclusion, this study shows that machine learning algorithms can be used in agricultural irrigation with very high performance.</p>

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(2): 497-503, 2025

## Sulama Durumu Tahmini için Makine Öğrenimi Algoritmalarının Karşılaştırmalı Analizi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 21.01.2025 Kabul : 27.02.2025</p> <p><b>Anahtar Kelimeler:</b> Sulama durumu tahmini Makine öğrenimi Tarımsal sulama Yapay zekâ Mekansal Sulama Tahmini</p>	<p>Geleneksel yöntemlere kıyasla, makine öğrenimi ile desteklenen sistemlerin, daha hassas sulama kararları verebildiği tespit edilmiştir. Bu çalışmada, veri bilimi alanında sıkça kullanılan veri paylaşım platformu olan Kaggle'dan faydalanılmıştır. Sulama durumu tahmini için “Weather Data” veri kümesi kullanılmıştır. Veri kümesinde eksik ve aykırı veriler düzeltilmiş, bağımlı (sulama durumu) ve bağımsız (hava sıcaklığı, nemi, toprak nem değeri, yağış durumu) parametreler elde edilmiştir. Güneydoğu Anadolu Bölgesi'ndeki illere (Adıyaman, Batman, Diyarbakır, Gaziantep, Kilis, Mardin, Siirt, Şanlıurfa ve Şırnak) odaklanılarak farklı koşullarda algoritmaların doğrulukları test edilmiştir. Her bir il için ayrı ayrı yapılan analizlerde, makine öğrenimi algoritmalarından Karar Ağaçları, Destek Vektör Makineleri, Rastgele Orman, Naive Bayes, Gradyan Artırma, Lojistik Regresyon, K-En Yakın Komşu ve Yapay Sinir Ağı modelleri kullanılarak sulama durumu tahmini gerçekleştirilmiştir. Veri kümesi üzerinde yapılan tahminler sonucunda algoritmalar, doğruluk (accuracy) metriği kullanılarak karşılaştırılmış ve en etkili algoritmaların Rastgele Orman (%95), Karar Ağacı (%97), Gradyan Artırma (%93) ve Yapay Sinir Ağı (%98) modeli ile tüm şehirlerde %90'ın üzerinde sulama durumu tahmini doğruluğu elde edilmiştir. Diğer algoritmalar da yüksek doğruluk oranları ile (%75 üzeri) dikkate değer performans sergilemişlerdir. Her bir il için yapılan analizlerde, algoritma performans sıralamasının benzer olduğu belirlenmiştir. Sonuç olarak, bu çalışma makine öğrenimi algoritmalarının tarımsal sulamada oldukça yüksek bir performansla kullanılabileceğini göstermektedir.</p>

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## Giriş

Su, tarımsal üretimin temel yapı taşlarından biridir. Bitkiler, kökleri aracılığıyla topraktan suyu emerek ihtiyaç duydukları besinleri alır ve bu besinler, temel metabolik süreçlerin sağlıklı bir şekilde gerçekleşmesi için kullanılır (Sargıncı ve ark., 2022). Sulama suyunun doğru yönetimi, su güvenliğinin sağlanması ve tarımsal üretimin sürdürülebilirliğinin korunması açısından kritik bir öneme sahiptir.

Kuraklık, dünya nüfusunun hızla artması, tatlı su kaynaklarının sınırlı olması tarımsal üretimi tehdit eden önemli etmenler arasındadır (Türkeş, 2012). Türkiye'nin yıllık su potansiyeli yaklaşık 112 milyar metreküptür. Bu suyun %74'ü tarımsal sulama için kullanılmaktadır. Tarımsal sulamada kullanılan suyun %50'si ise verimsiz kullanım ve geleneksel yöntemler nedeniyle israf edilmektedir (Çakmak & Gökalp, 2013). Bu nedenle Türkiye gibi birçok ülke, yenilikçi çözümler geliştirme ihtiyacı ile karşı karşıyadır. Akıllı sulama sistemleri, bu ihtiyaca yanıt olarak, suyun doğru zamanda, doğru miktarda ve doğru noktaya uygulanmasını sağlayarak su kullanımını optimize eder (Şahin, 2024). Sulama planlaması; toprağın su tutma kapasitesi, bitki türü, iklim koşulları ve sulama yöntemine göre değişkenlik gösterir. Bu nedenle, sulama kararları alınırken birçok faktörün göz önünde bulundurulması gerekmektedir.

Geleneksel sulama kararları genellikle çiftçilerin tecrübelerine ve gözlemlerine dayanır (Çakmak ve ark., 2008). Çiftçiler, bitkilerdeki yaprak solgunluğu, renk değişimi veya büyüme duraklaması gibi belirtileri inceleyerek su ihtiyacını tahmin eder. Toprağın yüzey rengine bakarak veya el ile sıkarak, toprağa bir çubuk batırarak, bir su kabının buharlaşma oranına bakarak, bölgenin yağış ve sıcaklık düzenlerini göz önünde bulundurarak sulama zamanlamasını belirler ve önceki sulamaların ürün verimi üzerindeki etkilerini inceleyerek kararlar alır. Geleneksel yöntemler, düşük maliyetli ve uygulanabilir olmaları nedeniyle hala yaygın olarak tercih edilmekle birlikte, modern tekniklerle karşılaştırıldığında hassasiyet ve esneklik açısından sınırlamalar göstermektedir (Şahin, 2024). Bu durum, tarımsal üretimde daha yenilikçi yaklaşımların benimsenmesine zemin hazırlamıştır. Sensörlerle yapılan ölçümler ve veri tabanlı sulama tekniklerine kısmen geçilmiş olsa da, bu teknolojilerin sulama kararlarında istenen doğruluk seviyesine ulaşmadığı ve tarımsal verimliliği tam anlamıyla optimize edemediği gözlemlenmektedir. Bunun temel nedenleri arasında, sensörlerin sınırlı kapsama alanı, toprak ve bitki türlerine göre değişkenlik gösteren nem ihtiyacının tam olarak belirlenememesi, altyapı ve veri analizinde yaşanan eksiklikler ile çiftçilerin bu teknolojilere erişim ve adaptasyon konusundaki zorlukları yer almaktadır.

Çeşitli koşullarda en iyi sulama kararlarını sağlayan yöntemler arasında; FAO Penman-Monteith yöntemi, Hargreaves-Samani denklemi gibi evapotranspirasyon modelleri, tansiyometre ve toprak nem sensörleri gibi doğrudan ölçüm teknikleri, su dengesi modelleri, iklim tahmin modelleri, zaman serisi analizleri, regresyon modelleri ve makine öğrenimi tabanlı karar destek sistemleri bulunmaktadır (Taştan, 2019). Bu yöntemler, sulama kararlarının daha hassas bir şekilde alınmasına

katkı sağlarken, uygulamada karşılaşılan veri eksikliği ve bölgesel değişkenlikler gibi faktörler nedeniyle beklenen doğruluk seviyesine tam olarak ulaşamamaktadır.

Allen ve ark. (1998) tarafından geliştirilen FAO Penman-Monteith yöntemi, evapotranspirasyonu hesaplamak için kullanılan ve dünya genelinde yaygın kabul gören bir modeldir. Bununla birlikte, tansiyometre ve toprak nem sensörleri ile toprak nemine dayalı sulama kararları alınabilir (Doğru ve ark., 2012). Çiftçiler, bu sensörlerden gelen verilerle bitkilerin su ihtiyacını tahmin eder ve sulama zamanını optimize eder. Bitkilerdeki yaprak solgunluğu, renk değişimleri veya büyüme duraklamaları gibi anormal durumlar gözlemlenerek sulama gereksinimleri belirlenebilir.

İstatistiksel yöntemler, geleneksel yaklaşımların önemli bir parçasıdır. Yılmaz ve ark. (2007), geçmiş sulama verilerinin analizine dayalı regresyon modellerinin sulama ihtiyaçlarını tahmin etmede etkili olduğunu göstermiştir. Benzer şekilde, zaman serisi analizleri, yağış ve sıcaklık gibi iklim parametrelerinin de sulama zamanı tahmini için kullanıldığı bilinmektedir. Ancak bu yöntemlerin doğruluğu, veri kalitesine ve ölçüm sıklığına bağlı olarak sınırlı kalabilmektedir.

Makine öğrenmesi, sulama kararlarının verilmesinde geleneksel yöntemlere kıyasla daha hassas ve veriye dayalı çözümler sunarak tarımsal süreçlerde önemli bir dönüşüm yaratmıştır. Destek Vektör Makineleri (SVM), farklı parametreler arasındaki ilişkileri modelleyerek sulama ihtiyaçlarının tahmini için sıkça kullanılan yöntemlerden biridir (Demir ve ark., 2024). Bu yöntem, özellikle karmaşık ve çok değişkenli verilerin analiz edilmesinde etkili bir araç olarak öne çıkar. Benzer şekilde, Karar Ağacı ve Rastgele Orman algoritmaları gibi yöntemler, toprak koşulları ve iklim verileri gibi çok boyutlu verilerle sulama planlamasının otomasyonu için başarıyla uygulanmıştır (Zhang ve ark., 2015). Hosseini ve ark. (2019), Yapay Sinir Ağları (ANN) kullanarak geliştirdikleri modelle sulama zamanlamasının daha yüksek doğrulukla tahmin edilebileceğini göstermiştir. Ayrıca, Konvolüsyonel Sinir Ağları (CNN) gibi yöntemler, görüntü verilerini analiz ederek bitki sağlığı ve toprak durumunun değerlendirilmesini sağlayarak sulama kararlarına destek olmaktadır. Optimizasyon tabanlı yöntemlerden Genetik Algoritmalar, sulama sistemlerinde su dağıtımını optimize etmek için kullanılırken, Karınca Kolonisi Optimizasyonu gibi yöntemler, sulama yollarının verimliliğini artırmak için uygulanmıştır. Bu yaklaşımlar, sulama sistemlerinin enerji ve su tasarrufunu maksimize etme potansiyeline sahiptir (Rahman ve ark., 2020). Makine öğrenimi yöntemleri, sulama kararlarının daha hassas ve etkili bir şekilde verilmesine olanak tanımaktadır. Geleneksel yöntemlerin sınırlarını aşan bu teknikler, büyük veri setlerinin analiz edilmesi ve kararların optimize edilmesi süreçlerinde önemli bir rol oynamaktadır.

Türkiye'de de makine öğrenimi tabanlı sulama sistemleri giderek daha fazla kullanılmakta, verimliliğin artırılması için önemli bir araç olarak değerlendirilmektedir (Bayrakçı ve ark., 2021). Ancak sulama planlamasında hangi makine öğrenimi algoritmasının daha iyi sonuçlar verdiği değişkenlik göstermektedir. Her algoritmanın belirli veri setlerine ve

koşullara göre performansı farklılık gösterebilir. Bu çalışmanın amacı, sulama planlamasında hangi algoritmanın daha uygun olduğunu tespit ederek, doğru planlama kararlarının alınmasına fayda sağlamaktır. Su kaynaklarının daha etkin yönetilmesi ve tarımsal verimliliğin en üst düzeye çıkarılması için büyük önem taşımaktadır.

Bu çalışmada Güneydoğu Anadolu bölgesi çalışma alanı olarak seçilmiştir. Bölgedeki toplam su potansiyeli, yaklaşık 53 milyar metreküp civarındadır ve bunun büyük bir kısmı tarımsal sulama için kullanılmaktadır (GAP BKİ, 2023). Sulamanın büyük ölçüde geleneksel yöntemlerle (salma sulama vb.) yapılması, su kayıplarına ve gereksiz su kullanımına neden olmaktadır. Tarımsal sulamanın modern yöntemler ve makine öğrenimi tabanlı teknolojilerle optimize edilmesi hem su kaynaklarının korunması hem de tarımsal üretimin sürdürülebilirliği açısından büyük bir fırsat sunmaktadır. Bu süreçte kullanılacak makine öğrenimi algoritmalarının doğru seçimi, suyun verimliliğinin artırılmasında kritik bir rol oynayacaktır.

## Materyal ve Yöntem

Makine öğrenmesi, girdi olarak aldığı verileri kullanarak anlamlı bilgiler üreterek tahminler yapan algoritmalar geliştiren bir bilim dalıdır (Bayrakçı ve ark., 2021). Makine öğrenmesi, sulama yönetiminde, geçmiş verilere dayanarak su kullanımının tahmin edilmesi ve tarımsal verimliliğin artırılması için kullanılır.

Tarımsal sulama yönetiminde kullanılan makine öğrenmesi algoritmaları, veri setinin özelliklerine göre seçilir ve su yönetimini optimize etmek, çiftçilerin karar alma süreçlerini iyileştirmek için güçlü araçlar sunar.

Bu çalışmada kullanılan makine öğrenimi algoritmaları aşağıda sunulmuştur.

**Lojistik Regresyon (Logistic Regression):** Bağımsız değişkenlerin doğrusal kombinasyonunu lojistik (sigmoid) fonksiyon aracılığıyla bir olasılık değerine dönüştüren ve çıktı değişkeninin belirli bir sınıfa ait olma ihtimalini hesaplayan bir sınıflandırma yöntemidir. Genellikle iki sınıflı (binary) sınıflandırma problemlerinde kullanılır ve modelin çıktısı belirlenen bir eşik değerine göre sınıflandırılır. Lojistik regresyon, kolay yorumlanabilir olması ve hesaplama açısından verimli çalışması nedeniyle tercih edilen yöntemlerden biridir (Bircan, 2004).

**Karar Ağaçları (Decision Tree):** Veri setindeki özelliklere göre karar kuralları oluşturur ve bu kuralları kullanarak tahminler yapar. Basit ve sezgisel bir yapıya sahiptir. Kök düğüm olarak adlandırılan başlangıç noktasından başlayarak, her düğüm bir özelliği temsil eder ve dallara ayrılır. Bu dallar, karar ağacının oluşturduğu karar kurallarını temsil eder. Sonuç olarak, veri setinin her bir girdisi, ağacın dallarını takip ederek bir sonuca ulaşır ve böylece tahmin yapılır (Özcan & Özer, 2021). Karar Ağaçları algoritması, karmaşık algoritmalarla birlikte kullanılır veya birleştirilerek daha güçlü modeller oluşturulur.

**Rastgele Orman (Random Forest):** Birçok karar ağacının bir araya gelerek oluşturduğu bir modeldir. Her bir karar ağacı, veri setinin farklı alt kümeleri üzerinde eğitilir ve farklı kararlar alır. Daha sonra, bu karar

ağaçlarının tahminleri bir araya getirilerek, son tahmin yapılır. Bu yaklaşım, modelin aşırı öğrenme (overfitting) yapma olasılığını azaltır ve genellikle yüksek doğruluk oranları ile sonuçlanır (Sevgen & Aliefendioğlu, 2020). Rastgele Orman algoritması, bireysel karar ağaçlarının sınırlamalarını aşarak, büyük veri setlerinde bile etkili bir performans sergiler. Birden fazla ağacın kullanılmasıyla model, her bir ağacın yaptığı hataları minimize eder. Bu sayede, özellikle büyük ve karmaşık veri setlerinde yüksek tahmin doğruluğuna ulaşabilir.

**Destek Vektör Makineleri (SVM):** Veriler arasında bir ayrım çizgisi oluşturarak sınıflandırma yapan bir algoritmadır (Demirci, 2019). SVM, özellikle karmaşık verilerde yüksek doğruluğu ile geleneksel yöntemlere kıyasla daha etkili sonuçlar sunar.

**K-En Yakın Komşu (KNN):** Bir veri noktasını sınıflandırmak veya tahmin etmek için veri setindeki en yakın komşularını analiz eder. Algoritma, yeni bir veri noktasının etrafındaki K sayıda en yakın komşuya bakarak bu noktanın hangi sınıfa ait olduğunu veya hangi değeri alacağını belirler. Küçük veya orta ölçekli veri setlerinde oldukça hızlı analiz yapabilir. Daha büyük veri setlerinde veya çok fazla değişken içeren veri setlerinde, KNN'nin yavaş çalışması ve performans kaybı yaşaması muhtemeldir. Bu gibi durumlarda, veri boyutunu küçültmek için özellik seçimi veya boyut indirgeme teknikleri kullanılabilir, ya da daha uygun bir algoritmaya geçiş yapılabilir (Bütüner ve ark., 2023).

**Gradyan Artırma (Gradient Boosting):** Zayıf modelleri ardışık olarak eğitip birleştirerek güçlü ve yüksek performanslı tahmin modelleri oluşturur. Bu süreçte her bir yeni model, önceki modelin yaptığı hataları düzeltmeye odaklanarak nihai tahmin doğruluğunu artırır (Aydın ve ark., 2023).

**Yapay Sinir Ağları (YSA):** Yapay sinir ağları (YSA), biyolojik sinir sistemlerinden esinlenerek tasarlanmış yapay modellerdir (Öztürk & Şahin, 2018). Giriş, gizli ve çıkış katmanlarından oluşur. Giriş katmanı verileri alır, gizli katmanlar bu verilerdeki desenleri öğrenir ve çıkış katmanı tahminleri üretir. Model, ileri besleme (feedforward) ile veri akışını sağlar ve geri yayılım (backpropagation) yöntemiyle hatayı azaltarak öğrenme sürecini geliştirir. Geri yayılda, hata geriye doğru yayılıp ağırlıklar güncellenerek modelin doğruluğu artırılır (Akkaya, 2007).

## Çalışma Alanı

Güneydoğu Anadolu Bölgesi, yarı kurak ve sıcak iklim özelliklerine sahiptir. Yazlar oldukça sıcak ve kurak geçerken, kışlar nispeten ılıman ve az yağışlıdır. Yıllık yağış miktarı ortalama 400-700 mm arasında değişmekte olup, bölgenin tarımsal üretimi büyük ölçüde sulamaya dayalıdır. Yaz aylarında sıcaklıklar 40°C'yi bulabilirken, kış aylarında sıcaklık nadiren 0°C'nin altına iner. Bu durum, bölgedeki tarımsal faaliyetlerde su yönetiminin kritik öneme sahip olmasına yol açmaktadır. Özellikle yaz aylarında aşırı sıcaklık ve yetersiz yağış, su kaynaklarının dikkatli ve verimli bir şekilde kullanılmasını zorunlu kılmaktadır.

Bu çalışmada, Güneydoğu Anadolu Bölgesi'ndeki tarımsal sulama yönetiminin optimizasyonu amaçlanmış, bölgedeki su kaynaklarının verimli kullanımı ve sulama süreçlerinin iyileştirilmesi için makine öğrenimi

algoritmaları kullanılmıştır. Bu algoritmalar; iklimsel veriler, toprak nem durumu gibi parametreleri analiz ederek en uygun sulama kararlarını verir. Projede, farklı makine öğrenimi algoritmalarının performansları karşılaştırılarak hangi algoritmanın daha iyi sonuçlar verdiği değerlendirilmiştir. Bu projede, veri toplama aşamasından itibaren kapsamlı bir yöntem uygulanmıştır.

### **Veri Toplama ve Veri Analizi**

Sulama durumu tahmini için “Weather Data” veri kümesi kullanılmıştır (Meena, 2024). Hava durumu ile ilgili çeşitli özellikler içeren bu veri seti, sıcaklık (Temp\_C), bağıl nem (Rel Hum %), toprak nem oranı (soilmoisture), hava durumu (Weather) ve pompa durumu (pump status) olmak üzere beş farklı özelliği kapsamaktadır. Toplamda 10.000 satırdan oluşan bu veri seti, tarımsal sulama sistemlerinin otomatik hale getirilmesi amacıyla sensörlerden alınan verilerin analiz edilmesi için kullanılmıştır. Bu çalışmada pompa durumu (pump status), tahmin edilmesi gereken bağımlı değişken olarak tanımlanırken; sıcaklık (Temp\_C), bağıl nem (Rel Hum %), toprak nem oranı (soilmoisture) ve hava durumu (Weather) ise bağımsız değişkenler olarak kullanılmıştır.

### **Veri Tanımlama ve Düzenleme**

Veriler öncelikle eksiklikler, aykırı değerler ve tutarsızlıklar açısından incelenmiştir. Hedef özellik olan “pompa durumu” ile bağımsız değişkenler arasındaki ilişkiler değerlendirilmiş, kategorik ve sayısal değişkenler ayrıştırılarak veri temizliği yapılmıştır. Eksik veri noktaları, veri setindeki sürekli değişkenler için önceki ve sonrakilerin ortalaması (interpolasyon) yöntemi kullanılarak tamamlanmıştır. Kategorik değişkenler için ise en sık görülen (mod) değerler doldurma yöntemi tercih edilmiştir. Veri setindeki aykırı değerler, IQR (Interquartile Range) yöntemiyle tespit edilmiş ve bu değerler eşik değerler doğrultusunda düzeltilmiştir. Tutarsız veriler, manuel inceleme ve veri doğrulama kuralları kullanılarak düzeltilmiştir. Kategorik Değişkenler: Pompa Durumu: “Açık (1)”, “Kapalı (0)”; Yağış Durumu: “Fog (0)”, “Cloudy (1)”, “Rain (2)”, “Clear (3)”, “Snow (4)”

### **Veri Dönüşümü**

Bağımsız değişkenler arasında yer alan sıcaklık, bağıl nem, toprak nem oranı ve hava durumu gibi veriler, analiz öncesinde uygun formatlara dönüştürülmüştür. Özellikle kategorik değişken olan “Weather”, etiketleme (label encoding) yöntemiyle sayısal verilere dönüştürülmüştür. Ayrıca, modelin genelleme yeteneğinin test edilebilmesi için veri seti, eğitim (%80) ve test (%20) verisi olarak ikiye ayrılmıştır. Bu ayırım, modelin yeni verilere nasıl tepki verdiğini ölçmek ve aşırı öğrenmenin (overfitting) önüne geçmek amacıyla yapılmıştır.

### **Model Eğitimi**

Toprak nemi, hava sıcaklığı, bağıl nem ve hava durumu gibi bağımsız değişkenler kullanılarak pompa durumunu tahmin edebilmek amacıyla Materyal ve Yöntem bölümünde sunulmuş olan makine öğrenmesi algoritmaları uygulanmıştır. “scikit-learn” kütüphanesi kullanılarak seçilen makine öğrenmesi algoritmalarıyla modeller oluşturulmuştur. Veri seti, eğitim (train) ve test setlerine

ayrılarak model eğitimleri gerçekleştirilmiştir. Eğitim sürecinde bağımsız değişkenler (sıcaklık, bağıl nem, toprak nemi, hava durumu) kullanılarak pompa durumu tahmin edilmiştir.

### **Model Doğrulama**

Modellerin performanslarını değerlendirebilmek için doğruluk metriği (accuracy\_score) kullanılmıştır. Doğruluk, modelin doğru sınıflandırdığı örneklerin tüm örneklerle oranını ifade eder ve aşağıdaki formül ile hesaplanır:

$$\text{Doğruluk (Accuracy)} = \frac{TP + TN}{TP + TN + FP + FN}$$

TP (True Positive): Doğru pozitif tahmin sayısı

TN (True Negative): Doğru negatif tahmin sayısı

FP (False Positive): Yanlış pozitif tahmin sayısı

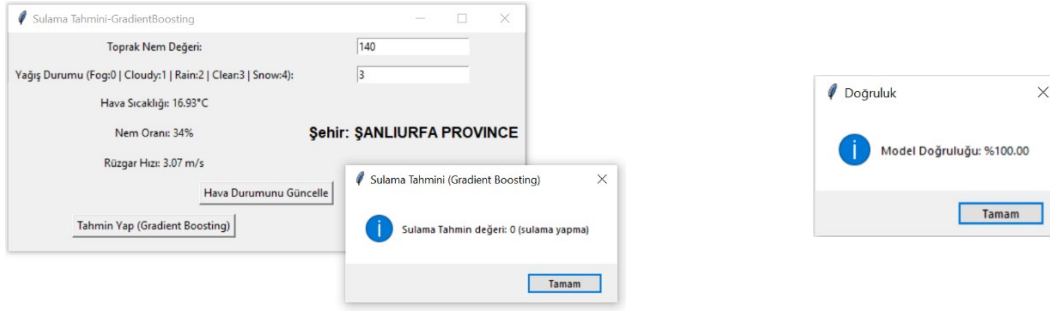
FN (False Negative): Yanlış negatif tahmin sayısı

Veri kümesi üzerinde yapılan tahminler sonucunda elde edilen doğruluk (accuracy) oranları karşılaştırılmış ve en etkili algoritmaların; Rastgele Orman (%95), Karar Ağacı (%97), Gradyan Artırma (%93) ve Yapay Sinir Ağı (%98) modeli ile tüm şehirlerde %90'ın üzerinde tahminleme doğruluğu elde edilmiştir. Diğer algoritmalar da yüksek doğruluk oranları ile (%75 üzeri) dikkate değer performans sergilemişlerdir.

### **Sonuçların Yorumlanması ve Raporlama**

Yapılan analiz sonucunda, toprak nemi 300'ün üzerindeyse, hava sıcaklığı 30,3°C'yi aşıyor ve yağış yoksa sulama önerisi yapılmıştır. Bu koşullar altında “Sulama Tahmin Değeri: 1 (sulama yap)” şeklinde karar verilmiştir; aksi durumda “Sulama Tahmin Değeri: 0 (sulama yapma)” olarak belirlenmiştir. Bu karar destek sistemi, hem otomatik sulama sistemlerinde hem de manuel sulama süreçlerinde çiftçilere rehberlik edebilir. Otomatik sulama sistemleri, makine öğrenmesi modelleriyle entegre edilerek sulama işlemini tamamen otonom hale getirebilirken; manuel sulama yapan çiftçiler, önerilen tahminlerle sulama zamanlamalarını optimize edebilirler. Elde edilen sonuçlar raporlanmış ve hangi algoritmaların en etkili olduğu, hangi durumlarda kullanılması gerektiği belirlenmiştir. Sonuçlar, çiftçilerin mevcut meteorolojik parametreleri dikkate alarak tarımsal sulama işlemlerini optimize edebilmek için makine öğrenmesi modellerini hem manuel hem de otomatik sistemlerde kullanabileceklerini göstermektedir.

Ayrıca, Open Weather Map API kullanılarak anlık hava durumu verileri sistemde entegre edilmiştir. “update\_weather” fonksiyonu ile şehirlerin hava durumu güncel verilere göre çekilmiş ve değerlendirilmiştir. Bu fonksiyon, sulama sisteminin hava durumunu gerçek zamanlı güncelleyebilmesine olanak tanımaktadır. Tahmin işlemi ise “predict” fonksiyonu ile yapılmış ve toprak nemi ve hava durumu bilgileri dayanarak sulama kararı alınmıştır. Bu süreç, Tkinter kullanılarak bir grafiksel kullanıcı arayüzü (GUI) üzerinden kullanıcıya sunulmuştur (Şekil 1). Arayüzde, hava durumu güncellemesi ve tahmin yapılması için butonlar eklenmiş, kullanıcılar kolayca güncel durumu görebilmiş ve tahmin sonuçlarına erişebilmiştir. Bu çalışmada kullanılan algoritmanın kaba kodu Çizelge 1’de sunulmuştur.



Şekil 1. Kullanıcı arayüzü  
Figure 1. User Interface

Çizelge 1. Geliştirilen algoritmanın kaba kodu

Table 1. Pseudocode of the developed algorithms

Adım	Açıklama
Başla	Uygulamanın çalışmaya başlaması.
Veri Kümesini Yükle	Weather-Data1.csv dosyasından verileri yükle.
Modeli Oluştur ve Eğit	Karar Ağaçları modelini oluştur. Bağımsız değişkenleri (X) ve hedef değişkeni (y) belirle. Modeli eğit.
Pencereyi Başlat	Tkinter arayüzünü oluştur.
Etiketleri ve Giriş Kutularını Oluştur	Hava durumu bilgileri için etiketler, kullanıcı girişleri için kutular oluştur.
Butonları Ekle	“Hava Durumunu Güncelle” ve “Tahmin Yap” butonlarını ekle.
Fonksiyon: hava_durumu_al(api_anahatari, sehir)	API anahtarı ve şehir adı ile URL oluştur. API’den hava durumu verilerini al ve JSON formatında döndür.
Fonksiyon: hava_durumu_guncelle()	Hava durumu verilerini almak için hava_durumu_al fonksiyonunu çağır. Sıcaklık, nem, şehir, rüzgar hızı bilgilerini ekranda göster.
Fonksiyon: tahmin_yap()	Kullanıcıdan toprak nemi ve yağış durumu bilgilerini al. Model için giriş oluştur ve tahmin yap. Tahmine göre sulama mesajını göster. Modelin doğruluk oranını hesaplayıp ekranda göster.
Pencereyi Çalıştır	Tkinter ana döngüsünü başlatarak uygulamanın çalışmasını sağla.
Biti	Uygulama sonlanır.

Bu çalışmada kullanılan makine öğrenmesi algoritmaları, Türkiye’nin Güneydoğu Anadolu Bölgesi’ndeki Şanlıurfa, Mardin, Diyarbakır, Gaziantep ve Adıyaman illerine ait tarımsal sulama verileri üzerinde uygulanmış ve elde edilen sonuçlar karşılaştırmalı olarak analiz edilmiştir. Bu veriler, sulama yönetimini optimize etmek amacıyla çeşitli bağımsız değişkenlerden (örneğin: hava sıcaklığı, toprak nem oranı, bağıl nem, yağış durumu gibi meteorolojik veriler) ve bağımlı değişken olan “pompa durumu”ndan (sulama yapılıp yapılmadığı bilgisi) oluşmaktadır.

Algoritmaların performansı, bu veriler kullanılarak değerlendirilmiş ve hangi algoritmaların sulama yönetiminde daha etkili olduğu incelenmiştir. Veriler, sulama yönetimini iyileştirmeyi amaçlayan tahminler yapmak için işlenmiş ve karşılaştırmalı analizlerde hangi modelin daha yüksek doğruluk sağladığı belirlenmiştir.

## Bulgular

Bu çalışmada, Güneydoğu Anadolu Bölgesi’nin hava koşulları ve toprak yapısı değerlendirilerek tarımsal sulama tahmini için farklı makine öğrenmesi algoritmalarının performansları karşılaştırılmıştır. Bölgenin yarı kurak ve sıcak iklimi, su kaynaklarının sınırlı olması ve tarımsal üretimin büyük ölçüde sulamaya bağlı olması nedeniyle doğru sulama yönetimi hayati bir öneme sahiptir.

Çalışmada, Weather Data veri kümesi kullanılarak sulama durumu tahmini gerçekleştirilmiştir. Bu veri kümesi, hava sıcaklığı, nem, yağış miktarı ve toprak nemi gibi değişkenleri içermektedir. Bölgedeki iller üzerinde yapılan deneylerde, Karar Ağaçları (Decision Tree), Rastgele Orman (Random Forest), Gradient Boosting, Yapay Sinir Ağı (Artificial Neural Network - ANN), Destek Vektör Makineleri (Support Vector Machine - SVM), K-En Yakın Komşu (KNN), Naive Bayes ve Lojistik Regresyon algoritmaları değerlendirilmiştir.

Her bir algoritma, aynı eğitim ve test veri seti kullanılarak eğitilmiş ve sonuçları Çizelge 2’de sunulmuştur. Elde edilen bulgular, Yapay Sinir Ağı (ANN), Karar Ağaçları ve Gradient Boosting algoritmalarının en yüksek doğruluk oranlarına ulaştığını göstermektedir.

Yapay Sinir Ağı, %98 doğruluk oranıyla en yüksek performansı göstermiş, Karar Ağaçları algoritması ise %97 doğruluk oranıyla oldukça başarılı sonuçlar vermiştir. Şanlıurfa, Diyarbakır ve Mardin gibi tarımsal üretimin yoğun olduğu illerde bu iki algoritma özellikle öne çıkmıştır. Diğer algoritmalarla il bazında yapılan karşılaştırmalar da Çizelge 3’de gösterilmiştir. Buna göre, Rastgele Orman algoritması %95 doğruluk oranına ulaşmış, ancak K-En Yakın Komşu (KNN) ve Naive Bayes gibi daha basit algoritmaların doğruluk oranları %88 ve %84 civarında kalmıştır. Bu bulgular, daha karmaşık ve büyük veri setlerinde basit algoritmaların etkinliğinin sınırlı olduğunu göstermektedir.

Çizelge 2. Makine öğrenmesi algoritmalarının performans analizi  
Table 2. Performance analysis of machine learning algorithms

Algoritma	Doğruluk (%)
Karar Ağaçları	97
Rastgele Orman	95
Destek Vektör Makineleri	88
Gradient Boosting	93
Yapay Sinir Ağı (ANN)	98
K-En Yakın Komşu (KNN)	83
Lojistik Regresyon	75

Çizelge 3. İl bazında makine öğrenmesi algoritmalarının doğruluk metriği performansı (%)  
Table 3. Accuracy metric performance of machine learning algorithms for cities

İl	Karar Ağaçları (%)	Rastgele Orman (%)	SVM (%)	Gradient Boosting (%)	Yapay Sinir Ağı (%)	KNN (%)	Lojistik Regresyon (%)
Adıyaman	96	94	87	92	97	88	85
Batman	95	93	86	91	96	87	84
Diyarbakır	98	96	89	94	99	90	86
Gaziantep	96	94	89	92	97	88	85
Kilis	94	92	89	90	95	86	83
Mardin	97	95	89	93	98	89	84
Siirt	95	93	89	91	96	87	83
Şanlıurfa	97	95	89	93	98	89	86
Şırnak	94	92	89	90	95	86	83

Çizelge 3'deki sonuçlar incelendiğinde, ANN ve Karar Ağaçları'nın, Şanlıurfa ve Diyarbakır gibi geniş tarım arazilerine sahip illerde en iyi sonuçları verdiği görülmektedir. Bu illerde tarımın büyük ölçüde sulamaya bağımlı olması ve su kaynaklarının dikkatli yönetilmesi gerekliliği, bu algoritmaların önemini bir kez daha ortaya koymaktadır.

Bu algoritmalar, sulama planlamasında verimliliği artırarak su israfını minimuma indirmiştir. Özellikle toprak nemi, hava sıcaklığı, bitki türü ve yağış miktarı gibi çevresel faktörler analiz edilerek, her tarım alanı için en uygun sulama zamanı ve miktarı belirlenmiştir. Yapay Sinir Ağı, sulama yönetimi için büyük veri setleriyle çalışabilmesi ve değişkenler arasındaki karmaşık ilişkileri başarılı bir şekilde modelleyebilmesi sayesinde en iyi sonuçları sağlamıştır. Karar Ağaçları ise, daha anlaşılır ve takip edilebilir karar süreçleri sunması nedeniyle tarım üreticileri için pratik bir karar destek aracı olarak kullanılabilir.

## Tartışma

Güneydoğu Anadolu Bölgesi'nde sulama yönetimi, makine öğrenmesi algoritmaları sayesinde daha etkin hale getirilebilmektedir. İklimsel koşulların kurak ve düzensiz olması, tarımda su yönetiminin önemini artırmakta, ANN ve Karar Ağaçları algoritmaları bu ihtiyaçlara en uygun çözümleri sunmaktadır. ANN, karmaşık ve büyük veri setlerini başarıyla işleyerek yüksek doğruluk oranlarına ulaşmıştır. Karar Ağaçları ise, açıklayıcı yapısı ile tarım yöneticilerinin sulama kararlarını daha net bir şekilde almasına olanak sağlamaktadır.

Makine öğrenmesi algoritmalarıyla elde edilen bu bulgular, tarımsal üretimde sürdürülebilirlik ve su tasarrufunun sağlanması için önemli bir fırsat sunmaktadır.

Gelecekte IoT tabanlı veri toplama sistemlerinin yaygınlaşması ve bu verilerin makine öğrenmesi algoritmalarıyla işlenmesi, sulama yönetimini daha dinamik ve veriye dayalı bir yapıya kavuşturabilir. Böylece, su kaynakları daha verimli kullanılacak ve tarımsal üretim verimliliği artırılacaktır. Bu çalışmalar, özellikle Güneydoğu Anadolu gibi su kaynakları sınırlı olan bölgelerde büyük bir potansiyel taşımaktadır. Makine öğrenmesi algoritmaları ile entegre edilen bu sistemler, suyun ekonomik ve sürdürülebilir bir şekilde yönetilmesine katkıda bulunacaktır.

## Sonuç ve Öneri

Bu çalışma, Güneydoğu Anadolu Bölgesi'nde tarımsal sulama yönetimi için farklı makine öğrenmesi algoritmalarının performanslarını değerlendirmiştir. Elde edilen bulgular, Yapay Sinir Ağı (ANN) ve Karar Ağaçları algoritmalarının sulama tahminlerinde en yüksek doğruluk oranlarına ulaştığını göstermektedir. Şanlıurfa, Diyarbakır ve Mardin gibi tarımsal üretimin yoğun olduğu illerde ANN algoritması %98, Karar Ağaçları algoritması ise %97 doğruluk oranı sağlamıştır. Bu sonuçlar, gelişmiş algoritmaların tarımsal sulama yönetiminde etkin bir karar destek sistemi olarak kullanılabilirliğini ortaya koymaktadır.

Makine öğrenmesi algoritmalarının tarımsal sulama sistemlerine entegrasyonu, su kaynaklarının daha verimli yönetilmesini ve israfın önlenmesini sağlayabilir. Özellikle hava durumu, toprak nemi, bitki türü ve yağış miktarı gibi değişkenler analiz edilerek, her bölge için optimum sulama stratejileri geliştirilebilir. ANN gibi karmaşık modeller, büyük veri setlerinden anlamlı sonuçlar çıkararak iklim değişikliği gibi çevresel faktörlere uyumlu dinamik sulama yönetimi sunabilir.

Gelecekteki çalışmalar için şu öneriler getirilebilir:

- *IoT ve sensör teknolojileri ile entegrasyon:* Toprak nem sensörleri ve hava durumu istasyonlarından gerçek zamanlı veri toplanarak modellerin doğruluk oranları artırılabilir.
- *Bölgesel ve ürün bazlı optimizasyon:* Farklı bitki türleri ve toprak yapıları göz önünde bulundurularak bölgeye özel sulama planları geliştirilebilir.
- *Derin öğrenme modellerinin karşılaştırılması:* ANN'nin yanı sıra derin öğrenme tabanlı algoritmalar (CNN, LSTM, Transformer vb.) test edilerek, uzun vadeli tahminlerdeki başarısı değerlendirilebilir.
- *Karar destek sistemlerinin geliştirilmesi:* Çiftçilerin ve tarım yöneticilerinin kolayca kullanabileceği, mobil veya web tabanlı arayüzler oluşturularak, sulama tahminlerinin sahada uygulanması sağlanabilir.

Bu araştırmanın sonuçları, su kıtlığıyla mücadelede ve tarımsal verimliliğin artırılmasında makine öğreniminin büyük bir potansiyel taşıdığını göstermektedir. Gelecekte daha geniş kapsamlı çalışmalar ve saha uygulamaları ile bu sistemlerin tarım sektörüne entegrasyonu hızlandırılabilir.

## Beyanlar

### *Yazar katkısı*

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder

### *Çıkar Çatışması*

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan eder.

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## Exploring Farmers' Resilience: Climate Change and Sustainable Adaptation Strategies in the Agricultural Sector of Nepal

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### ABSTRACT

Agriculture is a cornerstone of the economy, providing livelihoods for a significant portion of population. However, climate change significantly affects people, their lifestyles, and the ecosystems posing a critical challenge to the global community, particularly the underprivileged in developing nations. Recognizing the indispensable role of agriculture and the challenges posed by a changing climate, this paper emphasizes the paramount need for proactive adaptation strategies. Central to these strategies is the pivotal concept of Climate-Smart Agriculture (CSA), a multifaceted approach that encompasses a range of practices, including agroforestry, conservation agriculture, and the adoption of climate-resilient crop varieties. Delving deeper, the paper navigates through the farmer's perceptions, unraveling their understanding of climate change, and the complex barriers like social barriers, institutional limitations, financial barriers, and limited awareness that impede effective adaptation, and illuminates the instrumental roles that governmental bodies and institutions, and extension agents play in shaping and fostering climate-resilient practices. Collaboration between local communities, governments, and non-governmental organizations is essential to ensure the successful implementation of sustainable adaptation strategies. Embracing sustainable and forward-thinking approaches, particularly CSA, including agroforestry, conservation agriculture, water management techniques, climate-resilient crop varieties, ICT, and climate-smart pest management, the agricultural sector gains the potential to bolster its resilience against climate-induced disruptions, ensuring consistent agricultural output that contributes significantly to broader food security initiatives.

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### Introduction

Agriculture is a vital part of the economy in Nepal, with about 65.1% of the population relying on it for their livelihoods. It is a major source of employment and sustenance, and its contribution of 24.1% to the GDP highlights its economic importance (MoF, 2023). However, the susceptibility of agriculture to climate change underscores the urgency of implementing effective adaptation strategies to ensure its continued stability and productivity. Climate change involves the prolonged transformation of average climatic conditions, driven by both natural processes and human activities. It impact lives, ecosystems, and global development, particularly affecting vulnerable populations in developing nations (Khanal, 2009; Weber, 2010). By 2030, South Asia is expected to witness substantial negative impacts on crop production due to climate change, with staple crops such as rice potentially facing yield reductions of around 10%, while maize and millet might experience even more significant reductions exceeding 10% (Lobell et al., 2008).

Undoubtedly, climate change poses substantial challenges to global food security, projected to exacerbate by factors like population growth, urbanization, economic development, and natural hazards. An estimated 9 billion people may have worsening living conditions by 2050, increasing poverty and hunger (FAO, 2017). Climate change poses a significant risk to African countries, especially in drylands where 70% of the population depends on these areas for everyday survival. By 2055, there might be an estimated 10% decrease in maize production in Latin America and Africa, translating into \$2 billion in losses annually (Altieri & Koohafkan, 2008). These yield declines are predicted to be worse as temperatures rise and rainfall patterns change (Altieri & Koohafkan, 2008; Jones & Thornton, 2003). To address the looming challenges and guarantee the resilience of food systems in the face of changing climate conditions, it is essential to deploy effective adaptive techniques in agricultural production (Teklewold, 2019).



The concept of climate-Smart Agriculture (CSA) emerges as a critical strategy that aligns with the growing need for sustainable and adaptable agricultural solutions in the face of climate change. It addresses climate change by implementing adaptive techniques that mitigate the adverse effects on crops while simultaneously enhancing agricultural resilience and sustainability (Barasa et al., 2021). The key focus of climate smart agriculture is on food security, productivity, profitability, sustainability, GHG emission reduction, vulnerability reduction, and capacity building (Adesipo et al., 2020). It aims to find trade-offs and synergies among the three core pillars of CSA: food security, adaptation, and mitigation to optimize balance between improving agricultural output and reducing environmental impacts (Nagothu & Kolberg, 2016; Campbell et al., 2014). To achieve this balance, CSA builds on the principles of sustainable intensification that not only enhance the farm production but also maintain good ecological sustainability. As a part of CSA, sustainable intensification combines local knowledge, and land conservation measures that boosts the productivity and enhance the soil health and farm profitability. This strategy is beneficial against the climate risk while simultaneously lowering GHG emission (Engel & Muller, 2016). Furthermore, CSA prioritizes sustainable methods in low-income agricultural systems, encompassing practices such as conservation agriculture, agroecology, ecosystem services utilization, small-scale irrigation, aquaculture, and agroforestry, while also advocating for integrated approaches like combined crop-livestock systems, landscape management, and reduced tillage techniques (Chandra et al., 2018).

This review paper explores the challenges posed by climate change to agriculture and provides an overview of climate change trends in Nepal, elucidates the climate-related challenges faced by farmers, examines their perceptions of climate change, and discusses sustainable adaptation strategies, the role of government and institutions, and the barriers to effective implementation. Lastly, it explores the benefits of climate adaptation practices and offers recommendations for future prospects. By comprehensively addressing these aspects, this paper aims to provide a holistic understanding of climate-smart agricultural approaches for building resilience and ensuring sustainable food systems.

## Materials and Methodology

The content of this review is solely based on information from qualitative sources of existing literature like articles, journals, and books. The relevant information was gathered through extensive exploration of platforms like Google Scholar. The review's findings incorporate a range of analyses conducted across various articles. The methods and tools used by different researchers for analysis are mentioned below:

- Multivariate probit model (MVP) and the random-effects ordered probit model (Teklewold et al., 2019)
- Leximancer™ Version 4 (Chandra et al., 2018)
- Multi-regional computable general equilibrium (CGE) models (Chalise et al., 2017)
- Questionnaire, FGD, KII (Bom et al., 2023)

- Climate trend analysis (Manandhar et al., 2011)
- Statistical Software 'R' (version 3.4.3) and Spearman correlation test (Amir et al., 2020)
- Snowball sampling, RQDA computer program, R package for analysis (Ghimire et al., 2022)

## Findings

### *Overview of Climate Change Trends in Nepal*

Nepal, with its diverse geography in a relatively small area and rugged terrain (Shrestha & Aryal, 2011), faces the impact of varying climatic elements like temperature, solar radiation, and rainfall, all of which can significantly affect its agricultural productivity. Despite ongoing efforts to mitigate climate change repercussions, Nepal's agricultural sector remains beset by these challenges. Over three decades from 1975 to 2006, Nepal has witnessed a gradual temperature rise of 1.8°C, with an annual average increase of 0.06°C, leading to recurring issues including droughts, intense flooding, landslides, and adverse effects on crop yields, highlighting the impact of climate change in agriculture (Malla, 2008). Increasing temperatures and shifting precipitation patterns across various region due to climate change are expected to impact the availability of water resources in the future (Dahal et al., 2020). Globally, Nepal ranks 4<sup>th</sup> in vulnerability to climate change, 11<sup>th</sup> for earthquakes, and 13<sup>th</sup> for food-related risks (DCA, 2021). Highlighting this vulnerability, a recent climate change vulnerability and risk assessment (MoFE, 2021b) indicate that a substantial portion of Nepal's districts (50 out of 77 districts) exhibit high levels of vulnerability to climate change impacts.

### *Climate change-related Challenges Faced by Farmers*

The effects of climate change are not limited to just environmental concerns but have significant social and economic implications, particularly for vulnerable populations. Extreme events like floods and droughts can have profound and far-reaching impacts on crop productivity and food supply in South Asia, and these effects can indeed lead to upward pressure on food prices (Chalise et al., 2017). Rising temperatures and unpredictable rainfall led to poor crop growth, causing many farmers to switch from growing grains to cultivating vegetables (Shrestha & Nepal, 2016). The study conducted by Rayamajhee et al (2021) on the impact of climate change on rice production has revealed that 1°C rise in average summer temperature has led to a substantial reduction of 4183 kg in rice production, highlighting the tangible impact of climate change on agricultural outcomes. It was reported that the severe winter drought during 2008-2009 led to a 14.5% decrease in wheat production and a 17.3% reduction in barley production, significantly impacting local food security and livelihoods in Nepal (Sapkota & Rijal, 2016). In 2005/06, Eastern Terai faced a rain deficit causing a 12.5% reduction in national crop production as 10 % of agricultural land remained fallow due to insufficient rainfall, while heavy rain and floods in the Midwest Terai led to a 30% decrease in production (Regmi, 2007). Climate change not only directly impacts production but also exacerbates issues through increased pest infestations (Gruda et al., 2019).

Research shows that there was 57% more damage in soybean field by different pests when it was grown under the elevated CO<sub>2</sub> level as compared to normal atmospheric condition (Hamilton et al., 2005). Similarly, as compared to normal condition, the population of wireworms was high in the upper portion of soil due increased summer rainfall (Gregory et al., 2009). Moreover, it can even influence conditions within protected environments. Teitel et al. (2005), for example, showed that in a naturally ventilated greenhouse used for pepper cultivation, the population of whiteflies was more significant near the roof opening, where wind entered the greenhouse. This suggests that the introduction of insects into greenhouse conditions may be impacted by modifications in wind patterns brought on by climate change.

#### ***Farmer's Perception on Climate Change***

According to Bom et al. (2023), Farmers' attitudes about climate change significantly impact how they respond to its effects in various economic and social contexts within a given area. The willingness of producers to implement climate change adaptation strategies can be strongly influenced by their perception of changes in local weather patterns (Khanal et al., 2018). Many farmers recognize climate change and use their indigenous knowledge and personal experience to adapt, which is a positive step. Indigenous knowledge act as a useful resource that may inform and improve formal methods for climate adaptation (Manandhar, 2011). At the local level, collaborative methods that fuse scientific ideas with traditional knowledge might improve the sustainability and efficacy of climate resilience initiatives. More and more people are realizing that the best ways to deal with the problems caused by climate change are to embrace local knowledge, include agricultural communities in managing their rural landscapes, and use bioresources sustainably (Negi et al., 2017). In response to issues like erratic rainfall and water shortages, farmer have adopted strategies such as growing improved varieties, raising goats to diversify income, and planting fodder trees to ensure stable supply to animal feed. These actions demonstrate initiatives to improve resilience and adjust to the effects of climate change (Baul & McDonald, 2014).

Farming communities are well-informed about weather and climate change, they understand how it affects their crops, livestock, and overall well-being. Amir et al. (2020) report that agricultural output declines have impacted livelihoods and increased vulnerability. However, they see opportunities for adaptation through technical and financial help. According to Abid et al. (2015), the majority (58%) of farm households have altered their agricultural practices in response to climate change because of widespread awareness. The opinion of farmer coincided with short-term weather data, which shows greater temperatures and less precipitation. Their readiness to change was most influenced by perceived behavioural control elements, followed by attitude and subjective standards (Arunrat et al., 2017).

Budhathoki and Zander (2020) found that farmers' perceptions of maximum temperature trends across stations and planting seasons matched actual climate data. Over a 30-year period, 80.7% of respondents in rural farmlands reported rising temperatures, while more than 90% reported decreasing rainfall. According to Devkota et

al. (2018), 73% of farming households have employed adaptive methods to offset the detrimental effects of climate change on rice cultivation, with higher adoption rate observed in the terai region compared to hilly areas.

#### ***Sustainable Adaptation Strategies for Climate Change Mitigation***

In the global context, Nepalese agriculture faces increasing challenges due to the impacts of climate change. Farmers are experiencing shifts in weather patterns, uncertain rainfall, and more frequent extreme events, which affects the traditional farming practices in many ways. In order to cope up with these challenges, it is crucial to develop and implement sustainable adaptation strategies that enhance both climate resilience and contribute to the long-term sustainability of agricultural systems (Rasul & Sharma, 2016).

*Agroforestry* is the key sustainable adaptation strategy, which is an integrated land-use approach that combines trees, crops, and livestock on the same land. Agroforestry systems provide a wide array of benefits, like biodiversity enhancement, carbon sequestration, microclimate regulation, and diversified income sources (Nair et al., 2021). Alley cropping, as an agroforestry practice helps to foster not only ecological resilience but also the economic resilience by minimizing the risks of crop failure and offering alternative income streams (Quinkenstein et al., 2009). Also, by integrating tree species with traditional crops, farmers can create beneficial microclimates that protect the crops from extreme heat and wind, mitigate soil erosion, as well as improve soil health. Additionally, trees serve as carbon sinks, which contribute to climate change mitigation (Aba et al., 2017). Globally, agroforestry systems are known for their dual benefits like protecting farmers from climate changes and sequestering carbon, resulting in lower net greenhouse gas emissions compared to other agricultural intensification methods (Altieri & Koohafkan, 2008).

*Water management and irrigation practices* are also a crucial practice that falls under sustainable adaptation practices especially at a changing precipitation pattern. Implementing modern irrigation techniques, such as drip or sprinkler systems, along with rainwater harvesting, can significantly enhance water efficiency (Handy et al., 2003). Proper water management ensures sufficient moisture for crops by minimizing water wastage, especially during periods of scarcity (Zahoor et al., 2019). Moreover, there are some advanced techniques which detects the plant water requirement rather than just soil moisture content (Erdem et al., 2010). This offers more precise irrigation solutions during the period of scarcity. Nepalese farmers can better manage water resources and enhance resilience against the erratic rainfall by integrating traditional knowledge with modern technology, ultimately fostering agricultural resilience.

*Soil health management and conservation techniques* form another vital pillar of sustainable adaptation. To maintain good crop productivity and climate resilience, different soil management techniques like cover cropping, no-till farming, and organic soil amendments, are highly important. These practices improve soil structure, moisture retention, and nutrient cycling. Additionally, managing carbon and nitrogen cycle by applying the strategy of soil

amendments and precision agriculture can help to manage issues like soil erosion, low soil quality, and reduced crop productivity (Cordovil et al., 2020; Handayani & Folz, 2021). Mulching, in particular, serves as a good agricultural technique with multiple benefits, particularly in the context of climate-smart farming. Mulching mitigates water stress by retaining soil moisture and reducing evapotranspiration losses. Also, in case of organic mulch, it enriches the soil with organic matter. In climate-resilient approach, mulching stands out as a good strategy as farmers often use ground-covering plants or straw mulches to lower radiation and heat, prevent moisture loss, and absorb the impact of rain and hail on newly planted surfaces (Altieri & Koohafkan, 2008). Additionally, by embracing practices like zero tillage in integration with mulching and other soil management methods farmers enhance carbon sequestration within the soil. Natural mulch materials offer the advantages of moderating soil temperatures, minimizing disease and pest outbreaks, and safeguarding soil moisture levels. This integrated approach of mulching as a climate resilient strategy helps to promote resource-efficient and sustainable farming practices (Mulumba & Lal, 2008; Nyong et al., 2007).

*Conservation agriculture* offers the potential to reverse the degradation caused by conventional tillage. By reducing soil disturbance, promoting crop diversity, and maintaining soil cover, conservation agriculture improves soil health and mitigate greenhouse gas emissions and fertilizer usage, and enhance carbon sequestration (Pisante et al., 2015). The foundation of sustainable agricultural practices lies in the principles of reducing soil disturbance, practicing crop rotation, and maintaining soil cover (Malhi et al., 2021). Several factors influence the adoption of conservation agriculture, including perceived individual benefits, effective market exchange for resources, economic incentives, farmer organization development, and collaborative efforts between farmer groups and institutions (Malhi et al., 2021).

*Climate-smart pest Management (CSPM)* is another strategy of sustainable adaptation that helps to addresses the complex challenges posed by climate change in agriculture. When climate change occurs, the shifts in climate results shifts in temperature, precipitation patterns, and extreme weather events, which influence the dynamics of pests. CSPM enables effective management of new and existing crop pests, reduces crop losses and enhances farmer livelihoods by addressing the challenges posed by climate change-induced pest impacts. It entails various components like prevention, early warning systems, and adaptive strategies, which allow timely intervention and reduce the likelihood of pest outbreaks. By utilizing the climate and pest monitoring, coupled with risk forecasting, CSPM helps prevent the buildup and emergence of pest issues (Bouri et al., 2022), thereby increasing agricultural system resilience and reducing vulnerability to climate-induced disruptions. Furthermore, it promotes tailored pest control methods, such as integrated pest management (IPM) and agroecological practices. CSPM is the best strategy that benefit farmers directly by increasing income and food security. A study by N'dakpaze (2022) has reported that using CSPM technique in tomato production has resulted in improved production with reduction in chemical use, pest density, and environment pollution. As

it has broader positive impacts on ecosystems, economy, and society, it requires coordinated efforts from extension, research, and the public and private sectors for successful implementation (Heeb et al., 2019; Ayoub et al., 2022).

*Use of climate-resilient crop varieties* is also one of the climate-smart farming technique to withstand the challenges posed by changing climatic conditions, such as increased temperatures, altered precipitation patterns, and more frequent extreme weather events. In the face of climate uncertainties, these varieties are bred for traits like drought tolerance, heat resistance, and disease resilience, to ensure stable and productive agricultural systems (Acevedo et al., 2020; Maheshwari et al., 2019). In the area with the high problem of drought, a drought-resistant variety could enhance resilience in the water scarcity period. For instance, wheat can be cultivated in the water scarce area rather than rice, as wheat requires less irrigation. Smallholder farmers have also explored drought-resistant crop varieties as adaptive measures to climate change (Ngigi, 2009). These climate-resilient crops help to ensure stable yields and contribute to resource efficiency by reducing the need for excessive water or inputs. Moreover, they reduce dependence on chemical pesticides by incorporating natural resistance mechanisms against pests and diseases.

*Information and Communication Technology (ICT)* has emerged as a powerful enabler of climate adaptation strategies within the agricultural sector with wide array of technologies, from traditional ones like landlines and television to rapidly advancing systems such as the internet, mobile communication, social networks, and remote sensing (Eakin et al., 2014). By using different kinds of sensor networks and remote sensing technologies, farmers can gather real-time data on various environmental factors, such as temperature, humidity, soil moisture, and rainfall patterns. When they have access to such data, they can make informed decisions on when to plant, irrigate, and harvest, aligning their practices with prevailing climate conditions. This helps in two ways; to optimize resource utilization and helps mitigate potential losses caused by extreme weather events, such as droughts or heavy rains (Jayaraman et al., 2016; Ajwang & Nambiro, 2022).

ICT also plays an important role in knowledge-sharing and capacity-building among farming communities. There are various channels such as online platforms, mobile apps, and virtual networks for farmers to access information about climate-resilient practices, innovative cultivation techniques, and pest management strategies (Gbangou et al., 2020; Eakin et al., 2014). Moreover, ICT supports supply chain management and market access (Zhang et al., 2016). Also, digital platforms help to facilitate the direct connection between producers and consumers, as a result there will be less intermediaries which ultimately improve the efficiency of agricultural markets (Kos & Kloppenburg, 2019). Farmers can secure their better prices for their output, and it would help to reduce the post-harvest losses, which will contribute to overall sustainability. Moreover, ICT has its role in mitigation of carbon emissions by automating and optimizing various sectors, reducing energy use, promoting electric vehicles, and minimizing travel through virtual alternatives, contributing to significant climate change mitigation (Ajwang & Nambiro, 2022).

### **Factors Affecting the Adoption of Different Strategies**

According to Fosu-Mensah et al. (2012), factors such as access to extension services, availability of credit, soil fertility, and land tenure play a critical role in shaping the perceptions and decisions of farmers regarding adaptation. Similarly, according to Abid et al. (2015) education, farm experience, household size, land area, tenancy status, tube well ownership, market information access, weather forecasting, and agricultural extension services are the influential factors in farmers' adaptation decisions. Similarly, age, years of education, climate change training, and participation in cooperatives were identified as significant factors affecting individuals' awareness of climate change (Adhikari et al., 2022). When the household head is educated there was a positive and significant influence on adaptation. Likewise, it was positive with variables like household head's education, household size, male household head, livestock ownership, received advice on production, credit access, and temperature. On the other hands, larger farm size and high annual precipitation were negatively correlated with adaptation (Deressa et al., 2011). Moreover, the situation like government extension services, participation in farmer field school, subsidies, access to energy, and perceptions of climate shocks also influence the farmer's adoption of different strategies (Tanti et al., 2022).

### **Challenges and Barriers**

There are multiple challenges and barriers associated with the climate change adaptation strategies which hinders to mitigate the impacts of changing climatic conditions (Hamin et al., 2014). A diverse range of barriers such as social, institutional, cognitive, and financial barriers are associated. A comprehensive understanding of these barriers is essential for crafting robust and successful adaptation measures (Biesbroek et al., 2013). In social barriers the cognitive behavior and normative practices, often lead to an underestimation of future climatic impacts and a preference for traditional methods over innovative solutions (Jones & Boyd, 2011). Institutional barriers, on the other hand, are associated with unequal political power and caste-related neglect, limiting the access to resources for marginalized communities and hindering their ability to respond effectively to climatic challenges (Jones & Boyd, 2011).

Uncertainty and lack of awareness pose another formidable barrier. The lack of certainty regarding the impacts of climate change and the effectiveness of adaptation strategies can hinder motivation to become more aware of the risks. On the other hand, limited awareness can negatively affect the efforts aimed at reducing uncertainty, underscoring the intricate bidirectional relationship between awareness-building and uncertainty reduction (Eisenack et al., 2014). The situation of no adequate budgets, financial crises, or competing priorities falls under the financial constraints which persistently hinder adaptation efforts. These financial challenges furthermore magnify other barriers related to information gaps, coordination challenges, and personal beliefs, in turn exacerbating the complexities faced in implementing climate adaptation strategies (Eisenack et al., 2014; Antwi-Agyei et al., 2015).

Additionally, the land tenure status and farm size also emerge as a crucial determinant in the adoption rate of climate change adaptation measures. Constraints related to land availability, high farmland costs, and communal land ownership systems all contribute to the intricate web of barriers to a climate change adaptation strategy (Ozor et al., 2010).

### **Government and Institutional Roles in Climate-Smart Agriculture**

The Government of Nepal plays a crucial role in climate adaptation through the implementation of programs like the National Adaptation Programme of Action, Climate Change Policy, Local Adaptation Plans of Action, and the promotion of climate-friendly agricultural practices, demonstrating its commitment to enhancing resilience and sustainability in the face of climate challenges (Paudel et al., 2017). The objective of the Agriculture Development Strategy (ADS) is to improve climate resilience and sustainability by strengthening the capacities of farmers and extension workers in Climate-Smart Agriculture (Khatri-Chhetri et al., 2017). In addition to tackling climatic concerns through capacity-building and enhanced information sources (radios, television), extension agents help implement government plans at the field level by providing advice on farming methods, pest control, and sustainability (Antwi-Agyei & Stringer, 2021).

The availability of meteorological information, farmer training, and the ability to access Extension services are all elements that affect how effective climate adaptation strategies—such as Direct Seeded Rice (DSR), enhanced crop varieties, and sophisticated irrigation techniques—are. These factors work together to influence how well these methods work to solve the climatic issues in agriculture. Access to Extension services, training, and meteorological data all have a favourable impact on the adoption of these techniques and improve their execution (Regmi et al., 2023).

Multiple institutions work together under Nepal's Climate Smart Village (CSV) approach to address how climate change affects agriculture. Interventions are customised to the local environment, and sustainable farming practices are promoted (Ghimire et al., 2022). Global organisations such as the FAO and the World Bank promote the use of contemporary agricultural techniques in order to improve adaptive capacity and climate-smart agriculture (CSA) (Iqbal et al., 2021). Climate-smart solutions for widespread adoption in agriculture in the face of climate change are being researched by the CGIAR initiative CCAFS (Vernooy & Bouroncle, 2019). A key component of the Climate Smart Village (CSV) concept, CCAFS integrates socio-economic and CSA components, highlighting the necessity of improving the capacities of local leaders and government representatives (Pudasaini et al., 2019).

### **Benefits**

The adoption of sustainable climate smart adaptation practices has a crucial role in improving crop yields and increasing the income for farmers (Ghosh, 2019). Furthermore, modest investments in small-scale infrastructure, such as the enhancement of irrigation

systems and the establishment of facilities for seed storage, present an economical motivation for policymakers and donors to support farmers in enhancing their productivity and ensuring more effective harvest protection (Azadi et al., 2021). Adoption of zero tillage technique for wheat cultivation is gaining traction in South Asia as it saves 15-16 % cost. Also, there is a higher and more consistent outputs in wheat and maize when farmers use this practice (Powlson et al., 2014). Similarly, when the farmers apply diversified farming system along with cropping drought-resistant varieties, there was greater stability and profitability in yield (Singh & Singh, 2017). Utilizing resistant varieties through crop breeding offers numerous benefits. Some of them are tolerance to thermal stresses, vernalization needs, heat shocks, and drought conditions. Besides tolerance, these varieties also exhibit resistance to pests and diseases, maintain high protein and nutritional levels, and ensure efficient irrigation even in water-scarce environments, thereby contributing directly to the mitigation of climate change impacts (Gruda et al., 2019).

The Resilient Mountain Village pilot project in Kavrepalanchowk district (2014-2016) demonstrated climate-smart practices such as bio-fertilizer ("Jholmal"), green manuring, and mulching. These methods in particularly improved crop yields, with jholmal and green manuring increasing rice yields by 10.1% and 8.1% respectively, and mulching enhancing the bitter-gourd yield by 18.1%, highlighting the effectiveness of these techniques in enhancing agricultural resilience (Subedi et al., 2019). Furthermore, in agroforestry practices there are low external input requirements, and effective integration of trees, crops, and animals reduces the vulnerability of agricultural systems to climate-related risks as well as enhances the agroecosystem diversity (Mbow et al., 2014). Additionally, ecosystem-based approaches leverage nature to protect communities from climate impacts by delivering services such as food security, and livelihood diversification (Munang et al., 2013).

## Discussions

Farming communities are aware of the effects of weather and climate change on their crops, animals, and general well-being (Amir et al., 2020). This is in line with the study of Adaawen (2021) where he mentioned that in the rural study area, most farmers possess intuitive perceptions of changing climatic and environmental conditions. These perceptions stem from their direct experiences with shifting weather patterns, altered growing seasons, variable crop yields, and challenges with pests and water availability. Focusing on a drought-resistant variety in areas prone to drought could enhance resilience in water scarcity periods. For instance, wheat, requiring less irrigation than dry-season rice, could be cultivated (Ngigi, 2009). Similar studies conducted by Bikila (2013) and Saguye (2016) have documented comparable findings with Ngigi (2009), they observed that farmers often employ specific climate change adaptation strategies, such as utilizing drought-resistant improved crop varieties and opting for short-maturing crop varieties. Around 80.7% of respondents in rural farmlands noted a temperature rise over a 30-year span, while over 90% believed rainfall had decreased during that period. In response, 73% of farming

households have implemented adaptive measures to mitigate climate change's negative effects on rice cultivation, with higher adaptation rates in the Terai region and lower rates in the hilly region (Devkota et al., 2018). Across various incidents, a consistent pattern emerges where a minimum of 80% or more respondents acknowledged experiencing climatic changes with potentially adverse effects on agricultural practices. Notably, a substantial majority (84%) of participating farmers affirmed adopting one or more recognized adaptive measures (Uddin et al., 2014). This is in line with the study of Dhaka et al. (2010), where a substantial portion of farmers were observed employing diverse adaptation strategies in response to the challenges posed by climate change. Besides the negative impact of climate change on agriculture given by (Lobell et al., 2008), Aydinalp & Cresser (2008) argue that there are some positive impacts of climate change on agriculture like Elevated atmospheric CO<sub>2</sub> levels are anticipated to positively impact certain plants by accelerating their growth rates and reducing transpiration, thereby enhancing water efficiency. Additionally, a noteworthy consequence of rising temperatures, especially in regions with limited agricultural production due to cold conditions, is the potential extension of the available growing season for plants. This change may result in shorter maturation times for crops. According to a cross-country study of factors impacting adaptation decisions to perceived climate change, farmers were more likely to adjust when they had access to extension services, credit, and land (Baryan et al., 2009). The findings of Fosu-Mensah et al. (2012)'s logit regression analysis match the findings of Baryan et al. (2009), who found that the four main relevant factors influencing farmers' perspective and adaptation are land tenure, credit availability, soil fertility, and access to extension services.

## Conclusion

Climate change poses serious problems to Nepal's agriculture, affecting food security and crop output. Farmers' adaptation methods are significantly shaped by their perceptions. Climate Smart Agriculture (CSA) provides a promising pathway for adaptation, which includes techniques like agroforestry, conservation agriculture, and climate-resilient crops. However, barriers like limited awareness, financial constraints, and institutional limitations hinder implementation. Government and institutions must collaborate to foster an enabling environment. The benefits of sustainable practices are evident through improved yields and resource efficiency. Integrating CSA is crucial for building resilience and securing sustainable food systems in Nepal's changing climate landscape.

## Future Prospects and Recommendations

Considering insights from multiple studies, a holistic perspective on future prospects for sustainable adaptation strategies emerges. Strengthening local institutions emerges as a crucial step in enabling communities to adopt and sustain climate-resilient practices (Tiwari et al., 2014). Likewise, Information and Communication Technologies

(ICTs) showcase the potential to enhance prevention efforts, ensuring fairness, informing policies, and bolstering overall social and environmental sustainability of adaptation measures (Eakin et al., 2014). Moreover, policymakers have a pivotal role in supporting producers' climate adaptation efforts. This involves integrated approaches that consider diverse needs, existing programs, and adaptable policies to address growing climate challenges while enhancing adaptive capacity in the agricultural sector through sustainable practices (Wall & Smith, 2008). On a global scale, the expansion of organic agriculture presents an opportunity for advancing climate-friendly farming practices. However, unlocking its full potential requires increased investments in research and development (Sciababba & Muller-Lindenlauf, 2010). Considering these findings, fostering collaborations between local communities, governments, and non-governmental organizations is essential to successfully implement sustainable adaptation strategies. Moreover, investing in education and awareness campaigns can empower individuals to actively participate in climate-resilient practices and contribute to the collective effort to mitigate climate change impacts.

## Declarations

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### Conflicts of Interest

The author here declares that no conflicts of interest are involved with this article.

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## Antibiotic Resistance in Food Pathogens: New Threats and Preventions Strategies

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ARTICLE INFO	ABSTRACT
<p><i>Revis Article</i></p> <p>Received : 18.10.2024 Accepted : 02.12.2024</p> <p><b>Keywords:</b> Antimicrobial resistance Food pathogens Food safety Public safe Antibiotic residue</p>	<p>Foodborne pathogens are a major threat for food production and safety, causing serious health problems, including illness and death, and contributing to food spoilage. Antimicrobial agents, which include both natural and synthetic chemicals, are commonly used to control the growth and survival of these microorganisms. However, the misuse of antimicrobial agents, particularly in animal food production, can lead to the contamination of the food chain and facilitate the spread of antibiotic-resistant genes. These resistance genes enable pathogenic bacteria to survive antibiotic treatment, posing a significant threat to global health. The acquisition of antibiotic resistance by foodborne pathogens has become an issue of major concern, contributing to the emergence of infections that are increasingly difficult to treat. This review aims to assess the current knowledge on antibiotic resistance in foodborne pathogens, emphasizing its global impact and the situation in Türkiye. By reviewing the literature on antibiotic resistance genes, this study highlights the urgent need for strategies to combat the rise of resistant pathogens in the food chain.</p>

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(2): 514-528, 2025

## Gıda Patojenlerinde Antibiyotik Direnci: Yeni Tehditler ve Önlemler

MAKALE BİLGİSİ	ÖZ
<p><i>Derleme Makalesi</i></p> <p>Geliş : 18.10.2024 Kabul : 02.12.2024</p> <p><b>Anahtar Kelimeler:</b> Antibiyotik direnç Gıda patojenleri Gıda güvenliği Halk sağlığı Antibiyotik kalıntıları</p>	<p>Gıdanın bozulma sürecinde etkili olan pek çok mikroorganizma arasında yer alabilen patojen bakteriler, gıda üretimi ve güvenliği açısından kontrol altında tutulması gereken kritik unsurlardır. Gıda kaynaklı patojen bakteriler akut, kronik ve ölümcül hastalıklara yol açabilir. Antimikrobiyal ajanlar, mikroorganizmaların hem çoğalmalarını hem de hayatta kalmasını engelleyen doğal veya sentetik kimyasallar olarak patojenlere de etki eder. Antibiyotikler hayvansal üretim süreçlerinde ve tedavi amaçlı olarak sıkça başvurulan antimikrobiyal ajanlardır. Antibiyotiklerin kontrolsüz ve aşırı kullanımı bakterilerde antibiyotik direnci sağlayan genetik değişimlere neden olabilmektedir. Direnç genleri edinen patojen bakterilerin epidemiyolojik olgulara neden olma potansiyeli kaygı yaratmaktadır. Gıda kaynaklı patojen antibiyotik direnci kazanması dünya sağlığını ve güvenliğini tehdit eden başlıca sorunlardan biri haline gelmiş, yüksek görülme oranları akademide ve toplumda endişe yaratmıştır. Bu derlemede konuya ilişkin literatürün özetlenmesi ve özellikle ülkemizde antibiyotik direnç genlerinin varlığı üzerine yapılmış çalışmalar incelenerek gıda kaynaklı patojenlerin antibiyotik direnci, konunun önemi ve potansiyel etkileri hakkında bilgilerin değerlendirilmesi amaçlanmıştır.</p>

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## Giriş

Gıda kaynaklı patojen bakteriler, gıda üretimi ve güvenliği açısından kritik öneme sahiptir. Patojenlerle kontamine olmuş gıdaların tüketilmesi bireylerde ishal, baş ağrısı, kusma, bulantı, karın krampları vb. akut belirtilerle gelişen hastalıklara, kronik hastalıklara, hatta ölüme neden olabilmektedir. Farklı mikrobiyal etmenlerle ilişkili 200'den fazla gıda kaynaklı hastalık olduğu bilinmektedir (Aladhadh, 2023; Lee ve Yoon, 2021). *Salmomella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Shigella* spp., *Yersinia enterocolitica*, *Vibrio* spp., *Brucella* spp. ve *Aeromonas* spp. gıda kaynaklı hastalıklara sebep olduğu bilinen başlıca patojen bakteri türleridir (Sağlam ve Şeker, 2016). Patojen bakterilerde, diğer bozucu mikroorganizmalarda olduğu gibi, buldukları ortama salgıladıkları enzimlerle protein, nişasta vb. polimer bileşiklerin hidrolizine neden olarak veya ortama bıraktıkları ekzopolisakkaritlerin etkisiyle gıdanın yapısal, besinsel ve görsel niteliğinin olumsuz yönde değişmesine sebep olurlar (Bhunua, 2018). Bakteriler gıda işleme süreçlerinde yaşamsal faaliyetleri sona ermiş olsa dahi ortama bıraktıkları bazı metabolitleri veya toksinleri ile hastalık yapma özelliklerini sürdürebilmektedir (Martinović ve ark., 2016).

Antimikrobiyal ajanlar, mikroorganizmaların hem çoğalmasını hem de hayatta kalmasını engelleyen doğal veya sentetik kimyasallar olarak tanımlanmaktadır (Kumar ve ark., 2020). Bu madde grubu mikroorganizmaların tamamına etki eden maddeleri kapsar ve aralarında antibiyotiklerin yanı sıra antifungal, antiparaziter, antiviral, antimikrobiyal peptit ve kozmetikte kullanılan bir takım katkı maddelerini de kapsar. Hayvancılık sektöründe kullanılan antimikrobiyal ajanların büyük bir bölümü antibiyotiklerdir. Amerika Birleşik Devletleri'nde (ABD) kullanılan antibiyotiklerin %80'i zirai üretimde hayvansal ilaç olarak uygulanmaktadır. Tiseo ve arkadaşları (2020) dünyada tavuk, sığır ve domuzlardaki (tüm gıda hayvanlarının %94'ünü oluşturan) antimikrobiyal madde kullanımı 2017 yılında 93309 ton aktif madde olduğunu söyleyerek 2030 yılına kadar antimikrobiyal madde kullanımında %11,5'lik bir artış öngörerek 104079 ton aktif maddeye ulaşacağını tahmin etmişlerdir. Ülkemiz hayvancılığında ne düzeyde antibiyotik kullanıldığına dair sağlıklı veriler bulunmamasına rağmen 2017 yılında 1 kilo et için 65,1 mg antibiyotik kullanıldığı tahmini edilmektedir (Şık, 2018). Bilinçsizce veya ticari amaçlar güdülerek kullanılan antimikrobiyal ajanlar ile atık su arıtma süreçlerinde varlığını sürdürebilen bu ajanların etkisiyle direnç genleri kazanmış patojen bakterilerinin gıda zincirine bulaşmasına sebep olabilmektedir. Ayrıca, çiftlik hayvanlarına uygulanan ilaçlar sonucunda, et, kümes hayvanları ve balık ürünlerinde antibiyotiklere dirençli genler gelişebilmektedir (Serwecińska, 2020). Direnç genlerini kazanmış patojen bakteriler, sindirim sisteminde canlılığını korumakla birlikte akut hastalıklara yol açabilmekte veya bağırsak mikrobiyomunda asemptomatik olarak çoklu ilaca dirençli, fırsatçı, ekstraintestinal enfeksiyonlar için kaynak teşkil edebilmektedir. Böylece antibiyotiklere dirençli gıda patojen bakterileri, sağlık sektöründe önemli sorunlara yol açabilmektedir (Skandalis ve ark., 2021). Amerika Birleşik

Devletleri, Hindistan, Tayland ve Avrupa Birliği'nde antibiyotiklere dirençli bakterilerin neden olduğu ölümlerin sırasıyla yıllık yirmi üç bin, elli sekiz bin, otuz sekiz bin ve yirmi beş bin'den fazla olduğu bildirilmiştir. Antibiyotiğe dirençli mikrobiyal patojenlerden kaynaklanan ölümlerin 2050 yılına kadar katlanarak artacağı, yıllık 10 milyona ulaşabileceği ve küresel sağlığı tehdit edebileceği tahmin edilmektedir (Hayden ve ark., 2016; Kumar ve ark., 2020).

Prokaryot organizmalar olan bakteriler, yapı bakımından görece basit görünseler de gelişmiş hücrelerde pek rastlanmayan bir adaptasyon ve evrimleşme kapasitesine sahiptirler. Bakteriler kompleks hücre tiplerinin yapamayacağı şekilde buldukları ortamda karşılaştıkları DNA uzantılarını (nükleotit dizilerini) hücre içine alarak bu parçaları plazmit veya genomik donatılara ekleyebilir. Antibiyotik direnci kazanmış bakteriler, antibiyotik bileşiklerinden etkilenmeyerek yaşam döngülerini sürdürürler (Cole ve Singh, 2017). Direnç mekanizmaları doğal, çevre ve şartlara bağlı olarak gelişen ve sonradan kazanılmış direnç olmak üzere 3 kategoride incelenir. Doğal direnç mekanizmasında bakterinin genetik özelliği sebebiyle antimikrobiyal ilaçlar bakteriyi etkilemez. Çevre ve şartlara bağlı olarak gelişen direnç ise oksijen basıncı değişikliği, pH değişikliği ve antimikrobiyal ajanın hedef bölgeye ulaşmaması sonucu *in vivo* ortamda yanıt vermemesi durumu olarak tanımlanır. Sonradan kazanılmış direnç mekanizmasında ise yeni direnç genleri bir mutasyon sonucu veya gen aktarımı yolu ile gelişebilir (Cole ve Singh, 2017; Kayış, 2019).

Gıda kaynaklı patojen bakterilerin antibiyotik direnci kazanması, dünya sağlığı ve güvenliğini tehdit eden önemli bir sorun haline gelmiştir (Ge ve ark., 2022). Bu durum insanlarda tedavisi zor enfeksiyonların ortaya çıkmasına yol açarak, küresel çapta araştırmacıların ilgisini çekmektedir (Meral ve Korukluoğlu, 2014; Sharma ve ark., 2014). Derlemenin temel amacı gıda kaynaklı patojenlerin antibiyotik direnç kazanmasına ilişkin yapılmış çalışmaları özetlemektir. Bu sayede konunun önemi vurgulanarak, muhtemel sorunların önlenmesi için alınabilecek önlemlere ilişkin bilgi ve önerilere yer verilmiştir.

## Antimikrobiyal Ajanlar ve Antimikrobiyal Direnç: Kısa Bir Tarihçe

Robert Koch tarafından antraks basili (1877), tüberküloz basili (1882) ve kolera basili (1883) tanımlaması başarılı ve enfeksiyonlarla mücadele için aşılama gibi yöntemler üzerinde çalışılmıştır (Lakhtakia, 2014). Bilim insanları, hastalığa ve ölüme yol açan mikroorganizmaları etkisiz hale getirecek antibakteriyel ajanları bulmak için araştırmalar yürütmüşlerdir. Enfeksiyona karşı kimyasalların kullanımı olan kemoterapinin temelini atan bilim adamı Paul Ehrlich tarafından 1910 yılında, tamamen sentetik bir antimikrobiyal ilacın ilk örneği olan arsenik içeren salvarsan başarıyla geliştirilmiştir. Salvarsan çok çeşitli bakteriyel enfeksiyonlara karşı etkili olmasa da, uyku hastalığı olarak da bilinen trypanosomiasis ve sifilizin spiroket gibi protozoal hastalıklara karşı etkili olduğu kanıtlanmıştır (Kumar ve Kumar, 2016). İlaç, Antibiyotik

Çağı olarak bilinen dönemde, 1928 yılında Alexander Fleming'in keşfettiği penisiline yer değiştirmiştir (Aminov, 2010). Ardından etkili antimikrobiyal ajan olan sülfonamid 1932'de keşfedilmiş ve 1935'te insan kullanımı için onaylanmıştır. İlk sülfonamid direnci ise 1939 yılında belirlenmiştir (Helmy ve ark., 2023).

Proflavine 1934 yılında piyasaya sürülerek kullanılmaya başlanana kadar antibakteriyel ajanların bulunmasında çok az ilerleme kaydedilmiş, ancak özellikle proflavin İkinci Dünya Savaşı sırasında derin yüzey yaralarındaki bakteriyel enfeksiyonlara karşı büyük bir etkiyle kullanılmıştır (Kumar ve Kumar, 2016). 1940 yılında penisiline dirençli ilk *Staphylococcus* türleri tanımlanmıştır. İlk beta-laktamazlara karşı koymak için 1959'da metisilin bulunmuş ve bir yıl sonra, 1960'ta metisiline dirençli bir *Staphylococcus* suşu rapor edilmiştir (Harkins ve ark., 2017).

Vankomisin antimikrobiyal ajanı metisiline dirençli stafilkokların tedavisi için geliştirilmiş ancak birkaç on yıl sonra, 1979'da vankomisine dirençli koagülaz negatif stafilkoklar rapor edilmiş ve on yıl sonra enterokoklarda direnç tanımlanmıştır (Salam ve ark., 2023). Ayrıca levofloksasin 1996 yılında klinik uygulamaya girmiş ve 2002 yılında Critchley ve arkadaşları tarafından gerçekleştirilen çalışmada on üç ülkeden beş bin on beş *Streptococcus* izolatının %57'sinin 1,1 mg/L konsantrasyonda levofloksasine dirençli olduğunu rapor etmişlerdir. 1960 ile 1980 yılları arasındaki yirmi yıl boyunca ilaç endüstrisi birçok antimikrobiyal ajan üretimi gerçekleştirmiştir. 1980'lerden sonra yeni antibiyotik sınıflarının keşfedilme oranı önemli ölçüde azalmıştır

(Ventola, 2015). Artan antimikrobiyal direncin sonucu olarak, ilaca dirençli patojenlerin neden olduğu bakteriyel enfeksiyonlar dünya genelinde klinik uygulamalarda önemli bir endişe haline gelmiştir. Çizelge 1'de antibiyotiklerin onaylandığı veya kullanımlarına izin verildiği ve bunu takiben ilk kez antibiyotik direncinin gözlemlendiği mikroorganizmalar ve tarihlerini içeren akış verilmiştir.

### Gıda Kaynaklı Patojenlerin Antibiyotik Direnç Mekanizmaları

Antimikrobiyal ajanların kontrolsüz veya uzun süreli kullanımı, antibiyotiğe dirençli patojenlerin sayısında endişe verici artışlara yol açabilmektedir (Reygaert, 2018). Bakterilerde antibiyotik direnci doğal direnç veya sonradan kazanılmış direnç şeklinde olabilmektedir. Doğal direnç mekanizmasında belirli kalıtsal özellikler sergileyen bakteride genetik özelliklerin değişimi transpozon veya plazmid DNA'da meydana gelen mutasyonlar sonucu gözlenmektedir (Kayış, 2019). Şekil 1'de bakterilerin direnç geni kazanma yolları özetlenmiştir.

Bakteriyel lipopolisakkarit duvarı, başta Gram-negatif bakteriler olmak üzere dış zarın koruyan ana biyolojik aktif bileşen olarak, antimikrobiyal ajanların bakteri hücrelerine nüfuzunu sınırlayan fiziksel bir bariyerdir ve antimikrobiyal direnç mekanizmasında önemli bir rol oynar. Bu durum, Gram-pozitif bakterilerin antibiyotik unsurları hücre içine alma konusunda Gram-negatif bakterilerden daha korunaklı oluşunu da kısmen açıklamaktadır (Ghai, 2023; Helmy ve ark., 2023).

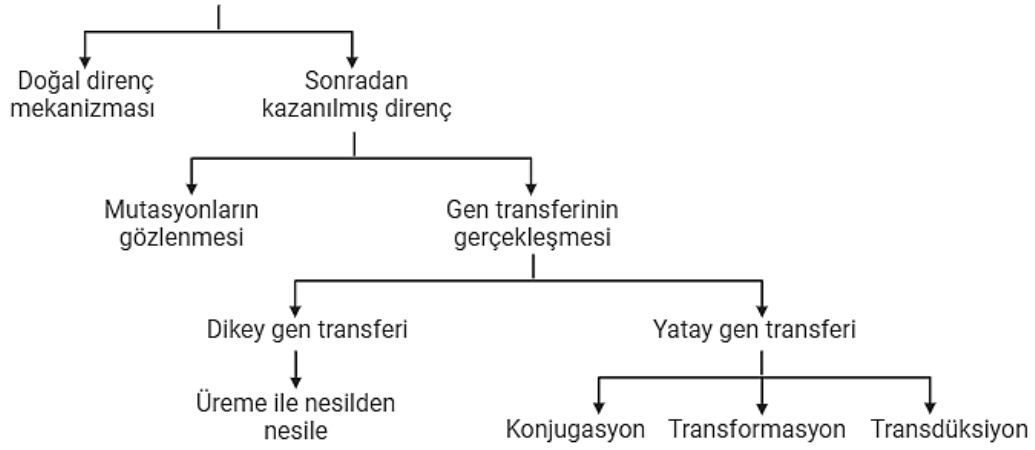
Çizelge 1. Antibiyotiklerin onaylandığı veya kullanıma izin verildiği ve bunu takiben ilk kez antibiyotik direncinin gözlemlendiği mikroorganizmalar ve tarihleri

Table 1. Historical flow chart including the microorganisms and dates when antibiotics were approved or authorized for use, followed by the microorganisms in which antibiotic resistance was first observed

AKY	Keşfedilen antibiyotik	Keşfedilen antibiyotiğe direncin gözlemlendiği ilk mikroorganizma ve gözlemlendiği yıl	Kaynaklar
1943	Penisilin	<i>Staphylococcus aureus</i> (1942)	(Rammelkamp ve Maxon, 1942)
1950	Tetrasiklin	<i>Shigella dysenteriae</i> (1953)	(Roberts, 1996)
1953	Eritromisin	<i>Streptococcus pneumoniae</i> (1968)	(Leclercq ve Courvalin, 2002)
1958	Vankomisin	<i>Enterococcus faecalis</i> (1988)	(Akpaka ve ark., 2016)
1959	Amfoterisin B	<i>Candida auris</i> (2016)	(Lockhart ve ark., 2017)
1960	Metisilin	<i>S. aureus</i> (1960)	(Harkins ve ark., 2017)
1967	Gentamisin	<i>Enterococcus faecalis</i> (1979)	(Patterson ve Zervos, 1990)
1972	Vankomisin	<i>Enterococcus faecium</i> (1986)	(Zanella ve ark., 1999)
1980	Geniş spektrumlu sefalosporinler	<i>E. coli</i> , geniş spektrumlu beta-laktamaz üreten (1983)	(Knothe ve ark., 1983)
1980	Azitromisin	<i>Salmonella enterica</i> (2011)	(Sjölund-Karlsson ve ark., 2011)
1985	İmipenem ve Seftazidim	<i>Klebsiella pneumoniae</i> (1987)	(Rice ve ark., 1990)
1986	Karbapenem grubu antibiyotikler	<i>K. pneumoniae</i> (1996)	(Chen ve ark., 2014)
1987	Siprofloksasin	<i>E. coli</i> (2004)	(Gagliotti ve ark., 2007)
1996	Levofloksasin	<i>S. pneumoniae</i> , penisiline dirençli (1996)	(Klugman ve ark., 1996)
2000	Linezolid	<i>S. aureus</i> , linezolid dirençli (2001)	(Michalik ve ark., 2021)
2001	Kaspofungin	<i>Candida</i> spp. (2004)	(Perlin, 2015)
2003	Daptomisin	<i>S. aureus</i> , metisilin dirençli (2004)	(Mangili ve ark., 2005)
2010	Seftarolin	<i>S. aureus</i> (2011)	(Long ve ark., 2014)
2015	Seftazidim/ Avibaktam	<i>K. pneumoniae</i> , KPC üreten (2015)	(Humphries ve ark., 2015)
2018	Meropenem/ Vaborbaktam	<i>E. coli</i> ve <i>K. pneumoniae</i> (2018)	(Sabet ve ark., 2018)
2019	Delafloksasin	<i>E. coli</i> ST43 (2023)	(Gulyás ve ark., 2023)
2020	Sefiderokol ve Lefamulin	<i>E. coli</i> (2022)	(Zalas-Wiecek ve ark., 2022)

AKY: Antibiyotiğin keşfedildiği yıl

## Antibiyotik direnci kazanımı



Şekil 1. Bakterilerin direnç geni kazanma yolları (Sharma ve ark., 2020)

Figure 1 Mechanisms by which bacteria gain resistance

Sonradan kazanılmış antibiyotik direnç, patojen bakterilerin genlerinde meydana gelen mutasyonlar veya direnç genlerinin yatay gen transferi yoluyla kazanılmasıyla ortaya çıkmaktadır (Reygaert, 2018). Antibiyotik direnç kazanımı, bakterilerin genetik materyallerini transdüksiyon, konjugasyon, transformasyon ve adaptif direnç olmak üzere dört teknikten biriyle paylaşmasına olanak tanıyan yatay gen transferi aracılığıyla gerçekleşmektedir (Christaki ve ark., 2020). Sonradan kazanılmış direnç mekanizmasında, patojen organizma antibiyotiğe maruz kaldığında, antimikrobiyal ajanı modifiye edebilir, inaktive edebilir veya hücre dışına atmak suretiyle direnç geliştirebilirler. Bu mekanizmalar, mikroorganizmaların antibiyotiklere karşı yanıt olarak geliştirdiği savunma stratejileridir (Egorov ve ark., 2018).

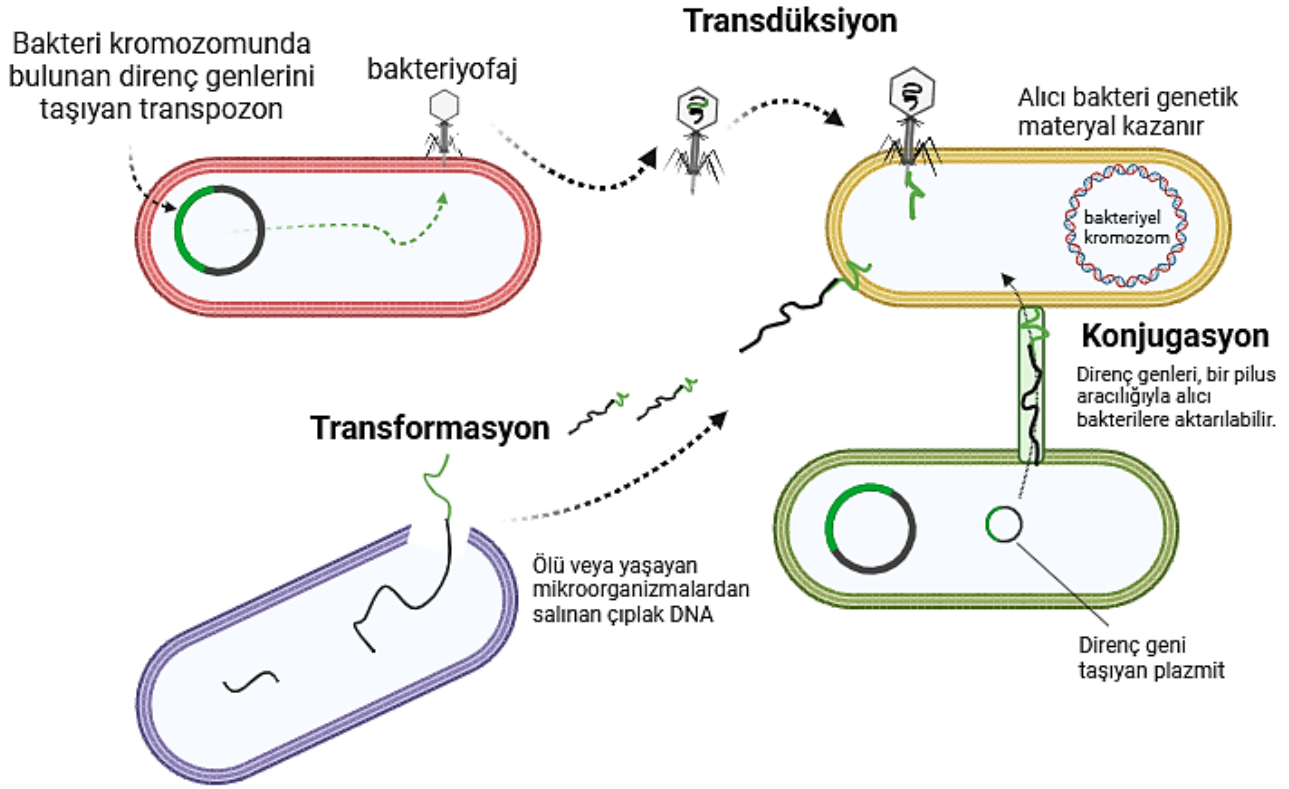
Transdüksiyonla ile gen aktarımı, bakteri hücrelerini enfekte eden bakteriyofajlar tarafından gerçekleştirilmektedir. Taşıyıcı faj dirençli bir bakteriden aldığı genetik materyali başka bir bakteriye aktarır. Temel olarak bakteriyofaj, verici bakteriye bağlanıp genetik materyalini ona enjekte ederek kendisini bakteri genomuna dahil eder. Bakteriyofajın kopyalanmasıyla direnç genlerini içeren genomu taşıyan birden fazla bakteriyofaj üretilmiş olur. Böylece yeni oluşturulan bakteriyofaj başka bir bakteriyi enfekte ettiğinde direnç genlerini içeren genomu da bu bakterilere aktararak direnç genlerinin yayılmasına neden olur (Christaki ve ark., 2020; Dibek ve ark., 2020; Helmy ve ark., 2023).

Bakterilerde genetik materyalin transferi olarak bilinen konjugasyonda direnç genini kodlayan bir plazmiti içeren verici bakteri hücresi alıcı bakteriye sitoplazmik köprü üzerinden aktarılır. Bu sürecin başarılı bir şekilde gerçekleştirilmesi için bir pilus (bakteriler arasındaki genetik materyalin transferini sağlayan proteinlerden oluşan supramoleküler yapı) ve tip IV salgı sistemi gerekmektedir. Tek bir konjugasyon olayında çoklu ilaç direncinin transferi mümkün olabilmektedir. Tek bir plazmit üzerinde çoklu direnç genleri bulunabilmekte ve çoklu direnç genlerinin tek bir plazmid üzerinde toplanmasına mobil genetik elementler (transpozonlar, integronlar ve ekleme dizisi ortak bölgeleri-ISCR-element) aracılık etmektedir (Christaki ve ark., 2020; Helmy ve ark., 2023).

Transformasyon, serbest direnç genlerini taşıyan bakterilerin ölmesinin ardından meydana gelmektedir. Bakteriyel transformasyon, bakteriyel lizis sonucu açığa çıkan genetik materyalin, saf DNA olarak başka bir bakteri tarafından alınarak kendi genomuna dahil edilmesiyle gerçekleşen bir genetik rekombinasyon şekli olarak tanımlanmaktadır. Transformasyonda tipik olarak benzer bakteri suşları veya aynı bakteri türünün suşları arasında gen aktarımı gözlenir (Chaguza ve ark., 2015; Kayış, 2019; Meral ve Korukluoğlu, 2014). Şekil 2’de bakterilerde görülen yatay gen transferi mekanizmaları verilmiştir.

Çevre ve koşullarına bağlı kazanılan direnç (adaptif direnç) için evrensel olarak kabul edilmiş bir tanım bulunmamaktadır. Bunun üzerine Fernández ve Hancock (2012) adaptif direnci, “çevresel bir tetikleyiciye maruz kalmanın bir sonucu olarak gen ve/veya protein ifadesindeki değişiklikler nedeniyle bir bakterinin antibiyotiklere karşı geçici direnç kazanması” olarak tanımlamışlardır. Adaptif direnç, belirli bir çevresel sinyal veya stresin varlığında, hücrenin kazandığı bir direnç mekanizmasıdır. Bu duruma çoğalma fazı, pH, ortamın iyon konsantrasyonu, hücrenin beslenme koşulları ve öldürücü olmayan dozlarda antimikrobiyallere maruz kalma örnek olarak verilebilir. İçsel ve sonradan kazanılmış direncin aksine, adaptif direnç geçicidir. Bakterilerin antibiyotik tehdidine daha hızlı yanıt vermesini sağlayan adaptif dirençte, genellikle tetikleyici sinyal ortadan kalktığında hücre normal durumuna geri dönmektedir (Fernández ve Hancock, 2012; Sandoval-Motta ve Aldana, 2016).

Adaptif direncin, çevresel değişikliklere yanıt olarak gen ekspresyonundaki modülasyonların sonucunda geliştiği düşünülmektedir. Çevresel uyaranlara yanıt olarak dirençteki artış, uyarının kaldırılmasının ardından her zaman eski haline dönmeyebilir. Bu durum, bakteriyel popülasyonların antibiyotiklerin düşük seviyelerde bulunduğu ortamlarda adaptif direnç yoluyla çoğalma yeteneği geliştirmelerine olanak tanır. Sonuç olarak, bu süreç daha etkili ve kalıcı direnç mekanizmalarının evrimleşmesine yol açabilir (Christaki ve ark., 2020; Giacometti ve ark., 2021; Sandoval-Motta ve Aldana, 2016).



Şekil 2. Bakterilerde yatay gen aktarımı (Lai ve ark., 2022'den uyarlanmıştır)  
Figure 2. Horizontal gene transfer in bacteria

### Antibiyotik Direnç Krizinin Patojenler Yoluyla Gıda Zincirinde Yayılma Yolları ve Nedenleri

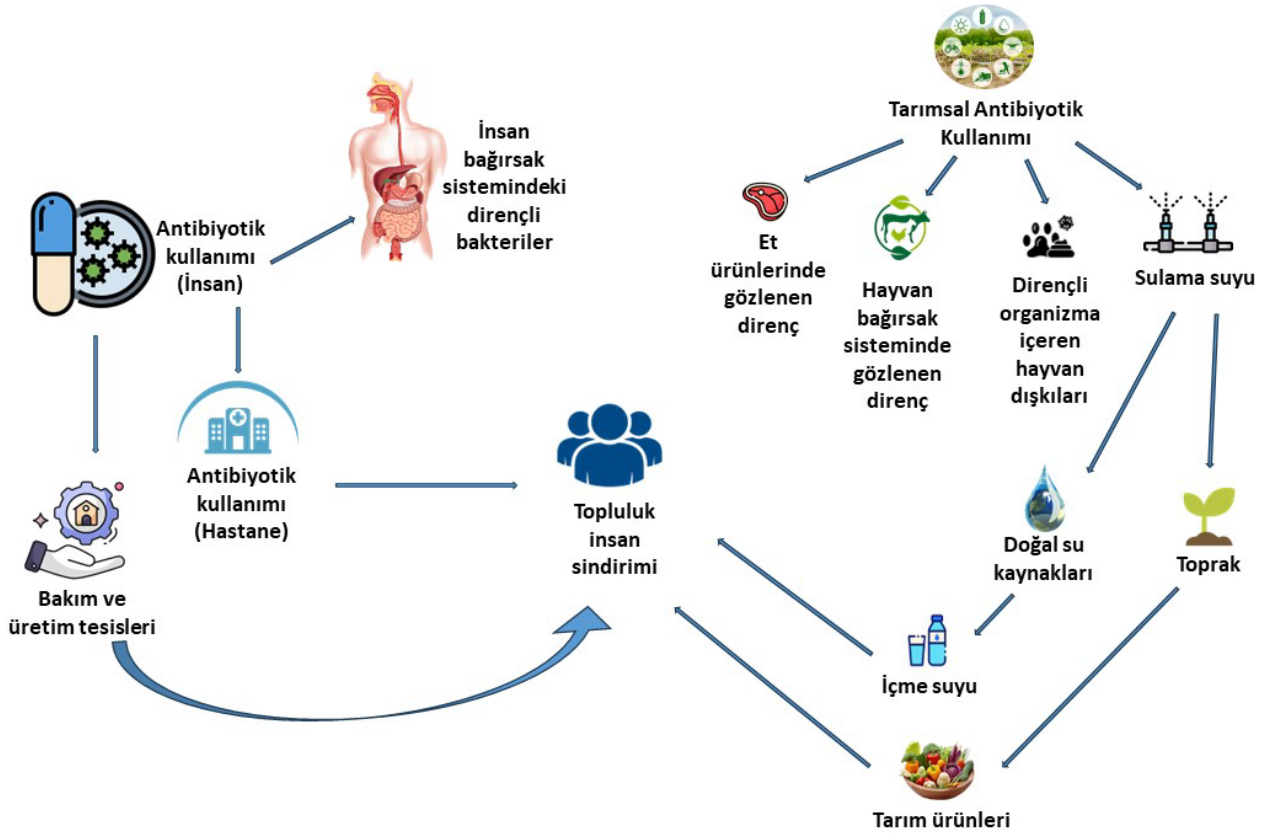
Antibiyotik direnç gelişen bir kriz durumudur ve bunun birçok sebebi vardır. Doğaya doğrudan atılan antibiyotikler, hayvancılıkta yanlış kullanılan antibiyotikler, antibiyotiklerin üretimi, kullanımı ve imhası üzerinde sıkı ve etkili denetim ve kontrol eksikliğinden dolayı antibiyotik direnç krizi oluşabilmektedir (Muteeb ve ark., 2023). Sanayileşmeye bağlı olarak insan faaliyetleri, gıdalarda ve doğal çevrede antibiyotik kalıntılarının bulunmasına yol açmaktadır. Bu durum, antibiyotiğe dirençli bakterilerin direnç genleriyle birlikte gelişimini ve yayılmasını artırarak, dirençli bakteri ve genlerin bolluğunun artmasına neden olmaktadır (Manyi-Loh ve ark., 2018).

Gıdalar, antimikrobiyal direnç kazanmış patojen bakterilerin yayılması için mükemmel bir ortam oluşturmaktadır. Antimikrobiyal direnç kazanmış patojen bakteriler, çiftlikten sofraya giden süreçte herhangi bir zamanda gıda işleme ve tedariğinde bulaşan olarak zincire dahil olabilmektedirler (Giacometti ve ark., 2021). Çiftlik üretimlerinde, gıdalara bulaşma, bu gıdalarda çoğalma ve bunlarla taşınma antimikrobiyal direnç kazanmış patojen bakterilerin çiğ ve az pişmiş ihtimali yüksektir. Antimikrobiyal direnç kazanmış patojen bakterilere maruz kalma yolları gıda tüketimi ile dolaylıdır ancak enfekte hayvanlarla, içilebilir ve kullanım suyu ile veya biyolojik bileşenler (kan, idrar, dışkı, tükürük vs.) temas yoluyla doğrudandır (Samtiya ve ark., 2022). Antibiyotiklere dirençli bakterilerin gıda aracılığıyla da dahil olmak üzere farklı kaynaklardan insan vücuduna geçiş yolları Şekil 3'te verilmiştir.

### Antimikrobiyal Direnç Kazanmış Patojenlerin Hayvansal Gıdalar Yoluyla Yayılımı

Hayvansal kaynaklı gıdalarda yayılım yollarının merkezinde çiftlik hayvanları ve bu hayvanlardan elde edilen et, süt, yumurta gibi ürünler yer almaktadır. Et ürünlerinde en büyük tehlike *Salmonella*'dır. *Salmonella*, 2.500'den fazla tanımlanmış serotipe sahip olup, 150'den fazla serotip gıda kaynaklı salmonelloza neden olabilir. Ancak, *S. typhimurium* ve *S. enteritidis*'in görülme sıklığı en yaygın olanlardır (Abebe ve ark., 2020). İnsanlarda salmonelloza neden olan bu patojenler, yaygın olarak kontamine olmuş kümes hayvanları, sığır eti, tavuk, hindi, domuz eti, yumurta, süt ve bunlardan üretilen ürünlerde görülür. Gıda hayvanı üretiminde, hastalıkların tedavisinde ve büyümenin teşvik edilmesi amacıyla antimikrobiyal ajanların artan kullanımı, antibiyotik dirençli *Salmonella* patojeninin ortaya çıkmasında önemli bir faktördür. Bununla birlikte *Salmonella* türleri arasında antimikrobiyal direncin artan görülme sıklığı nedeniyle tedavi başarısızlığı riski de bulunmaktadır (Arslan ve Eyi, 2010). Son zamanlarda ortaya çıkan çoklu ilaca dirençli *S. typhimurium* DT104, suşunun daha ziyade kontamine sığır eti tüketimi yoluyla bulaştığı tespit edilmiştir (Abebe ve ark., 2020).

Türkiye'de gerçekleştirilmiş başka bir çalışmada, *S. typhimurium*'un farklı suşlarında bulunan patojenite adaları ve direnç genleri Ekici ve arkadaşları (2021) tarafından *in silico* (bilgisayar destekli) analizlerle incelenmiştir.



Şekil 3. Antibiyotiklere dirençli bakterilerin gıda da dahil olmak üzere farklı kaynaklardan insan vücuduna geçiş yolları (Cole ve Singh, 2017 ve Samtiya ve ark., 2022'den uyarlanarak hazırlanmıştır)

Figure 3. Transmission pathways of antibiotic-resistant bacteria from various sources, including food, to human body (adapted from Cole and Singh, 2017 and Samtiya et al., 2022)

Çalışmada, on beş farklı *S. typhimurium* suşunun genomik verileri kullanılarak analiz edilmiş; genomik yakınlık CSI filogeni yazılımı ile değerlendirilmiştir. Antimikrobiyal direnç genleri ResFinder ile, patojenite adaları SPIFinder ile ve serotip analizleri SeqSero kullanılarak incelenmiştir. Yazarlar, suşların genomik benzerliğini %97'nin üzerinde bulmuş, %60'ının ST19 genotipinde olduğunu tespit etmişlerdir. Tüm suşlar SPI-1, SPI-2, SPI-3, SPI-4 gibi patojenite adalarını taşıırken, SGII patojenite adası yalnızca üç suшта bulunmuştur. Ayrıca, tüm suşlarda aminoglikozid direnciyle ilişkili aac(6)-Iaa geni tespit edilmiştir. SGII taşıyan suşların çoklu antibiyotik direnci sergilediği belirlenmiş ve bu direnç desenin halk sağlığı açısından kritik öneme sahip olduğu ortaya koyulmuştur. Yeni nesil dizileme tekniklerinin, *S. typhimurium* gibi önemli enfeksiyon etkenlerinin moleküler epidemiyolojisini anlamada ve enfeksiyonlardaki virülans mekanizmalarını aydınlatmada büyük bir rol oynadığı belirtilmiştir.

Bununla birlikte su ürünleri yetiştiriciliği sistemlerinde ve balık çiftliklerinde yetiştirilen deniz ürünlerine kontamine olmuş patojen bakterilerin direnç kazanmaya daha meyilli olması ve dirençli genlerinin su vasıtasıyla dolaylı olarak aktarılabilmesi sebebiyle "antimikrobiyal direncin sıcak noktası" olarak tanımlanmıştır (Caputo ve ark., 2023).

Kinolon dirençli *E. coli*'nin sığır ve çiftlik ortamında yaygın bir şekilde bulunduğu bilinmektedir. Bunun üzerine Duse ve arkadaşları (2016) yirmi üç süt çiftliğinde süttten kesilmiş buzağılardan üç yüz kırk beş dışkı örneği, doğum

sonrası ineklerden yüz on beş dışkı örneği ve onun haricinde iki yüz elli yedi çevre örneği toplamışlardır. Yapılan değerlendirmede dışkı örneklerinin %60 (buzağı) ve %28 (inek), çevre örneklerinin ise %44'ünde kinolon dirençli *E. coli* olduğu tespit edilmiştir.

Gıda sektöründe karşımıza çıkan direnç genleri ve bu direnci kazanmış patojenlerin bulaş kaynağı, özellikle hayvancılık sektöründe daha yaygındır. Kullanılan antibiyotik ajanlar (örneğin, tetrasiklin, penisin) ve büyüme faktörü olarak kullanılan hormonlar (örneğin, östradiol, projesteron ve somatotropin) direnç gelişimine neden olduğu konusunda endişeler, dirençli suşların tespit edilmesiyle artmıştır (Helmy ve ark., 2023). Ancak ilginç bir şekilde 1969 tarihli Swann tavsiyeleri antibiyotiklerin hayvanlarda ve tarımda tedavi amaçlı olmayan kullanımını yasaklanması yönündeki ilk çağrı olarak yapılmıştır. Bu öneri bugüne kadar pek çok ülkede uygulanması imkansız olan, makul ancak son derece tartışmalı bir öneri olarak literatüre geçmiştir (Davies ve Davies, 2010).

### Hayvansal Kökenli Olmayan Gıdalarla İlişkili Yayılma

Hayvansal kökenli olmayan gıdalarla ilişkili antimikrobiyal dirence ilişkin literatür verileri sınırlı sayıdadır (Samtiya ve ark., 2022). Dünya Sağlık Örgütü (WHO) ve Gıda ve Tarım Örgütü (FAO), meyve ve sebzelerin sağladıkları zengin besin içerikleri nedeniyle, sağlıklı beslenmenin bir parçası olarak günlük tüketimini teşvik etmektedir. Ancak, birçok taze yapraklı ve yapraksız sebzenin, kök sebzelerin, filizlerin ve meyvelerin çiğ

tüketimi, insanların antibiyotiğe dirençli bakteriler de dahil olmak üzere gıda kaynaklı bakteriyel patojenlere maruz kalmasına neden olabilmektedir. Gıda zinciri yoluyla antimikrobiyallere dirençli patojenlere maruz kalmanın daha fazla gıda kaynaklı hastalık salgılarına neden olduğu rapor edilmektedir (Holzel ve ark., 2018).

Taze meyve ve sebzeler, sulama yoluyla veya biyoyakıtların ve hayvan gübresinin gübre olarak kullanılması gibi çiftçilik sırasında dışkı atıklarıyla doğrudan temas yoluyla, üretim ve tedarik zinciri boyunca birçok noktada bakteriyel patojenlerle kontamine olabilmektedir. Bulaşı, ürünün depolanması ve taşınması sırasında da meydana gelebilmektedir (Rahman ve ark., 2022). Bu potansiyel kontaminasyon yolları et ve et ürünleri grubu gıda ürünlerinde iyi bir şekilde çalışılmış olsa da taze meyve ve sebzelerin antimikrobiyal direnç kazanmış patojenler ile kontaminasyonuna ait veri eksikliği bulunmaktadır. Usui ve arkadaşları (2019) Japonya'daki yedi süpermarketten toplam yüz otuz taze sebze örneği toplamış ve saptadıkları baskın cinsin, sebze numunelerinin %7,7'sinden izole edilen 10 genişlemiş spektrumlu  $\beta$ -laktamaz (GSBL) üreten *Pseudomonas* spp. olduğunu belirtmişlerdir. Yapılan değerlendirmede *Pseudomonas* suşlarının iki GSBL geni taşıdığı tespit edilmiş ve bazı suşların çoklu antibiyotiğe dirençli olduğu kanısına varılmıştır. Sonuç olarak taze sebze ürünlerin önemli bir bulaşma kaynağı olduğu ve bulaşmayı önlemek için daha sıkı hijyen önlemlerin alınması ve izleme yapılması gerektiği belirtilmiştir.

#### Antimikrobiyal Direncin Çevre ve Su ile Yayılımı

Gelişen sanayi ve ticaret faaliyetleri ve buna bağlı olarak artan antibiyotik tüketimi, hayvansal üretim ve gıda dışı ürünler haricinde de antibiyotik direnç genlerinin ve bu antibiyotiklere dirençli mikroorganizmaların hızla yayılmasına neden olmaktadır (Prestinaci ve ark., 2015).

İnsan ve hayvan dışkıyla dışarı atılan dirençli patojenler, doğal ekosistemlerde birikebilir ve çevreye yayılabilir. Bununla birlikte, ilaç kalıntıları kanalizasyon sularına veya içme sularına karışarak su kirliliğine yol açabilir. Bu nedenle, su sistemlerindeki doğal ekosistemlerde biriken antibiyotik kalıntılarını ve çevresel seçim baskısını azaltmak amacıyla, daha etkili atık su arıtma tekniklerinin geliştirilmesi ve hızlıca parçalanabilen antibiyotiklerin geliştirilmesi önerilmektedir (Jordan ve Gathergood, 2013; Samrot ve ark., 2023).

Üretim tesislerindeki atık su arıtma sistemleri antibiyotiklerin, antibiyotiğe dirençli bakterilerin ve genlerin (*bla* genleri, *mecA*, *tetA*, *erm*) seviyesini azalttığından dolayı önemlidir. Ancak diğer bir taraftan da atık su arıtma tesisleri antibiyotiklere direnç genlerinin veya mikroorganizmaların yayılmasına yönelik rezervuarlardır ve bunların yayılmasını önlemek veya risk yönetimini kontrol etmek zor olabilmektedir (Sambaza ve Naicker, 2023). Atık su arıtma tesislerinde bulunan direnç genlerinin ve bu genlere sahip patojenlerin tespiti üzerine farklı çalışmalar yapılmıştır. Godinho ve arkadaşları (2024) evsel ve endüstriyel atık su alan bir tesisten bakteri izole etmiş ve antibiyotik direncini kodlayan genleri moleküler yöntemlerle taramışlardır. Araştırmacılar izole ettikleri elli bakterinin genomik DNA izolasyonunu yapmış ve evrensel primerler 27F ve 1492R kullanılarak

16S rRNA geninin PCR amplifikasyonu gerçekleştirmişlerdir. Ardından tüm ampliconlar saflaştırılmış ve örnekler tanımlama yapılabilmesi için dizileme analizleri sonuçları NCBI Genbank veritabanında standart nükleotid BLAST yapılarak izole bakterilerin %50'sinin *Enterococcus* cinsine ait olduğunu tespit edilmiştir. İzolatların antibiyotiklere fenotipik direncinin araştırılması sonucunda en yaygın olarak tetrasiklin (%32,5) ve ampisiline (%25) dirençli oldukları bulunmuştur. *Citrobacter*, *Shigella* ve *Klebsiella* patojenlerinin ise çoklu ilaca dirençli olduğu tespit edilmiştir. Araştırma sonucunda, yazarlar ham atık sudan çevreye boşaltılan son atık suya kadar antibiyotiğe dirençli suşların bulunması nedeniyle atık su arıtma tesisi sistemlerinin antibiyotiklere dirençli bakterilerin yayılmasını engelleme konusundaki etkinliğinin önemini vurgulamışlardır.

#### Antibiyotik Direncinin ve Direnç Kazanmış Mikroorganizmanın Üzerine Gıda İşleme Yöntemlerinin Etkisi ve Gıda ile Temas Eden Çalışanlar ile Yayılım

Güvenli, besleyici gıda ve gıda ürünleri toplum sağlığının sürdürülmesi ve geliştirilmesinin anahtarıdır. Ancak gıdalar hijyenik bir şekilde işlenmediğinde kirlenmekte ve tüketim için sağlık riski taşıyan hale gelebilmektedir. Gıda ürünleri üretim ve dağıtım sürecinin herhangi bir noktasında kontamine olabilmektedir. Satılan gıdanın kontamine olmasının birincil sorumluluğu ise gıdayı işleyenlere aittir (Vicar ve ark., 2023). Gıda ürünlerinde antimikrobiyal direnç kazanmış mikroorganizmanın yayılması kötü hijyen uygulamaları veya kontamine gıdaların işlenmesinden kaynaklanan çapraz kontaminasyon ile ilişkilendirilmektedir (Samtiya ve ark., 2022).

Toplu gıda üretimi, özellikle yemek servisi yapan kurumlar, geniş çapta bulaşma olasılığı nedeniyle yüksek salgın riski altındadır. Yenew ve Tadele (2020) Debre Tabor Üniversitesi yemekhanesinde gıda işleyicilerinin antibiyotiğe dirençli bakterilerle kontaminasyonunu ve ilişkili faktörleri belirlemeyi amaçlayarak bir çalışma gerçekleştirmişlerdir. Bu kapsamda çalışanlardan izole edilen mikroorganizmaların (*E.coli*, *Salmonella* spp. *Shigella* spp.) ortalama çoklu ilaç direnç oranı  $\geq$ 85 olarak bulunmuştur. Dirençli suşların 3 veya daha fazla antibiyotiğe dirençli olduğu belirlenmiştir. Bunun yanında araştırmacılar gıda çalışanlarından izole edilen patojen *E. coli* suşlarının amoksisilin, kotrimoksazol ve vankomisine dirençli olduğunu belirlemişlerdir.

Gıda işleme ve muhafazaya yönelik dirençli suşların teknolojiler (yüksek basınç, iyonlaştırıcı radyasyon, ultraviyole radyasyon ve darbeli elektrik alanı) besinsel ve duyuusal nitelikleri korurken hücrelerin genetik materyali olan DNA'nın sarmal yapısını açarak mikrobiyal yükün azalmasına neden olabilmektedir. Bu teknolojiler mikrobiyal hücreleri kısa sürede öldürebilmektedir. Böylece gıdanın mikrobiyal güvenliği sağlanabilmektedir (Jadhav ve ark., 2021). Dirençli suşların hücre zarının parçalanmasına bağlı olarak, anti mikrobiyal direnç ile ilişkili genetik materyalin gıdanın temas ettiği ortamlara salınma ve aktarılma potansiyeli direnç geninin yayılması açısından tehlike arz etmektedir (Samtiya ve ark., 2022).



McMahon ve arkadaşları (2007) ölümcül olmayan gıda koruma stresinin (yüksek veya düşük sıcaklık, ozmotik stres ve pH stresi), gıdayla ilişkili üç patojen (*E. coli*, *Salmonella enterica* serovar typhimurium ve *S. aureus*) tarafından ifade edilen antibiyotik direnci üzerindeki etkilerini araştırmıştır. Araştırma sonuçlarına göre, yüksek sıcaklıkta (45 °C) inkübasyon antibiyotik direncini azaltırken, arttırılmış tuz (> %4,5) veya düşürülmüş pH (< 5,0) koşulları altında inkübasyon antibiyotik direncini arttırmıştır. Ayrıca, bazı patojenlerin stres faktörleri ortadan kalktıktan sonra bile daha yüksek seviyelerde antibiyotik direnç ifade etmeye devam ettiği gözlemlenmiştir. Böylece gıda ürününe uygulanan bakterisidal ve bakteriyostatik gıda işleme teknolojilerinin kullanımı, gıda kaynaklı patojenler arasında antimikrobiyal direncin gelişmesine ve yayılmasına yol açabilmektedir.

Antimikrobiyal direncin gıdalarda bulunmasının diğer olası yolları arasında fermentasyon sırasında veya probiyotik olarak gıdalara eklenen bakterilerde de yer alabilmektedir. Gıdada gözlenen konjugasyon deneysel çalışmalarda rapor edilmiş ve plazmit kaynaklı ampisilin direnç genlerinin *S. typhimurium*'dan aşılansız sterilize süt ve kıymadaki *E. coli* K12'ye transferi rapor edilmiştir (Walsh ve ark., 2008). Toomey ve arkadaşları (2009) laktik asit bakterilerinden (LAB) potansiyel patojen suşlara antimikrobiyal direncin aktarılabilirliğini *in vitro* yöntemler kullanılarak incelemişlerdir. Aktarılabilir DNA materyali üzerinden eritromisin veya tetrasiklin direnç belirteçleri içeren beş LAB verici tarafından (*E. faecalis*, *L. lactis*) patojenik suşlara (*Listeria* spp., *Salmonella* spp., *S. aureus* ve *E. coli*) direnç geni aktarılabilirliğini göstermişlerdir. Çalışmalar kapsamında, *in vitro* transfer deneyleri verici ve alıcı bakteriler arasında gerçekleştirilmiştir. Tüm aktarım suşlarının fenotipik ve moleküler yöntemlerle doğrulanması yapılmıştır. LAB suşlarından *Listeria* spp.'ye eritromisin direncinin yüksek aktarım frekanslarıyla (alıcı başına transkonjuganlar  $5,1 \times 10^{-4}$  kadar) gerçekleştiği gözlemlenmiştir. Ayrıca, eritromisin ve tetrasiklin direncinin LAB türleri arasında *in vitro* yöntemlerle yüksek sıklıkta (alıcı başına  $2,7 \times 10^{-8}$  ila  $1,1 \times 10^{-3}$  arasında değişen frekanslarla) aktarıldığı tespit edilmiştir. Ancak, *Salmonella* spp., *Staphylococcus aureus* ve *E. coli* 'ye herhangi bir direnç aktarımı gözlenmemiştir. Sonuç olarak yazarlar LAB'nin, direnç belirteçlerinin, LAB ile patojenik suşlar (örneğin *Listeria* spp.) arasında yayılmasında potansiyel bir kaynak olabileceğini ortaya koymuşlardır.

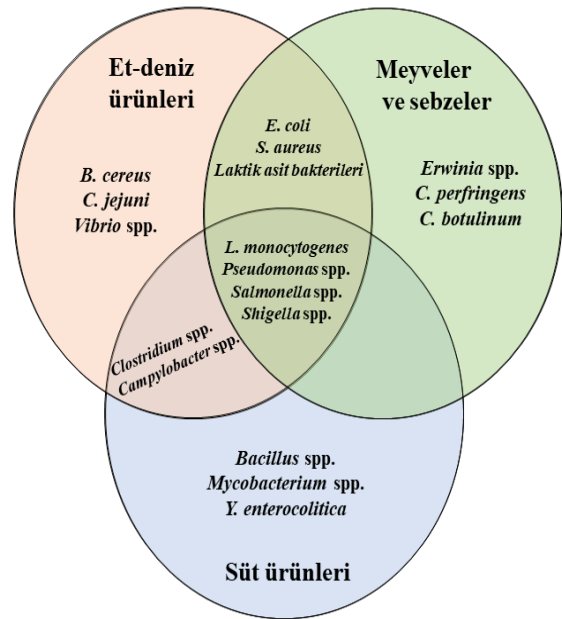
Avrupa Gıda Güvenliği Otoritesi'nin (EFSA) genetiği değiştirilmiş organizmalar (GDO) paneli, genetiği değiştirilmiş bitkilerde antibiyotik direnç genlerinin işaretleyici genler olarak kullanılmasına ilişkin bir görüş yayınlamıştır. Bu görüş, transgenik olayların seçiminde antibiyotik direnç işaret genlerinin güvenli kullanımına dair endişeleri ele almaktadır. Panel, genetiği değiştirilmiş bitkilerden bakterilere gen aktarımı yoluyla insan ve hayvanlarda antibiyotiklere karşı direncin artabileceği endişesine dikkat çekmiştir. Ancak yapılan değerlendirme, bu tür işaretleyici genlerin kullanımıyla antimikrobiyal direnç genleri için gen aktarımı riskinin çok düşük olduğunu ortaya koymuştur. Buna rağmen, EFSA, antimikrobiyal direncin yayılmasında bu işaretleyici genlerin bir risk oluşturabileceğini vurgulamıştır (European Food Safety Authority (EFSA), 2004).

## Antibiyotik Direnç Gösteren Gıda Kaynaklı Patojenler

Gıda kaynaklı patojen bakteriler, dünya çapında insanlarda görülen gıda kaynaklı hastalıkların üçte ikisinin etkenidir ve gelişmekte olan ülkelerde oran daha yüksektir (Abebe ve ark., 2020; Bhunia, 2018; Reygaert, 2018). Şekil 4'te gıda kaynaklı patojen bakteriler ve potansiyel olarak bulunabilecekleri gıdalar belirtilmiştir. Tıbbi tedavi amaçlı ve hayvan yetiştiriciliğinde kullanılan antibiyotikler dolaylı şekilde gıdalara bulaşabilmektedir. Bu gıdaların tüketimi sonrasında organizmada mutasyonlar gözlenebilmektedir. Bu durum, gıda ile bulaşan patojenlerin etkisinin değişmesine yol açmakta ve bu patojenler, yeni özellikler kazanmış organizmalar olarak değerlendirilmektedir (Manyi-Loh ve ark., 2018).

*S. aureus* koagülaz pozitif, Gram pozitif, hareketsiz ve endospor oluşturmeyen bir koktur. Büyüme için en uygun sıcaklık 30-37 °C, pH ise 7-7,5'tir. Ayrıca %15'e kadar NaCl konsantrasyonlarında da gelişebilmektedir (Kadariya ve ark., 2014). *S. aureus* nedeniyle gıda zehirlenmelerine yol açar. *S. aureus* enterotoksinleri yüksek sıcaklık da dahil olmak üzere stres faktörlerine karşı dirençli kalabilmektedir (Grispoldi ve ark., 2021).

*S. aureus* metisilin, oksasilin, sefoksitin, sefuroksim, oksasilin ve amoksisilin-klavulanik asite karşı direnci ile bilinmektedir. Metisiline dirençli *S. aureus*, enfeksiyonlara yol açan başlıca patojenlerden biridir ve dünya çapında insan sağlığı için büyük bir tehdit haline gelmektedir (da Silva ve ark., 2020; Naeim ve ark., 2023). İlk başta *S. aureus*'ın metisiline direnci klinik ortamlardan izole edilen suşlarda tespit edilmiştir, ancak son yıllarda hastane ortamı dışında metisiline dirençli *S. aureus* hücreleri et, kümes hayvanları, süt, salatalar, hazır yiyecekler ve peynirlerde tespit edilmiştir (Naeim ve ark., 2023).



Şekil 4. Gıda kaynaklı bakteriler ile gözlemlendiği gıdalar (Cole ve Singh, 2017'den uyarlanmıştır)

Figure 4. Foodborne bacteria and the foods they have been observed in (adapted from Cole and Singh, 2017).

Tanaman ve Vural (2023) ülkemizde gerçekleştirdiği bir çalışmada İstanbul merkezli organik ürün satan marketlerden satın alınan süt, kefir, yoğurt, beyaz peynir, kaşar peyniri, dil peyniri, keçi peyniri ve tereyağından oluşan toplam yüz dört adet örnekten izole edilen *Staphylococcus* spp.'nin antibiyotik duyarlılığını incelemiştir. Araştırmacılar *S. aureus* izolatlarında sefoksitin (metisilin) ve gentamisin direncini sırasıyla %50 ve %25 düzeyinde bulmuşlardır. Bunun yanında *S. aureus*, *S. sciuri*, *S. carnosus*, *S. hominis* ve *S. xylosus* izolatlarında en az iki ve daha fazla antibiyotiğe karşı direnç (çoklu direnç) saptamışlardır.

Lv ve arkadaşları (2021) gıda örneklerinden ve gıda zehirlenmesi salgınlarından elde edilen yüz otuz sekiz *S. aureus* suşunda penisilin direncinin oluşmasını sağlayan *blaZ* geni ve metisilin direncinin oluşmasını sağlayan *mec* geni varlığını araştırmışlardır. Yazarlar 9 suşta metisiline dirençli *mec* geninin varlığını doğrulamıştır ve bunların yüz otuz üçü *blaZ* genine sahip olduğunu tespit etmişlerdir.

Saber ve arkadaşları (2022) yemeye hazır et ve gıda işleyicilerinden izole edilen yüz yetmiş altı örnekten altmış koagülaz pozitif *S. aureus* izolatı tespit etmişlerdir. Tüm izolatların *mecA* pozitif olduğunu belirlemişler ve dolayısıyla metisiline dirençli *S. aureus* olarak sınıflandırmışlardır. İzolatlar 0,25-0,92 arasında değişen çoklu antibiyotik direnç indeksleriyle çoklu direncin varlığını ortaya koymuşlardır. Bunun yanında araştırmada genellikle sefepime (%96,7), penisiline (%88,3) direnç yaygın olmakla birlikte ampisilin-sulbaktam (%65), siprofloksasin (%55), nitrofurantoin (%51,7) ve gentamisin (%43,3) dirençleri olduğunu da belirtmişlerdir. Bununla birlikte, metisiline dirençli *S. aureus* enfeksiyonlarının tedavisinde kullanılan vankomisin antibiyotiğine dirençli *S. aureus* suşunun Saber ve arkadaşları (2022) tarafından tespit edilmesi endişe verici bir sonuç olarak karşımıza çıkmaktadır.

*E. coli* insanların ve hayvanların sindirim sisteminde yaşayan fakültatif anaerob ve Gram negatif bir bakteridir. Bununla birlikte, genomun yüksek evrimsel değişkenliği, daha önce bilinmeyen özelliklere sahip türlerin ortaya çıkmasına neden olmuştur. Enfeksiyonları genellikle dışkıyla kontamine olmuş su veya gıda tüketiminden kaynaklanmaktadır. Patojen *E. coli* zehirlenmesinde en sık görülen ürünler arasında meyve ve sebzeler, kümes hayvanları, domuz eti, sığır eti, balık ve süt yer almaktadır (Grudlewska-Buda ve ark., 2023).

Taniş ve arkadaşları (2021), Kahramanmaraş'ta satışı sunulan 30 lor peynirini mikrobiyolojik açıdan incelemiştir. İnceleme sonucunda otuz örneğin yirmi dokuzunda *E. coli* üremesi tespit etmişlerdir. Yaptıkları araştırma sonucunda yetmiş dört adet *E. coli* suşu tanımlamışlardır. İzole edilen suşlarda en yüksek direnç oranı sefuroksime (%88), en düşük direnç oranı ise sefepime (%1) karşı belirlenmiştir. Bununla birlikte levofloksasine karşı herhangi bir direnç bulunmadığını belirtmişlerdir. Yazarlar bu sonuçları değerlendirdiklerinde lor peynirlerinin üretimden tüketiciye ulaşana kadar kontaminasyona maruz kaldığını ve izole edilen *E. coli* suşlarında antibiyotik direncinin arttığını vurgulamışlardır. Araştırmacılar, gıda üretiminde ve depolama süreçlerinde daha dikkatli olunması gerektiğine, antibiyotik kullanımının kontrol altına alınmasının önemine ve satış koşullarının daha uygun şartlarda sağlanması gerektiğine vurgu yapmışlardır.

Babines-Orozco ve arkadaşları (2024) Latin Amerika ve Meksika'da gıdalardan izole edilen çoklu ilaca dirençli *E. coli*'yi analiz etmek amacıyla 2015'ten 2022'ye kadar yayınlanan literatürün sistematik bir taramasını gerçekleştirmişlerdir. Sonuç olarak bakıldığında gıda kaynaklı hastalıklara neden olan en önemli patojenlerden biri olan Shiga toksini üreten *E. coli* (STEC), *E. coli* O157:H7 olduğu tespit etmişlerdir. Bu enfeksiyonlar çoğunlukla az pişmiş kıyma, çiğ süt, salatalar, pırasa, çiğ patates, sebzeler, meyveler ve genellikle kötü hijyenik koşullarla ilişkilendirilen yiyecekler yolu ile bulaştığı belirlenmiştir.

Dünya Sağlık Örgütü'nün (WHO), 2017 yılında yayınlanan, insan sağlığına en büyük tehdidi oluşturan antibiyotiklere direnç kazanmış 12 bakteri ailesini içeren öncelikli patojenlerin ilk listesini açıklamıştır. Öncelikli antibiyotik dirençli patojenler listesine göre kritik öncelikli veya ilk dikkat edilmesi gereken patojenler arasında karbapenem dirençli *Acinetobacter baumannii*, karbapenem dirençli *Pseudomonas aeruginosa* ve karbapenem dirençli GSBL üreten *Enterobacteriaceae* türleri (*K. pneumoniae*, *E. coli* ve *Enterobacter* spp.) olarak yayınlanmıştır. Antibiyotiğe direnç kazanmış bu patojenler ciddi ölümcül enfeksiyonlara neden olabileceği belirtilmiştir (WHO, 2017). Hayvanlardan izole edilen ilk karbapenemaz gen belirteci, Almanya'da bir domuzdan elde edilen *bla<sub>VIM-1</sub>* olmuştur. *E. coli*'de tespit edilen diğer karbapenemazlar arasında *bla<sub>NDM-1</sub>* ve *bla<sub>NDM-5</sub>* bulunmaktadır. *NDM-1*, Amerika Birleşik Devletleri ve Çin'de köpeklerden, kedilerden ve domuzlardan izole edilmiştir. *NDM-5* ise Çin, Hindistan ve Cezayir'de, sığır, kümes hayvanları, köpek, kedi ve balıklardan tespit edilmiştir (Poirel ve ark., 2018). Antimikrobiyal direncin kontrol altına alınamayıp artması ile WHO 2019 yılında, insan sağlığı üzerindeki etkisi nedeniyle küresel sağlığa yönelik en büyük on tehditten biri olarak kabul ettiğini bildirmiştir (Mancuso ve ark., 2021).

*Salmonella* spp., *Enterobacteriaceae* ailesine ait, Gram negatif, spor oluşturmeyen, fakültatif anaerob ve çubuk şeklindeki bakterilerdir. Bu bakteriler, genellikle gastroenterit ve enterik ateşe yol açar. *Salmonella*'nın başlıca kaynakları insan ve hayvanlardır. Enfeksiyon, taşıyıcı durumundaki hayvanların ve insanların dışkısından yayılmaktadır. İnsanlarda enfeksiyonlar, kontamine olmuş hayvansal gıdalar aracılığıyla meydana gelebilir. Bunlar arasında kanatlı etleri, yumurta ve yumurtadan yapılan ürünler, kırmızı et ve et ürünleri, kontamine süt ve süt ürünleri ile kabuklu deniz ürünleri bulunmaktadır (Asal, 2021).

Ağay ve Kimiran (2017) İstanbul ilinde satışı sunulan kıyma örneklerinden izole edilen on sekiz izolat, deniz suyu örneklerinden on dört izolat ve İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi Temel Bilimler Bölümü Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı'ndan temin edilen dışkı örneklerinden izole edilen üç farklı *Salmonella* spp. izolatı üzerinde bir çalışma gerçekleştirmiştir. Bu çalışmaya göre, gıda ve deniz suyu kaynaklı *Salmonella* bakterilerinin antibiyotik dirençlilik oranının, klinik ve standart bakterilere göre daha yüksek olduğu gözlemlenmiştir. Ayrıca yazarları gıda kaynaklı örneklerden izole edilen izolatlarda çoklu antibiyotik direncinin tespit edilmesi sonucunda, besi hayvanlarında kullanılan antibiyotiklere karşı bakterilerin direnç kazandığı sonucunu ileri sürmüşlerdir.

Talukder ve arkadaşları (2023) Güney Asya'daki insan, hayvan ve çevresel izolatlardaki antimikrobiyal direnç durumunu belirlemek için Ocak 2010'dan Haziran 2021'e kadar çalışılmış veriler sistematik olarak incelenerek genel antimikrobiyal direncin 10 yıl içinde %53'ten %77'ye çıktığını belirtmişlerdir. Antibiyotiğe dirençli on sekiz farklı *Salmonella* serotipi arasında *S. enterica* en yaygın olarak belirlenmiş ve ardından *S. pullorum*'un geldiğini vurgulamışlardır. *Salmonella* izolatları en çok nalidiksik asit (%74,25) ve tetrasikline (%37,64) dirençli bulunurken, seftriakson (%1,07) ve sefiksim (%1,24) duyarlı olduğunu belirtmişlerdir.

*L. monocytogenes* Gram-pozitif, fakültatif anaerobik, kapsülsüz ve sporsuz bir bakteridir. Hücreler kısa, yuvarlak uçlu çubuk veya kokobasil şeklindedir. Optimum gelişme sıcaklığı genellikle 35-37 °C olup, suşlar 1-45 °C gibi geniş bir sıcaklık aralığında da gelişme gösterebilmektedir. Halotolerant olan *L. monocytogenes* yüksek konsantrasyonlardaki NaCl (%10-12) varlığında ve geniş pH aralığında (4,1-9,6) çoğalabilmektedir. Çiğ süt, önemli bir *L. monocytogenes* kaynağı olarak bilinmektedir. Bunun yanında süt ve süt ürünleri, sığır ve domuz eti ile fermente sucuk gibi et ve ürünleri, turp, lahana gibi taze ürünler ile deniz ürünlerinden ve gıdaların hazırlandığı yerlerde bulaşan olarak gözlenmektedir (Yavuz ve Korukluoğlu, 2010).

Pehlivanlar Onen ve Elmalı (2016) tarafından yapılan bir çalışmada, Hatay'da üretilen yüz on altı yöresel peynir örneğinde *L. monocytogenes* varlığı ve antibiyotik dirençlilikleri incelenmiştir. Sonuç olarak, peynir örneklerinin yirmisinde *L. monocytogenes* tespit edilmiştir. Tüm izolatlar streptomisine dirençli bulunmuş, bunların bir kısmında ise kloramfenikol (2 suş), tetrasiklin (6 suş), ampisilin, vankomisin (bire suş), eritromisin (4 suş) ve gentamisin (5 suş) direnci tespit edilmiştir. Araştırmacılar, yöresel peynirlerde *L. monocytogenes*'in varlığının ve bu bakteride antibiyotik direncin halk sağlığı için risk oluşturduğunu vurgulamış ve denetimlerin artırılmasını önermiştir.

Moura ve arkadaşları (2024) Fransa'da ve deniz aşırı bölgelerde 2012 ve 2019 yılları arasında toplanan klinik (2908) ve gıda (2431) örneklerinden izole edilen *L. monocytogenes* üzerinde yapılan fenotipik antimikrobiyal direnç değerlendirmeleri ile dizileme verilerini kullanmışlardır. Bu süreçte antimikrobiyal dirençte bir artış olmadığı ve tüm izolatların (%100) listeriyoz için birinci basamak tedavi olan ampisilin ve amoksisiline duyarlı olduğunu gösterilmiştir. Ayrıca yazarlar, tüm genom dizilemesinin (WGS) antimikrobiyal direnci tahmin etmede yüksek bir doğruluğa (>%99) sahip olduğunu ve bununla ilişkilendirilmiş genetik elementlerin (transpozonlar, profajlar veya plazmidler gibi) aydınlatılabileceğini de ortaya koymuşlardır. Dahası çalışma, kazanılmış direncin nadir olduğunu (%2,23) ancak gıda örneklerinde (%3,74) klinik örneklere (%0,98) göre daha yaygın olduğunu göstermişlerdir. *Campylobacter* spp., Gram negatif, hareketli, çubuk formda, spor üretmeyen küçük ve sarmal yapılu bakterilerdir. Bu bakterilerin başlıca bulaşma kaynağı kanatlıların bağırsak florasıdır. Bununla birlikte, sığır ve koyun gibi diğer çiftlik hayvanlarının bağırsaklarında da bulunabilmektedir (Kara ve Arpacı, 2023).

Hızlısoy ve arkadaşları (2020), Kayseri bölgesindeki üç farklı kesimhaneden kesim tahtası, kesimhane atık suyu, duvar, bıçak ve karkaslardan toplam 150 numune toplamışlardır. Bu numunelerden 17'si *Campylobacter* spp. açısından pozitif çıkmıştır. *Campylobacter jejuni* izolatlarının tamamı siprofloksasine dirençli bulunurken, enrofloksasine direnç oranı %87,5, neomisine direnç oranı %25, amoksisilin-klavulanik asite direnç oranı %25 ve eritromisine direnç oranı ise %12,5 olarak tespit edilmiştir. *C. coli* izolatında ise test edilen antibiyotiklere karşı direnç olmadığı belirtilmiştir. Benzer bir çalışma olan Al-Khresieh ve arkadaşları (2022) insanlardan, tavuk kloakasından, çiğ tavuk etinden, pastörize edilmemiş süttten ve sebzelerden alınan üç yüz altmış örnek incelenmiştir. Tavuklar, ruhsatlı ve ruhsatsız mezbahalardan temin edilmiştir. Çalışma, bu örneklerde *C. jejuni*'nin varlığını, biyokimyasal testler ve polimeraz zincir reaksiyonu (PCR) kullanılarak virülans genleri olan *hipO*, *asp*, *dnaJ*, *cadF*, *cdtA*, *cdtB* ve *cdtC* ile moleküler olarak tanımlamıştır. Sonuçlar değerlendirildiğinde, tavuklardan alınan örneklerin çoğunda *C. jejuni* ve *C. coli* tespit edilmiştir, bunlardan 5 izolatın *C. coli*, 15 izolatın ise *C. jejuni* olduğu belirlenmiştir. Özellikle, tavuk boyunları ruhsatlı mezbahalardan alındığında *C. jejuni* kolonizasyonu %6,66 gibi düşük bir oranla tespit edilmiştir; buna karşın ruhsatsız mezbahalardan alınan boyunlarda bu oran %33,3'tür. Antimikrobiyal duyarlılık testleri, tüm *C. jejuni* izolatlarının antibiyotiklere dirençli olduğunu ve %53,5'inin altı antibiyotiğe karşı direnç gösterdiğini ortaya koymuştur. Tüm izolatlar, özellikle siprofloksasin, tetrasiklin ve aztreonama karşı dirençli bulunmuştur. Ayrıca, Penner testi, insan örnekleri, tavuk boyunları ve kloaka örneklerinde en baskın serotipi P:21 olarak belirlenmiştir. Atımlı alan jel elektroforez (PFGE) analizi, *C. jejuni*'nin insan örnekleri ve tavuk boyunları arasında tam genetik homoloji, kloaca örnekleriyle ise kısmi homoloji göstermiştir. Bu çalışma, tavuk üretiminin güvenliğini sağlamak ve gıda kaynaklı patojenlerle, özellikle *Campylobacter* ile enfeksiyon riskini sınırlamak için etkili müdahale stratejilerinin önemini vurgulamışlardır.

*B. cereus*, *Bacillaceae* familyasında yer alan bir Gram-pozitif, çubuk şeklinde bir bakteridir. Hücrenin tamamını saran flajellumları (veya Peritrik flajellaları) sayesinde hareketli olan bu bakteri, aerofilik özellik gösterir ve kemoorganotrofik bir yapıya sahiptir. *B. cereus*'un çoğalma aralığı 10-45°C arasında olup, optimal çoğalma sıcaklığı 37°C'dir. Spor oluşumu için minimum sıcaklık -1°C, maksimum sıcaklık ise 59°C olup, optimum sıcaklık 30°C civarındadır. Bu bakteri, hem çiğ hem de işlenmiş veya pişirilmiş gıdalarda bulunabilir. Ayrıca, donmuş ve pastörize edilmiş ürünlerde de canlı sporların bulunması ve düşük sıcaklıklarda bile bu sporların aktifleşebilmesi *B. cereus* ile ilgili endişeleri artırmıştır (Halkman ve Çilak, 2018; Sağlam ve Şeker, 2016).

Can ve Sarı (2023) tarafından yapılan çalışmada, Malatya'daki köy tipi peynirlerden alınan yetmiş beş örneğin elli beşinde (%73,3) *B. cereus* tespit edilmiştir. Araştırmacılar tarafından elde edilen tüm izolatların (%100) sefoksitine dirençli olduğu ve ayrıca rifampin (%94,5), penisilin (%87,2), eritromisin (%58,1), gentamisin (%56,3) ve kloramfenikol (%12,7) gibi antibiyotiklere de direnç gösterdiği gözlemlenmiştir.

Fiedler ve arkadaşları (2019) Almanya pazarında satılan çiğ sebze ürünlerinde bulunan toksin üreten *B. cereus sensu lato* (s.l.) grubuna ait yüz kırk yedi suşu incelemiştir. Yapılan analizler sonucunda, bu suşların tamamının (%100) penisilin-G ve sefotaksim gibi  $\beta$ -laktam antibiyotiklerine ve %99,3 oranında amoksisilin/klavulanik asit kombinasyonu ile ampisiline karşı direnç gösterdiğini belirtmişlerdir. Bununla birlikte araştırma sonucunda bu suşların çoğu siprofloksasin (%99,3), kloramfenikol (%98,6), amikasin (%98,0), imipenem (%93,9), eritromisin (%91,8), gentamisin (%88,4), tetrasiklin (%76,2) ve trimetoprim/sülfametoksazol kombinasyonuna direnç (%52,4) gösterdikleri saptanmıştır.

## Sonuç

Patojenlerin gıdalara bulaşması, önemli ölçüde insan faaliyetleri ve bazen bilinçli veya hatalı insan kararlarının etkilerinin sonucu olarak gözlenmektedir. Antibiyotiğe dirençli gıda kaynaklı patojenlerin ortaya çıkmasının ve bulaşmasının ana nedenlerinden biri, tarımsal üretim, hayvancılık, gıda işleme süreçlerinde ve bazı fungal hastalıkların kontrol altına alınmasında kullanılan antibiyotiklerin yanlış yönetimi, yani yanlış seçim veya aşırı kullanımı olarak kabul edilmektedir. Gıdalardan izole edilen bakterilerin direncinin artması nedeniyle etkinliği giderek azalan antibiyotikler arasında beta-laktamlar, sülfonamidler, tetrasiklinler ve florokinolonlar yer almaktadır.

Bu derlemede özetlendiği üzere dünyanın farklı bölgelerinde yapılan birden fazla çalışma gıda kaynaklı bakterilerde çoklu ilaç direncine ilişkin sonuçları gözler önüne sermektedir. Özellikle gıda patojenlerinin antimikrobiyal direnç kazanımında endişe verici olan husus, direnç kazanmış organizmaya özgü özelliklerin yeterince tanınmaması ve neden oldukları enfeksiyonların sonuçlarının tahmin edilmesinin zorluğudur. Bu nedenle, antimikrobiyal direnç oluşumu dinamiklerini anlamak, takibi için gelişmiş metodolojiler geliştirmek, bulaşma yolları ve mekanizmaları hakkında fikir edinmek çok önemlidir. Antibiyotik kullanımı ve antimikrobiyal dirençle ilgili verilere erken ve sürekli erişim, antimikrobiyal direnç gelişimini önlemeye yönelik daha etkili müdahaleler yapılmasını sağlayacaktır.

Antibiyotik kullanımının azaltılması ve sadece gerekli durumlarda kullanılması konusunda hayvan yetiştiricilerinin, veterinerlerin, gıda üreticilerinin ve tarım işçilerinin bu alanda eğitilmesi de son derece önem arz etmektedir. Patojenlerin antibiyotik direnci göstermesi, ulusal ve uluslararası bir problem olarak görülmesi ve bu konuda alınacak önlemlere bağlı olarak halk sağlığının korunması için gerekli düzenlemelerin yapılması gerekmektedir. Bunun sonucu olarak, antimikrobiyal direnç kazanmış patojenler gıda güvenliği ve halk sağlığı kapsamında değerlendirildiğinde, gıda üretiminin ve işlenmesi için gerekli teknolojik ve hijyenik önlemlerin alınması önem arz etmektedir.

## Beyanlar

### Etik Kurul İzni

Etik kurul izni ve/veya yasal/özel izni gerekmemektedir.

## Yazar Katkı Beyanı

A.U.: Literatür taramasını gerçekleştirme, araştırma ve orijinal taslağın yazılması  
B.K.B.: Gözden geçirme ve düzenleme

## Çıkar Çatışması

Yazarlar arasında çıkar çatışması bulunmamaktadır.

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## Integrating Millets into Modern Agriculture: A Strategic Pathway to Advancing Sustainability, Climate Resilience, and Nutritional Security

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Hidden hunger

Climate-smart agriculture

Millets, including pearl millet, foxtail millet, and finger millet, among others, offer a complementary solution to traditional staple crops such as wheat, rice and maize, particularly in the context of climate change. Known for their resilience to heat, drought, and poor soil conditions, millets can be integrated into existing agricultural systems to enhance food security and nutritional diversity in regions increasingly affected by climate change. While millets may not replace staple crops, their cultivation alongside these staples provide several benefits, including improved nutritional outcomes and reduced environmental impact. Pearl millet, for instance, is rich in iron and zinc, addressing micronutrient deficiencies that are common in many developing regions. Finger millet's high calcium content makes it a valuable addition to diets in areas with limited access to dairy. These grains thrive in marginal environments, contributing to more sustainable farming practices with a lower environmental footprint. Incorporating millets into agricultural systems can reduce dependency on water-intensive crops, lower the risk of crop failure, and provide a buffer against the impacts of climate change. By diversifying cropping systems, millets could help to stabilize food production and improve nutritional outcomes without displacing the critical role of traditional staples in global diets. To maximize the benefits of millets, efforts should focus on improving value chains, supporting smallholder farmers, and increasing consumer awareness. Moreover, targeted research and supportive policies are critical to unlocking their full potential and integrating them effectively into global food systems. As the world faces the dual challenges of climate change and malnutrition, millets offer a viable pathway to enhance resilience and sustainability in agriculture, complementing staple crops and enriching global food systems.

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### Introduction

Addressing hunger and ensuring food security for the growing global population are two of the most critical challenges of the globe nowadays (Altaf et al., 2023). Several factors contribute to this issue, including shortages of both micro and macronutrients, imbalances between food supply and demand, and conflicts that disrupt food production in various regions across the globe (Saxena et al., 2018). Declining food production rates coupled with the need to feed more than 9 billion people by 2050, could leave 2 to 3 billion individuals facing hunger and nutritional insecurity (Wheeler & Von Braun, 2013; Godfray et al., 2010). Climate change, along with rising global temperatures, directly threatens agricultural productivity and the overall sustainability of food systems. While some regions may see productivity gains due to

climate change, these increases will be insufficient to meet global food demand (Liaqat et al., 2022). Many scientists agree that current levels of global warming and greenhouse gases (GHG) emission will sharply reduce crop productivity. To secure a sustainable food supply, controlling global temperatures by controlling GHG emissions is unavoidable. However, the agricultural sector itself is a major source of emissions, particularly methane, due to intensive farming operations (Chataut et al., 2023).

Agriculture is often viewed as a cornerstone of national and food security due to its essential role in human survival. However, the growing challenges posed by climate change and an expanding global population are causing widespread concern (Muluneh, 2021). Both human-made and natural disasters continue to disrupt

agricultural productivity and food systems, jeopardizing the ability to meet the rising demand for nutrition and energy (Mishra et al., 2021). Farmers now face the compounded difficulties of adapting to climate change alongside uncertainties in production, market fluctuations, transportation, and income stability (Rasul, 2021). These issues have a direct and/or indirect impact on agriculture and food systems, leading to socio-economic instability, especially among vulnerable populations. To address these pressing concerns, a shift is required from incremental adjustments to more transformative approaches that prioritize human health, nutrition, and environmental sustainability. This need is particularly urgent given the ongoing natural and man-made crises, emphasizing the importance of building a climate-resilient agricultural framework (Hossain et al., 2021). One promising approach is the integration of “orphan crops” into food systems which have the potential to diversify agricultural production while offering more nutritious food options (Talabi et al., 2022). Orphan crops are traditional crops that have been largely neglected in terms of scientific research, commercial development, and policy support, despite their significant potential to address nutritional and food security challenges, particularly in marginal environments (Mabhaudhi et al., 2019). Examples include millets, sorghum, and teff, among others, which thrive in diverse climatic conditions and offer high nutritional value. The rising interest in orphan crops is reflected in global efforts, such as the United Nations’ designation of 2023 as the “International Year of Millets,” aiming to promote their role in sustainable agriculture, climate resilience, and nutritional security.

Millets, among the oldest and most adaptable grains hold significant importance for ensuring food security, improving nutrition, and providing income for smallholder farmers in developing regions. Nevertheless, despite their historical significance, these grains have been largely neglected in recent years, with minimal scientific focus on enhancing their yields under stressful environmental circumstances (Kumar et al., 2018). As orphan crops, millets often outperform other crops in response to climate change, thanks to their unique characteristics such as thriving on marginal lands, requiring little water, and demonstrating resilience to both biotic and abiotic stresses (Kumar et al., 2023). The pressing challenges of climate change and pandemics call for swift and robust policies to safeguard food and nutritional security especially for communities already burdened by poverty (Bisoffi et al., 2021).

Millets play a critical role in addressing “hidden hunger,” a term used to describe micronutrient deficiencies that affect billions of people globally, often without visible symptoms. Unlike macronutrient deficiencies, hidden hunger impairs immune function, cognitive development, and overall well-being (Srivastava and Arya, 2021). Millets are a powerhouse of micronutrients such as iron, zinc, calcium, and magnesium, which are essential for preventing diseases caused by deficiencies, such as anemia and osteoporosis (Kumar et al., 2024). For instance, pearl millet is rich in iron and zinc, addressing common deficiencies in developing regions, where anemia is widespread. Finger millet, known for its exceptionally high calcium content, is particularly beneficial for children and lactating mothers who require higher calcium intake

(Anitha et al., 2021). Barnyard millet, with its high fiber content and low glycemic index, not only improves gut health but also supports better absorption of these micronutrients. Incorporating millets into regular diets can substantially alleviate hidden hunger by providing essential micronutrients in bioavailable forms (Mazumder et al., 2024). This is especially crucial in low- and middle-income countries, where diets often lack diversity and are predominantly composed of calorie-dense but nutrient-poor staples like rice and wheat.

This review focuses on how millets are important for a sustainable agricultural system. Furthermore, this review summarizes the unique properties of millets and their nutritional and health benefits. Additionally, we discuss strategies for integrating millets into mainstream agriculture through policy creation and research investment.

## Global Cultivation and Production of Millets

Millets are grown across 93 countries globally (Meena et al., 2021). Figure 1 illustrates the global distribution of millet cultivation, highlighting the regions where different types of millets are predominantly grown. Africa emerges as a major hub for millet cultivation, particularly in semi-arid and arid zones, where these crops thrive under rainfed conditions. The figure underscores millet’s role in ensuring food security in regions prone to drought and poor soil fertility. In Asia, millet cultivation is particularly significant in India and China, with these countries accounting for a substantial proportion of global production. The figure emphasizes millet’s adaptability to diverse climates, making it an indispensable crop in global agricultural systems. This spatial representation underscores the importance of millet in combating climate challenges and supporting smallholder farmers. In China, millets have long been a dietary staple, particularly in the cold, arid northern regions where foxtail, proso, and barnyard millet varieties are most commonly grown. In Latin America, millet cultivation is concentrated in Mexico, primarily for animal feed while in North America, proso millet is mainly grown as bird feed in the Great Plains and Midwest (Kheya et al., 2023). Globally, the top millet producers are India, Nigeria, and China which collectively account for over 60% of the world’s millet production (Table 1). India alone contributes 80% of Asia’s millet output and 20% of global production. Although India has historically been the leading producer, recent years have seen a surge in millet cultivation in Africa. Globally, sorghum accounts for 65% of the total millet production (Amit Tomar et al., 2023).

## Types of Millets

### *Pearl Millet (Pennisetum glaucum)*

Pearl millet ranks as the world’s sixth most significant cereal, predominantly cultivated across Central, Eastern, and Southern Africa, the Sahel region, Pakistan, and India. This resilient crop survives in arid environments and nutrient-poor sandy soils, tolerating minimal annual rainfall ranging from 200 to 500 mm (Prasad et al., 2020). It plays a critical role in ensuring food security, particularly in Africa and India (Nagaraj et al., 2013; Jukanti et al., 2016).

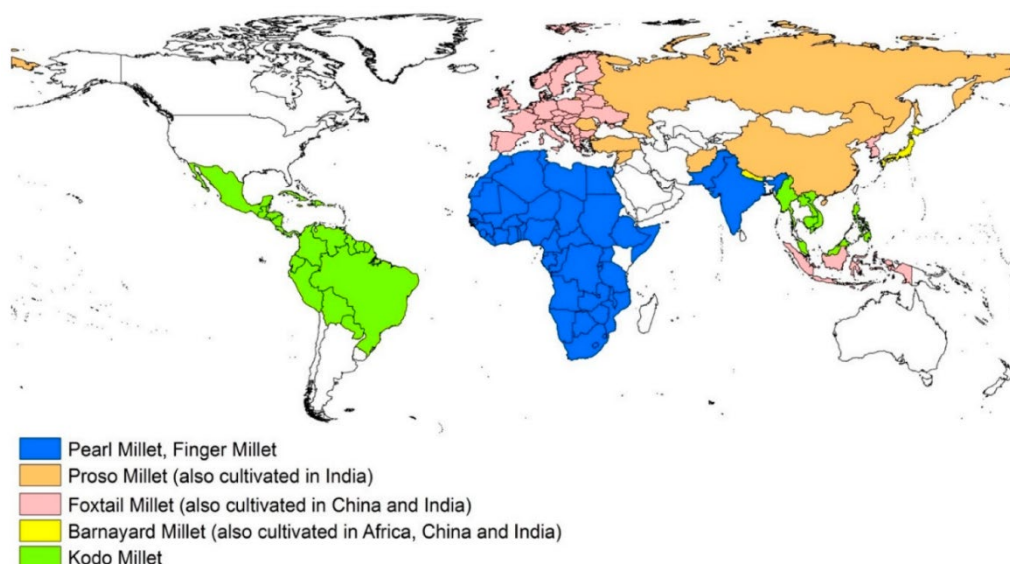


Figure 1. Cultivation of different types of millets around the world (Saxena et al., 2018).

Table 1. Top ten producing countries of millets in 2023 (USDA, 2024).

Country	% of global production	Total production (Metric Tons)
India	41%	12.2 million
Niger	11%	3.16 million
China	9%	2.7 million
Mali	7%	1.94 million
Nigeria	5%	1.56 million
Senegal	5%	1.35 million
Ethiopia	4%	1.1 million
Burkina Faso	3%	861,000
Sudan	2%	684,000
Chad	2%	634,000

#### ***Finger Millet (*Eleusine coracana*)***

Finger millet is primarily cultivated across regions of India and Africa, ranking as the sixth most important cereal grain in India. Known for its resilience, it tolerates higher temperatures and soil salinity better than most cereal crops. Optimal growth occurs in temperatures ranging from 11 to 27°C, with a soil pH preference between 5 and 8.2 (Upadhyaya et al., 2008; Devi et al., 2014).

#### ***Proso Millet (*Panicum miliaceum*)***

Proso millet, originally from Central and Eastern Asia, is now widely cultivated in areas such as India, Russia, the Middle East, and Europe. This crop thrives in short growing seasons, typically maturing within 60 to 75 days, and flourishes under minimal rainfall and moderate temperature conditions (Santra et al., 2019; Djanaguiraman et al., 2020).

#### ***Foxtail Millet (*Setaria italica*)***

Foxtail millet is extensively grown across Europe, China, India, and other regions. It stands out for its rapid maturation, superior photosynthetic efficiency, and greater water-use efficiency compared to crops like maize and sorghum (Singh et al., 2017; Moharil et al., 2019).

#### ***Barnyard Millet (*Echinochloa spp.*)***

Japanese barnyard millet (*Echinochloa utilis*) and Indian barnyard millet (*Echinochloa frumentacea*) are widely grown in countries such as India, China, Japan, and Nepal. Known for their drought resilience and quick maturation, these millets

are also highly valued for their nutritional richness (Jayakodi et al., 2019; Mohanapriya et al., 2024).

#### ***Kodo Millet (*Paspalum scrobiculatum*)***

The distribution of Kodo millet is extensive in moist environments throughout tropical and subtropical regions worldwide. This is an indigenous cereal originating from India. It contains a high protein level of 11%, as well as a low fat content of 4.2% and a very high fibre content of 14.3%. Easily digestible, Kodo millet is rich in lecithin and is highly beneficial for enhancing the functioning of the nervous system. Kodo millets are abundant in antioxidants, particularly niacin, B6, and folic acid, and with essential minerals like calcium, iron, potassium, magnesium, and zinc. Kodo millets are gluten-free and highly suitable for individuals with gluten intolerance (Dayakar Rao et al., 2017).

#### ***Little Millet (*Panicum sumatrense*)***

This millet was domesticated in India. It is an annual herbaceous species that grows either straight or with folded blades to a height ranging from 30 cm to 1 m. It can withstand both drought and waterlogging. It is capable of being grown at elevations up to 2000 metres above sea level. Considering its early maturity and ability to withstand unfavorable agro-climatic conditions, it is another dependable catch crop. The stover is an excellent animal feed for cattle (Dayakar Rao et al., 2017).

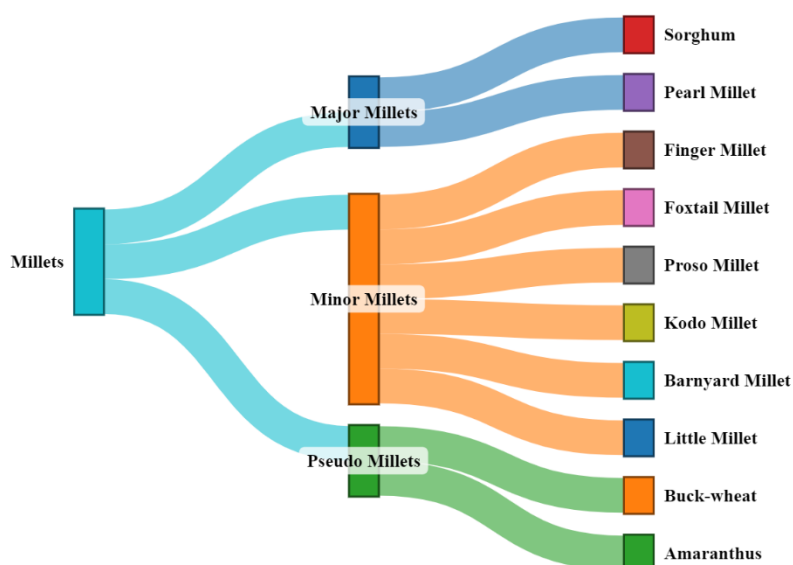


Figure 2. Types of millets.

### ***Sorghum (Sorghum bicolor)***

Sorghum is a highly adaptable and robust cereal crop primarily cultivated in the arid and semi-arid regions of Africa and Asia. Acknowledged for its exceptional drought resistance, sorghum is able to flourish in unfavorable soil conditions and endure high temperatures. It is also notably water-efficient compared to maize and wheat, which makes it an essential crop in water-scarce areas. Beyond its traditional use as food and animal feed, sorghum is gaining attention for its promising role in biofuel production (Liaqat et al., 2024; Liaqat et al., 2023). Figure 2 illustrates the classification of millets based on key characteristics, including seed size, plant type, and growth habits. Millets are broadly categorized into three groups: major millets, minor millets, and pseudo-millets (Amit Tomar et al., 2023).

### **Agronomic Advantages of Millets**

Millets are exceptionally well-adapted to combat the adverse effects of drought and other abiotic stresses commonly encountered in semi-arid and arid regions. These stresses, which encompass drought, extreme temperatures, flooding, and salinity, adversely impact crop yields. Millets exhibit a variety of morpho-physiological, molecular, and biochemical characteristics that enhance their resilience to these environmental challenges (Chellapilla et al., 2022). For example, pearl and proso millet require only about 20 cm of rainfall, a stark contrast to rice, which demands 120 to 140 cm (Kumar et al., 2018). Additionally, the relatively short growing period of millets (approximately 10 to 12 weeks) compared to major crops further aids in alleviating the impact of these stresses (Bandyopadhyay et al., 2017).

Millets possess a C4 photosynthetic pathway, a highly efficient mechanism that allows plants to thrive in high-temperature and low-moisture environments. This pathway enhances the concentration of carbon dioxide in the bundle sheath cells, significantly reducing photorespiration, a process that can waste energy and carbon. By improving water-use efficiency and carbon fixation, the C4 pathway

enables millets to grow optimally under stressful environmental conditions where C3 crops, such as wheat and rice, may struggle. For instance, *Setaria italica* requires only 257 grams of water to produce one gram of dry biomass, compared to 470 grams for maize and 510 grams for wheat (Li & Brutnell, 2011; Nadeem et al., 2020). This heightened photosynthetic efficiency allows millets to grow better, allocate biomass more effectively, and maintain lower hydraulic conductivity in warm conditions.

Millets display several adaptive characteristics, including the ability to alter their flowering patterns in response to varying rainfall and to adjust membrane dynamics to maintain water balance during stress (Bidingger et al., 2007; Bandyopadhyay et al., 2017). In response to abiotic stresses, millets accumulate antioxidants and osmolytes, which further bolster their resilience (Ajithkumar & Panneerselvam, 2014; Tiwari et al., 2022). These features render millets an excellent model for investigating stress-responsive traits and mechanisms, essential for enhancing their adaptability and overall improvement.

### **Nutritional and health advantages of millets**

Millets are integral to ensuring nutritional and health security, particularly in developing countries where vulnerable populations are disproportionately impacted by crises and pandemics. Enhancing the immune system is vital for protection against pathogens such as bacteria and viruses (Calder, 2020). Millets are distinguished by their high nutrient density (Table 2), which contributes significantly to their health benefits. In comparison to other major cereals, millets typically offer higher protein levels. For instance, finger millet contains between 11.64% to 13.6% protein (Onipe & Ramashia, 2022), foxtail millet ranges from approximately 11.13% to 18.75% (Sachdev et al. 2021), and proso millet provides 10.65% to 14.10% protein (Shen et al. 2018). Consequently, the substantial protein content of millets makes them a crucial source of plant-based protein in many dietary patterns.

Table 2. Nutritional value of millets and major cereals.

Crop	Protein (g/100 g)	Fibre (g/100 g)	Carbohydrate (g/100 g)	mg/100 g						
				K	Ca	P	Mg	Zn	Fe	Cu
Foxtail millet	12.3	8	60.9	299.3	38	422	81	2.9	5.3	1.6
Proso millet	12.5	7.2	70.4	177.3	23	281	117	2.4	4	5.8
Kodo millet	8.3	9	65.9	181.8	31	215	166	1.5	3.6	5.8
Finger millet	7.3	3.6	72	407	398	320	137	2.3	3.9	0.5
Barnyard millet	6.2	9.8	65.5	195	21	340	82	2.6	9.2	1.3
Pearl millet	11.8	2.3	67	275.7	46	379	137	3.1	8	1.1
Little millet	7.7	7.6	67	192.2	12	251	133	3.5	13.9	1.6
Sorghum	10.4	2	70.7	672	13.5	380	165	2.51	8.23	0.3
Wheat	6.8	1.2	71.2	107	34	357	137	2.6	3.6	0.4
Rice	11.8	0.2	78.2	35	21	433	177	6	2	0.2

Source: Muthamilarasan et al. (2016); Saleh et al. (2013); Choudhary et al. (2023)

Millets are abundant in both soluble and insoluble dietary fibers, which play a crucial role in regulating blood sugar levels and enhancing digestive health. Among these, pearl millet stands out as a particularly nutritious grain, offering superior levels of calories, protein, vitamins, and minerals compared to other major cereals. It also contains phenolic compounds with demonstrated antidiabetic properties, making them a viable ingredient for foods aimed at managing diabetes mellitus (Pei et al., 2022). Additionally, pearl millet provides various health benefits, including a reduced risk of cardiovascular diseases, diabetes mellitus, cancer, and decreased tumor incidence. It can also contribute to lower blood pressure and reduced cholesterol levels (Pei et al., 2022). Rich in B complex vitamins and high in antioxidants, millets support detoxification by eliminating harmful toxins and neutralizing free radicals, which may help prevent conditions such as heart diseases and cancer (Kumar et al., 2021).

Millets are a valuable source of essential nutrients, including calcium, iron, potassium, magnesium, phosphorus, manganese, zinc as well as important compounds such as vitamins, amino acids, and fatty acids (Muthamilarasan & Prasad, 2021). Finger millet stands out for its high levels of calcium, iron, and zinc, while pearl millet is especially rich in zinc and iron (Kumar et al., 2021; Kumar et al., 2022). Millets are also abundant in magnesium and potassium, which can help lower blood pressure by acting as vasodilators, thereby reducing the risk of cardiovascular diseases. Moreover, their high fiber and mineral content contributes to alleviating digestive issues such as constipation, bloating, excess gas, and cramping (Rodríguez et al., 2020). Similarly, millets offer a range of health benefits, including antioxidant, anticancer, anti-inflammatory, antifungal effects, and support for blood clot prevention.

Finger millet stands out among cereals for its exceptionally high calcium content, exceeding 0.3%, which makes it particularly advantageous for bone and dental health, especially for children (Ceasar et al., 2018; Antony Ceasar & Maharajan, 2022). In addition, its fiber and mineral levels surpass those found in rice and wheat (Gull et al., 2014). Moreover, finger millet is relatively rich in protein and provides a well-balanced amino acid profile, including lysine, threonine, and valine, which are frequently deficient in other starchy cereals (Anitha et al., 2020).

Barnyard millet, known for its high crude fiber content, supports better blood glucose regulation and has a lower glycemic index. In contrast, other types of millet, such as foxtail millet, are abundant in essential minerals and vitamins. Little millet is a good source of iron and fiber, while pearl millet is particularly high in protein. Including millet in the diet can help people in low and middle-income countries meet their nutritional needs, thereby contributing to sustainable development goals (Kumar et al., 2016; Goron & Raizada, 2015). Millets present a diverse range of micronutrients, addressing cereal needs while supporting long-term nutritional security. They also offer a viable substitute for wheat flour for those with celiac disease. Additionally, millets support sustainable agricultural practices and align with the United Nation's objective of ensuring healthy lives and promoting well-being for people of all ages.

### Millets As Climate-Smart Crops

Millets offer a sustainable alternative in the context of climate change due to their resilience against the negative effects of global warming. As C4 plants, millets are adept at utilizing atmospheric CO<sub>2</sub> more effectively, converting it into biomass while emitting fewer GHG, with emissions ranging from 3218 to 3358 kg CO<sub>2</sub> equivalent per hectare (Jain et al., 2016). They exhibit notable climate-resilient traits, such as adaptability to diverse environmental conditions, reduced irrigation requirements, and enhanced growth with minimal nutrient inputs and environmental stress (Rajendrakumar, 2022). Millets are capable of thriving in higher temperatures and with less water, thus having a lower carbon footprint compared to other crops, which contributes to mitigating the global carbon impact (Bisht et al., 2022).

Millets offer several advantages due to their rapid life cycle and stress-adaptive traits. Their short stature, small leaf area, thicker cell walls, and deep root systems contribute to their resilience (Kencharaddi et al., 2024; Patan et al., 2024). The C4 photosynthetic pathway further reduces photorespiration and enhances efficiency under stress conditions. For instance, pearl millet thrives in poor sandy soils and adapts well to arid climates, while finger millet withstands salinity, high temperatures, and fluctuating soil pH (Kheya et al., 2023). Additionally, millets require substantially less water than crops such as rice and sugarcane, making them ideal for water-scarce

regions. Their capacity to provide food, fodder, and income for smallholder farmers, combined with their potential for diverse products, underscores the importance of government support and further research in millet cultivation and market development.

### **Advances In Millet Research and Genomic Sequencing**

Millets, traditionally cultivated in South Asia and Central Africa, have gained significant global research attention due to their exceptional nutritional benefits and adaptability to climate change. Twenty years ago, millet research was primarily limited to Asia, particularly India, and attracted minimal interest from high-income countries. Recently, however, this focus has broadened, with researchers from regions including Europe and America actively engaging in millet studies. This shift has led to an increase in published studies in reputable journals, reflecting the growing recognition of millets' potential.

Advancements in genomic sequencing have further driven millet research, enabling the identification of genes and traits linked to stress resilience and nutritional enrichment. Genomes of various millet species, such as foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), and barnyard millet (*Echinochloa* spp.), have been sequenced, providing valuable insights (Zou et al., 2019; Hatakeyama et al., 2018; Hittalmani et al., 2017; Guo et al., 2017; Varshney et al., 2017; Bennetzen et al., 2012; Zhang et al., 2012). Foxtail millet was the first minor millet to have its genome fully sequenced, paving the way for understanding genes related to stress adaptation. The Phytozome database now includes a fully annotated genome for finger millet ([https://phytozome-next.jgi.doe.gov/info/Ecoracana\\_v1\\_1](https://phytozome-next.jgi.doe.gov/info/Ecoracana_v1_1)), further advancing the study of its stress-resilient and nutrient-rich traits. However, many other millet genomes remain in draft form, lacking full annotation, which limits their utility for genetic studies.

The availability of genome sequences, whether annotated or in draft form, holds great promise for advancing research on millets' nutrient enrichment and climate resilience mechanisms. For instance, studying quantitative trait loci (QTL) associated with calcium levels in finger millet offers a pathway to address global calcium deficiencies (Antony Ceasar and Maharajan, 2022). While progress has been made in identifying genes and QTL for biotic and abiotic stress in major cereals like rice, wheat, and maize, similar studies in millets remain limited. Genome-editing tools, such as CRISPR/Cas systems, represent a promising avenue to enhance millet productivity and stress resilience. However, challenges in genetic transformation and regeneration techniques must be overcome to fully leverage these tools. Future research should prioritize complete genome annotation and the development of effective transformation protocols to accelerate millet improvement and ensure their role in global food security.

### **Strategic Approaches for Mainstreaming Millets In Agriculture**

Addressing climate change and pandemics poses considerable challenges due to their unpredictable nature and the potential for severe nutritional and economic consequences. These crises underscore the necessity for robust risk governance strategies that incorporate scientific insights into policy decisions to effectively manage both human and natural disasters (Mishra et al., 2021; Priyadarshini & Abhilash, 2021). To fully realize the benefits of underutilized crops such as millets, a collaborative approach involving local, regional, and international stakeholders is crucial. Such efforts should be aligned with global sustainable development goals and encompass comprehensive engagement across the entire value chain i.e., from research and production to marketing and consumption (Babele et al., 2022). Effective policymaking is essential to bridge existing knowledge gaps and facilitate collaboration among various sectors, thereby promoting the integration of these crops into mainstream agriculture (Talabi et al., 2022). The growing recognition of millets for their climate resilience and health benefits highlights the need for increased research and development in this area. Policies should therefore shift focus towards supporting these underutilized crops rather than solely concentrating on major staples like maize, rice, and wheat.

### **Policy Development and Investment for Millet Crops**

Developing markets for orphan crops like millets necessitates the implementation of supportive policies and an enabling environment, which can be achieved through collaborations among national, regional, and international organizations. Governments need to craft integrated policies that highlight the importance of millet crops, fostering public-private partnerships to bolster agricultural sustainability in developing areas. It is essential to promote the adoption of advanced agricultural technologies by farmers and end-users and to enhance extension services that bridge research with farming practices (Bisoffi et al., 2021; Borelli et al., 2020). In addition, policies should address land use, marketing, and credit systems to facilitate the growth and commercialization of millet crops. Increasing millet adoption requires educational and awareness initiatives that emphasize the nutritional, environmental, and economic advantages of millets, coupled with programs aimed at diversifying diets.

Addressing challenges in post-harvest processing and storage is vital for scaling up millet grain production (Babele et al., 2022). The development of crop-specific equipment and storage facilities is crucial to minimize waste and preserve grain quality. Implementing effective storage solutions and enhancing supply chains will aid farmers and stakeholders by reducing losses and ensuring food security during adverse events (Baldermann et al., 2016; Muthamilarasan and Prasad, 2021).

Providing subsidies for smallholder farmers to invest in millet-specific machinery and storage infrastructure can further reduce production costs and enhance competitiveness in markets. Additionally, forming cooperatives or producer groups can empower farmers to access larger markets, negotiate fair prices, and reduce logistical barriers.

Investing in research networks and focusing on invasive species research are essential for improving the agricultural and economic value of less-studied crops. Additionally, the collection and management of germplasm from these crops are critical for preserving genetic diversity and mitigating the effects of biotic and abiotic stresses on crop productivity (Ye and Fan, 2021). Building interdisciplinary collaborations and obtaining funding from both national and international sources will enhance research into gene functions and stress resistance mechanisms. Engaging with government and non-governmental organizations for participatory research can harness indigenous knowledge and bolster millet research efforts. To integrate millets into modern food systems effectively, governments and organizations must promote millet-based products through public procurement programs, school feeding schemes, and awareness campaigns that highlight their health and environmental benefits (Shanker, 2024; Satyavathi and Bhat, 2024). The United Nations General Assembly designated 2023 as the International Year of Millets to spotlight their significance in food security and health benefits. This global acknowledgment underscores the importance of increasing awareness about the nutritional and climate-resilient properties of millets.

### Major Hurdles in Millet Research and Development

Millets have attracted growing attention in genomic and genetics studies in recent years. Nevertheless, high-resolution forward and reverse genetic studies, similar to those conducted on model plants, has not yet been utilized for millets. This limitation is largely attributed to the absence of comprehensive and annotated genome sequences for many millet species (Antony Ceasar and Maharajan, 2022). At present, annotated genome sequences are available only for foxtail millet and finger millet (<https://phytozome-next.jgi.doe.gov/>), as well as for green foxtail (*Setaria viridis*), a wild relative of foxtail millet (Lu, 2002). In contrast, other millets, such as pearl millet, proso millet, and barnyard millet, have only draft genome sequences that lack annotation (Zou et al., 2019; Varshney et al., 2017; Guo et al., 2017).

To make optimal use of excellent quality functional genomic investigations, particularly those utilizing the CRISPR/Cas system, it is essential to have access to fully annotated genome sequences. In order to precisely target genes and minimize off-target effects, these extensive sequences are essential (Ceasar, 2022). Nevertheless, a significant obstacle in progressing reverse genetic studies in millets is the lack of effective procedures for transformation and regeneration. This limitation hampers the application of gene-editing techniques like CRISPR in millets. In contrast to model plants such as *Arabidopsis*, which benefit from the straightforward floral dip transformation method due to their brief life cycle, millets encounter significant obstacles in this area (Antony Ceasar & Maharajan, 2022).

### Conclusions and future outlooks

The future of millet research and integration into modern agriculture is promising yet requires targeted efforts to overcome existing challenges. Continued advancement in genomic research and genome editing technologies such as CRISPR will be essential to enhance the nutritional quality and stress resilience of millet varieties. Additionally, interdisciplinary collaborations focused on improving post-harvest processing, storage, and market accessibility will further solidify millets as a sustainable solution for addressing global food security and nutritional challenges. Exploring millet-based dietary interventions in diverse regions could provide valuable insights into their role in alleviating micronutrient deficiencies and promoting health. The development of high-resolution genome sequences and effective transformation systems will facilitate deeper insights into their genetic makeup, enabling precise genetic improvements. Investments in research should focus on improving crop-specific technologies for planting, harvesting, and processing millets to boost their production and marketability.

Policy frameworks and market strategies must be developed to support the widespread adoption of millets. Governments and institutions should establish favorable policies and create partnerships to enhance the visibility and accessibility of millets. Public-private partnerships can play a significant role in bridging gaps between research and practical applications, promoting millet consumption through education and awareness campaigns, and supporting local farmers. Moreover, addressing post-harvest challenges is essential for the large-scale adoption of millets. Advancing genomic tools to accelerate the identification of stress-tolerant and nutrient-dense traits in millets can further support their integration into global food systems. Developing suitable storage technologies and optimizing supply chains will prevent significant losses and ensure the efficient distribution of millet products. The creation of robust markets and infrastructure will enhance the viability of millets as a staple crop, particularly in regions facing food security issues.

### Declarations

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### Author Contribution

All authors participated in the and design and completion of the study. W.L. and M.T.A. collected the material and wrote the first draft. C.B. and F.S.B. supervised, reviewed and made corrections to the article. All authors have reviewed and approved the final version.

### Conflict of Interest

The authors declare no conflicts of interest.

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## Use of Soybean Silage as A Forage Source in Dairy Cow Rations

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ARTICLE INFO	ABSTRACT
<p><i>Review Article</i></p> <p>Received : 08.11.2024 Accepted : 10.01.2025</p> <p><b>Keywords:</b> Ration Ruminant feeding Soybean, (Glycine max L) Soybean silage Concentrate feed</p>	<p>Rations for ruminant animals should contain a particular proportion of roughage and concentrate feed. Concentrated feeds, which are rich in nutrients, typically increase the cost of the ration. The cultivation of high-quality roughage is critical to reducing costs in cattle enterprises. Getting rid of this high cost in livestock enterprises depends on the production of quality roughage. Livestock firms in our country are looking for alternative roughage to cut feed costs and improve product quality. Soybean, the topic of this study, is a type of roughage that can be used as silage or grain in ruminant feeds. Soybean, a legume forage plant, is an important roughage feed due to its high protein content (about 20%). With this functionality, soybean can be substituted for some of the feed sources used as the basic protein source in ruminant rations. In fact, some studies have shown that soybean silage can be used, even partially, instead of soybean meal, which is considered the highest quality protein source. This article provides information on the potential of soybean plant to be used as a source of roughage (grain and silage) in ruminant rations.</p>

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## Süt Sığırı Rasyonlarında Kaba Yem Kaynağı Olarak Soya Silajı Kullanımı

MAKALE BİLGİSİ	ÖZ
<p><i>Derleme Makalesi</i></p> <p>Geliş : 08.11.2024 Kabul : 10.01.2025</p> <p><b>Anahtar Kelimeler:</b> Rasyon, Ruminant besleme Soya (Glycine max L) Soya silajı Kesif yem</p>	<p>Ruminant hayvanların beslenmesinde kullanılan rasyonlar belirli oranlarda kaba ve kesif yem içermelidir. Besin madde içeriği bakımından zengin olan kesif yemler genelde rasyon maliyetini artırmaktadır. Hayvancılık işletmelerinde bu yüksek maliyetten kurtulmak kaliteli kaba yem üretimine bağlıdır. Ülkemizde faaliyet gösteren hayvancılık işletmeleri yem (besleme) maliyetlerinin azaltılması ve ürün kalitesinin yükseltilmesi amacı ile farklı kaba yem arayışı içindedirler. Bu çalışmanın konusunu teşkil eden soya bitkisi, ruminant rasyonlarında hasıl veya silaj formunda kullanılma potansiyeli olan bir kaba yem türüdür. Bir baklagil yem bitkisi olan soya bitkisi protein içeriğinin yüksek olması (yaklaşık %20) ile kaba yem grubu yemler içinde önemli bir yere sahiptir. Soya bitkisi bu özelliği ile ruminant rasyonlarında temel protein kaynağı olarak kullanılan bazı yem kaynaklarının bir kısmı yerine ikame edilebilmektedir. Nitekim bazı çalışmalarda soya silajının en kaliteli protein kaynağı olarak kabul edilen soya küspesinin yerine bile kısmen de olsa kullanılabileceği ortaya konulmuştur. Bu derlemede soya bitkisinin ruminant rasyonlarında kaba yem kaynağı (hasıl ve silaj) olarak kullanılma potansiyeli hakkında bilgi verilmiştir.</p>

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## Giriş

Ruminant hayvanlar yaşamlarını sürdürebilmeleri (yaşama payı ihtiyaçları) ve et-süt gibi hayvansal ürünleri üretebilmeleri (verim payı ihtiyaçları) için ihtiyaç duydukları besin maddelerini farklı yem kaynaklarından temin ederler. Bahsedilen bu yem kaynakları genel anlamda kaba yemler ve kesif yemler olmak üzere 2 ana grupta incelenir. Kaba yemler daha ziyade yaşama payı ihtiyaçlarının karşılanması amacı ile kullanılan ve hayvanın sindirim sistemini çalıştıran bir yapıya sahip olan yem grubudur. Bir başka deyişle, kaba yemler besin maddelerin temini ve sindirim sisteminin sağlıklı çalışması bakımından önem taşımaktadır. Bahsedilen bu kaba yem kaynakları arasında yer alan silajlar bir yandan rasyon maliyetini düşürmesi, diğer yandan da hayvanın besin madde ihtiyaçlarını etkin bir şekilde karşılaması etkileri ile ön plana çıkmaktadır. Diğer yandan silaj, süt sığırlarında süt verimini artırıcı özellikleri ile rasyonların vazgeçilmez unsurları arasında yer almaktadır. Her ne kadar silaj denilince akla mısır bitkisi gelse de başta soya bitkisi olmak üzere diğer bazı alternatif silaj üretim kaynakları da bulunmaktadır. Mısır bitkisinin monokültür üretim tarzında yetiştirilmesi topraktan besin madde alımını olumsuz anlamda etkilemekte ve erozyon riskini artırmaktadır (Ueno ve ark., 2011). Tamamlayıcı bitki olarak soya ekiminin ekim nöbetine alınması yoluyla tarlanın boş kalması engellenmekte, toprağa azot bağlanmakta (azot fiksasyonu) ve toprak yüzeyinin yıl boyunca kaplı kalması sağlanarak erozyonun önüne geçilmektedir (Stagnari ve ark., 2017). Geleneksel hayvancılık (aile işletmeleri) işletmelerinde hayvanların besin madde ihtiyaçlarının karşılanamaması sonucunda verimde düşüklük ve canlı ağırlık kaybı gibi olumsuzluklar yaşanmaktadır. Bu olumsuzluklar hayvanların kızgınlık göstermesini dolayısıyla yavru elde edilmesini engellemektedir. Hayvanların ihtiyaç duyduğu besin maddeleri ve vitaminler fabrika yemleri ya da kaba yemler ile karşılanmaktadır. Soya silajı ruminantların protein ihtiyaçlarını karşılaması yanında yem maliyetlerini düşürmekte ve toprak verimliliğini artırıcı ve toprak yapısını düzenleyici etki göstermektedir (Vargas-Bello-Perez ve ark., 2008; Spanghero ve ark., 2015; Nkosi ve ark., 2016).

## Soya Bitkisi ve Soya Silajı Hakkında Genel Bilgiler

Soya ülkemizde daha çok hibrit tohumu kullanılarak sınırlı miktarda tarımı yapılan bir baklagil bitkisidir. Soya bitkisinden hayvan beslemede farklı şekillerde (dane, saman, hasıl, silaj ve küspe) faydalanılmaktadır. Soya danesi ve küspesi daha çok kanatlı hayvan rasyonlarında kullanılmaktadır. Hayvancılık sektörünün başta Güney Amerika ülkeleri olmak üzere farklı ülkelerden ithal edilen soya küspesine olan bağımlılığı ekonomik ve çevresel açıdan problemler ortaya çıkarmaktadır. Her şeyden önce bu yem hammaddesinin uzak ülkelerden taşınması küresel düzeyde sera gazı emisyonlarının artmasına yol açmaktadır. Ayrıca, Brezilya'da Amazon ormanlarının azımsanmayacak bir kısmı, soya ürünlerinin üretimi için gereken alan ihtiyacının karşılanması amacı ile kesilmektedir. Bu durum ise biyoçeşitliliğinin ve karbon stokunun kaybına yol açmakta ve sonuçta soya üretimine

bağlı olarak küresel çevre temizliği ile ilgili bir problem ortaya çıkmaktadır. Soya başta süt sığırları olmak üzere ruminant rasyonlarında da yer alabilmektedir. Ruminantlarda soya daha ziyade silaj ya da hasıl formunda kullanılmaktadır. Silaj üretimine yönelik geliştirilen soya çeşitleri ülkemizin farklı bölgelerinde (yüksek rakımlı bölgeler hariç) yetiştirilmektedir. Silajlık soya çeşitleri yüksek sıcaklık dereceleri ve yeterli sulama koşulları talep etmektedir. Nisan ayının sonundan Temmuz ayının sonuna kadar ekimi yapılabilen soya bitkisi ikinci ürün olarak da üretilebilmektedir. Yaklaşık 90 günlük süre sonunda hasat olgunluğuna gelen ve bu dönemde 2-2,5 metre kadar boylanabilen soyanın dane verimi oldukça yüksek düzeydedir. Soya bitkisi tarımında dekar başına yeşil hasıl ve kuru ot üretimi sırasıyla yaklaşık 5-6,5 ve 1,5-2 ton arasında değişmektedir. Soya bitkisinin silaj yapımı için en uygun hasat zamanı olarak baklaların tümüyle dolduğu ve alt yaprakların sarıya dönmeye başladığı gelişme dönemi kabul edilmektedir. Bu gelişme döneminde en yüksek düzeyde kuru madde verimi elde edilmektedir. Bu dönemden sonra yapılan hasat danelerdeki yağ oranında artışa neden olmakta ve bu durum soya bitkisinin silolanma yeteneğini düşürmektedir (Undersander, 1999). Silolama öncesinde kuru madde içeriğinin arzulanan seviyeye (%35-40) getirilebilmesi için soldurma işlemine ihtiyaç duyulmaktadır. Soldurma işlemi, silolama sürecinde silo suyu üretimi ve *Clostridial* bozulmaya bağlı olarak ortaya çıkan kalite düşüklüğünün önüne geçmektedir (Perez, 2007). Protein içeriği yüksek olan soya bitkisinin farklı inokulantlar veya kolay çözünebilir karbonhidrat içeriği yüksek olan arpa, mısır ve buğday gibi danelerle birlikte silolanması önerilmektedir (Koç ve ark., 1999). Nitekim, Gao ve ark. (2022), soya silajı gibi proteince zengin baklagil yem bitkilerinin tanik asit ve tanine toleranslı bakterilerle birlikte silolanması durumunda silaj fermentasyonunu olumsuz yönde etkileyen proteolizis olayının yavaşlamasına bağlı olarak silaj kalitesinin yükseldiğini bildirmiştir. Bu konuda önerilen diğer bir uygulama ise silajlık soya ve silajlık mısırın tarlaya 1:1 oranında ekilmesi ve birlikte hasat edilerek silolanmasıdır. Bu yolla silo ortamında arzulanan fermentasyonun (laktik asit fermentasyonu) gerçekleşmesi mümkün olabilmektedir. Arslan ve ark. (2016), farklı oranlarda soya ve mısır bitkileri ile hazırlanan silajların karşılaştırıldığı çalışmada, karışımda soya oranının artışına bağlı olarak kuru madde tüketiminin düştüğünü, silaj pH 'sı, silaj ham protein ve ADF içeriklerinin yükseldiğini bildirerek, en uygun karışım oranı olarak %50 mısır+ %50 soya karışımını önermiştir. Kızılsimşek ve ark. (2017), mısır ve soya karışımının kaliteli silaj üretimi açısından potansiyel taşıdığını ancak karışımda soya bitkisi oranının %40'ı aşması durumunda silajda laktik asit düzeyinin düştüğünü, pH, asetik asit, propiyonik asit ve bütirik asit düzeylerinin ise arttığını ve buna bağlı olarak silaj kalitesinin düştüğünü bildirmiştir. Bunun yanı sıra karışık olarak ekimi yapılan mısırın çeşitli baklagil bitkileri ile ekimi hem tarım alanlarının etkili kullanılmasını hem de daha yüksek ve kaliteli kaba yem elde edilmesini sağlamaktadır (Ayan ve ark., 2019). Erdal ve ark. (2016), %75 mısır+ %25 soya karışımıyla hazırlanan silajın sadece mısır ile hazırlanan silaja göre daha kaliteli olduğunu bildirmiştir. Aynı

şekilde, Serbester ve ark. (2016) mısır+soya karışımının yalın haldeki soya veya mısır silajına göre daha kaliteli silaj eldesine imkan verdiğini bildirmiştir. Soya silajının besin maddeleri içerikleri Çizelge 1'de sunulmuştur (Ayaşan, 2011). Soya bitkisinin verim ve besin madde içerikleri çeşit, sıra aralığı, dikim sıklığı ve gelişme devrelerine göre değişmektedir (Undersander, 1999; Çizelge 2). Soya bitkisinin bazı gelişme devreleri (R1, R3, R5 ve R7) Şekil 1'de gösterilmiştir.

Soya silajı üretimi için R5 ve R7 dönemlerinde yapılan hasadın daha uygun olduğu, çünkü bu devrelerde soya bitkisinin düşük lif içeriği, yüksek protein ve sindirilebilir enerji içeriğine sahip olduğu belirtilmektedir (Hintz ve Albrecht, 1994; Damosarkoro ve ark., 2001; Zhai ve ark., 2008; Açıköz ve ark., 2013). Kitapçı ve ark. (2024), kısa vejetasyon süresine sahip bölgelerde silajlık soya yetiştiriciliğinin kaliteli kaba yem üretimi konusunda etkili bir alternatif olacağını ileri sürmüştür. Aynı çalışmada, farklı soya çeşitleri arasında ekim zamanlarına bağlı olarak

belirgin bir fark belirlenmemiştir. Rosa ve ark. (2020), soya silajı yapımında mikrobiyel inokulant kullanımının ortamda laktik asit bakterisi sayısını artırmak suretiyle silolama sürecinde proteolizis (proteinlerin parçalanması) hızını ve buna bağlı olarak da silajda asitte çözünmeyen azot oranını düşürdüğünü bildirmiştir. Araştırmacılar proteolizis oranındaki azalmayı ise silolama sürecinde mikrobiyel inokulant kullanımına bağlı olarak arzulanan bakteriyel popülasyonunda (*Clostridium* ve *Enterobacteria*) ortaya çıkan azalma ile ilişkilendirmiştir. Kökten ve ark. (2013), 12 adet soya çeşidini karşılaştırdıkları çalışmalarında ham protein, NDF ve ADF içeriklerinin (% kuru maddede) sırasıyla %11,81 ve 18,86, %41,34 ve 46,,13 ve 28,16 ve 38.54 arasında değiştiğini belirlemiştir. Çalışmada, soya bitkisinin silaj üretiminde kullanılabileceği ve ele alınan çeşitler arasında Blaze ve Nova çeşitlerinin ham protein içeriklerinin yüksekliği ile ön plana çıktığı sonucuna varılmıştır.



R1:Çiçeklenme başlangıcı dönemi



R3:Çiçeklenme başlangıcı dönemi



R5: Tohum oluşum dönemi



R7:Fizyolojik olgunluk dönemi

Şekil 1. Soya bitkisinin bazı gelişim devreleri

Shape 1. Some developmental stages of the soybean plant

Kaynak: (Çırak ve Esenal., 2005)

Çizelge 1. Soya silajının besin maddeleri kompozisyonu

Table 1. The nutritional composition of soy silage

	Ortalama	Minimum	Maksimum
	Kuru madde üzerinden (%)		
Kuru madde	37,1	30,8	45,8
Ham protein	20,7	18,1	24,0
ADF	31,9	29,7	36,2
NDF	39,0	33,0	47,5
Kalsiyum	1,42	1,36	1,49
Fosfor	0,28	0,26	0,31

Kaynak: Ayaşan, 2011

Çizelge 2. Farklı çeşit, sıra aralığı, dikim sıklığı ve gelişme devrelerine göre soya bitkisinin verim ve bazı besin maddeleri içerikleri

Table 2. Yields and some nutrient contents of soybean plant according to different varieties, row spacings, planting density and growth stages

		Kuru madde verimi(ton/da)	Ham protein %	NDF %	ADF %
		Gelişim devresi	R1	1,07	20,11
R3	1,74		18,07	43,12	31,94
R5	2,53		18,21	45,67	33,69
R7	3,32		19,22	40,73	29,27
Çeşit	Corsoy79	3,02	20,48	40,53	28,70
	Pella	3,34	19,01	39,49	28,54
	Williams 82	3,59	18,17	42,16	30,56
Ekim aralığı	17.78 (cm)	3,55	18,80	40,91	29,58
	76.2 (cm)	3,08	19,63	40,54	28,96
Yoğunluk (bitki/dekar)	100,000	3,35	19,21	40,35	28,97
	300,000	3,28	19,22	41,10	29,64

Kaynak: Undersander, 1999.

Ayaşan ve ark. (2012), inokulantlı ve inokulantsız soya silajlarında pH değerinin (5,5) farklılık göstermediğini, ham kül değerinin ise inokulantlı ve inokulantsız silajlarda sırasıyla %14,80 ve 15,73 olarak belirlendiğini ve soya bitkisinin protein içeriğinin yüksekliğine ve karbonhidrat içeriğinin düşüklüğüne bağlı olarak zor silolanan yem bitkileri arasında yer aldığını bildirmiştir.

### Süt Sığırları Rasyonlarında Soya Silajı Kullanımı

Süt sığırlarında da diğer hayvanlarda olduğu gibi farklı dönemlerde farklı besleme uygulamalarına ihtiyaç duyulmaktadır. Örneğin gebeliğin son aşamalarında silaj kullanımı yavru atma riskini artırdığı için pek tercih edilmemektedir. Bu dönemde uterusda büyük yer kaplayan yavru rumeni baskılayarak rumenin küçülmesine neden olmaktadır. Doğum sonrası dönemde hemen süt vermeye başlayan hayvanlar yüksek düzeyde protein, enerji, vitamin ve mineral ihtiyacı içindedirler. Bu ihtiyaçları karşılamak için besin madde içeriği yüksek yem maddelerine ihtiyaç duyulmaktadır. Bu sayede hayvan kilo kaybetmeden rahimin kendisini toplaması mümkün olmakta ve hayvan 2-3 aylık sürede kızgınlık göstermektedir. Ayrıca, başta ketozis olmak üzere metabolik rahatsızlıkların önüne geçilebilmektedir. Bu dönemde kullanılan kaba yem rumenin eski hale gelmesini sağlar. Soya silajı, hem protein içeriğinin yüksek olması, hem de kaba yem olmasından dolayı bu dönemde kullanılması önem arz eden bir yemdir. Soya silajının verildiği bir denemede rumen florasının olumsuz yönde etkilendiği, mikrobiyal protein oranının düştüğü (rumende bulunan mikroorganizmaların enerjiye ihtiyaç duymaları ve soyanın tek başına bu ihtiyacı karşılayamaması sonucu) gözlemlenmiştir. Soya bitkisinin tüketimi sonucu açığa çıkan bütirik asidin ise süt kalitesini artırdığı görülmüştür (Garcia, 2020). Mısır silajı yerine soya silajının tek başına ya da mısır silajı ile birlikte kullanımının etkisinin araştırıldığı bir çalışmada %60 mısır + %40 soya silajı ile

besleme yapılmıştır. Mısır + soya silajı ile beslenen gruptaki ineklerin %11 civarında daha fazla süt verdiği, sadece mısır silajı ile beslenen ineklerin sütlerinde süt yağı ve protein oranının daha yüksek olduğu belirlenmiştir (Kudo ve ark., 2003). Perez ve ark. (2007), yonca silajı ile soya silajının karşılaştırdığı çalışmada soya silajı ile beslenen hayvanların yem tüketimi ve süt verimlerinin düştüğünü tespit etmişlerdir. Graziosi ve ark. (2022), süt sığırları ile yürütülen bir denemede rasyonda soya küpsesinin yerine rasyon kuru maddesinin %35'i oranında soya silajının kullanılması durumunda kuru madde tüketimi (kg/gün) ve süt veriminin (kg/gün) etkilendiğini, ancak süt protein içeriğinin düştüğünü, süt üre düzeyinin ise arttığını bildirmiştir. Çalışmada, rasyonda soya silajının kullanılması durumunda rasyon kuru madde, organik madde, NDF ve ham protein sindirilebilirliğinin de düştüğü belirlenmiştir. Bu bulgular soya silajı kullanımının yem tüketimi ve süt üretimini etkilemediği, ancak besin maddelerinin sindirilebilirliği ve azottan yararlanma etkinliğini düşürdüğünü göstermektedir. Soya silajı rumende hızlı çözünen protein içeriğini artırmak suretiyle ruminal mikrobiyal protein oranını artırıcı yönde etki yapmaktadır. Soya silajının süt sığırları rasyonlarında soya küpsesinin %35'i oranında ikame edilebileceği ve bu şekilde sera gazı emisyonunun azaltılması konusunda katkıda bulunulabileceği bildirilmiştir (Gislon ve ark., 2020; Graziosi ve ark., 2022). Rasyonun toplam kuru madde içeriğinin %35'in altında olduğu soya silajı içerikli rasyonlarda kuru madde tüketiminin yetersiz olmasına bağlı olarak süt veriminin düştüğü bildirilmiştir (Ghizzi ve ark., 2020). Silva ve ark. (2021), süt sığırları rasyonunda mısır silajı yerine soya + yulaf silajının kullanılması durumunda kuru madde tüketimi, besin maddelerinin sindirilebilirliği ve hayvanların yaşamsal faaliyetlerinde (yeme, içme ve geviş getirme) önemli derecede farklılık gözlemlenmediğini bildirmişlerdir.

Çizelge 3. Soya silajının süt sığırlarında süt verimi ve kalitesi üzerindeki etkileri

Table 3. Effects of soy silage on milk yield and quality in dairy cattle

Özellikler	Muameleler			
	Soya silajı	Yonca silajı	SH*	P değeri**
Yağ (%)	3.78	3.58	0.051	<0.05
Protein (%)	3.17	3.18	0.022	0.76
Laktoz (%)	4.69	4.69	0.012	0.89
Süt üre nitrojeni (mg/dl)	15.67	15.03	0.164	<0.05
Tüketim				
Kuru madde (kg/gün)	22.7	23.8	0.46	<0.05
Ham protein (kg/gün)	4.0	4.9	0.15	<0.05
NDF (kg/gün)	7.4	9.3	0.25	<0.05
Organik madde (kg/gün)	19.2	23.5	0.63	<0.05
Verim				
Süt verimi (kg/gün)	35.5	37.2	0.32	<0.05
Enerjice düzeltilmiş süt (kg/gün)	32.1	32.9	1.33	0.33
Yağ (kg/gün)	1.35	1.33	0.019	0.39
Protein (kg/gün)	1.09	1.16	0.006	<0.05
Laktoz (kg/gün)	1.67	1.74	0.006	<0.05

Kaynak: Ayaşan 2011; SH: Standart hata; \*\*p<0,05 istatistik olarak önemlidir

Ghizzi ve ark. (2020), mısır silajına (83.9 g/kg kuru madde) kıyasla daha yüksek ham protein içeriğine sahip olan soya silajı (134 g/kg kuru madde) kullanılan süt sığı rasyonlarında soya küspesi kullanım oranının, dolayısıyla rasyon maliyetinin düşeceğini bildirmiştir. Ayaşan (2011), yonca silajı yerine soya silajı kullanımının süt verimi ve kalitesi üzerindeki etkilerini tabloştürmüştür (Çizelge 3). Söz konusu çalışmada soya silajı kullanılan grupta süt verimi (kg/gün) ve protein veriminin (kg/gün) düşük olduğu tespit edilmiştir. Silva ve ark. (2021), süt sığı rasyonlarında mısır silajının soya silajı ile %16 oranında ikame edilmesi durumunda kuru madde, organik madde ve ham protein tüketimlerinin (kg/gün) düştüğünü, rumen amonyak konsantrasyonu, ruminasyon ve çiğneme aktivitelerinin ise artış gösterdiğini ve hayvanların verim düzeylerinin soya silajı kullanımı ile düşmediğini bildirmiştir.

## Sonuç

Bu çalışmada paylaşılan bilgiler soya silajının ruminant beslemede göz ardı edilemeyecek bir kullanım potansiyeline sahip olduğunu göstermektedir. Çalışmalar protein içeriği yüksek olan soya bitkisinin tek başına silolanması durumunda, silolama sürecinde pH ve amonyak düzeyindeki artışa bağlı olarak, silaj kalitesinde ciddi anlamda düşme olabildiğini ortaya koymuştur. Bahsedilen bu sakıncalar soya bitkisinin mısır gibi bitkilerle birlikte ekilerek veya bazı dane yemlerle (arpa, mısır ve buğday) birlikte silolanması yoluyla bertaraf edilebilmektedir. Başarılı bir şekilde hazırlanan soya silajının ruminant rasyonlarında kullanılması rasyon maliyetini önemli düzeyde artıran protein ek yemlerinin kullanım oranını düşürecektir. Bu şekilde bir yandan işletme ekonomisi olumlu yönde etkilenirken, diğer yandan da protein ek yemlerinin rasyonda daha düşük düzeyde kullanılmasına bağlı olarak çevreye daha düşük düzeyde N'lu bileşikler salınacak ve dolayısıyla çevresel kirlenmenin önüne bir nebze de olsa geçilebilecektir.

Bu gerçeklerden hareketle ülkemizde soya bitkisi üretiminin iklim şartları uygun olan bölgelerde yaygınlaştırılmasına yönelik tedbirlerin acilen alınması gerektiği önerilebilir. Bu sayede ruminant rasyonlarında kullanılan başta soya olmak üzere diğer yağlı tohum küspelerinin ithali zorunluluğu bir nebze de olsa azalacak ve ülke ekonomisine katkıda bulunulmuş olacaktır.

## Beyanlar

### Yazar Katkısı

ADY (%50), AVG(%50): Çalışmanın Tasarlanması

ADY (%50), AVG(%50): Veri Toplanması

ADY (%50), AVG(%50): Veri Analizi

ADY (%50), AVG(%50): Makalenin Yazımı

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