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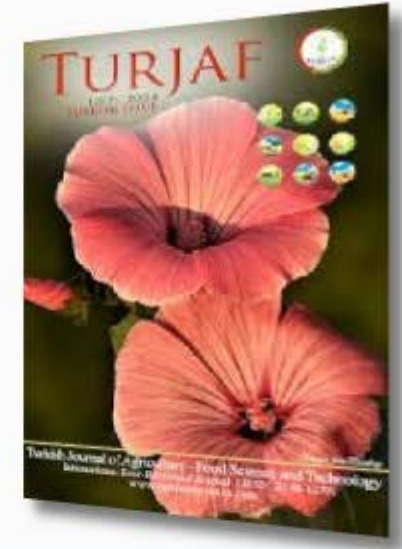
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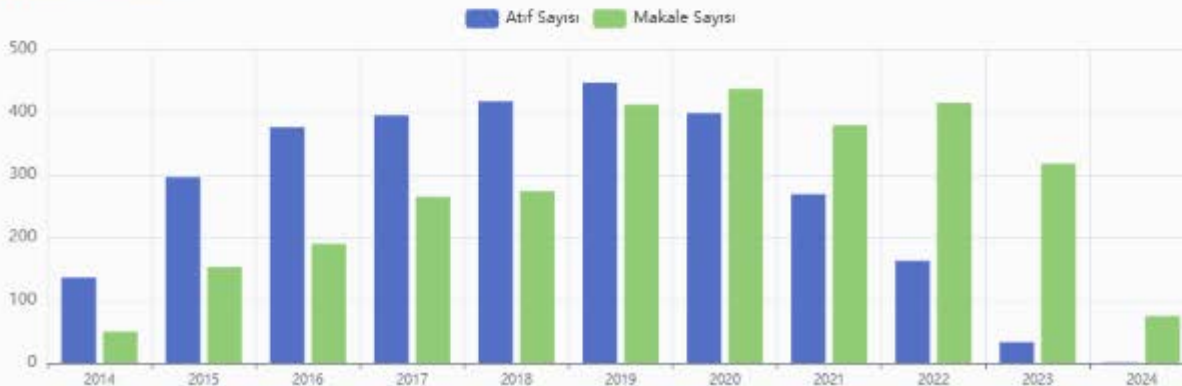
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## Makale & Atıf Sayısı





## Effect of Different Doses of Ethephon on Vegetative Characters, Sex Expression and Yield of Cucumber [*Cucumis Sativus*] In Rainas Municipality, Lamjung, Nepal

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### ABSTRACT

#### Research Article

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This study was carried out at the research field of Rainas Municipality, lamjung, Nepal from February to June 2022. It was conducted to evaluate the effect of different doses of ethephon on vegetative character, sex expression and yield of cucumber. The experiment was laid out in Randomized Complete Block Design (RCBD) with 4 replications and 5 treatments (four different doses of ethephon @ 100 ppm, @ 300 ppm, @ 400 ppm and @ 500 ppm as well as water spraying as control). Spraying was done twice, the 1<sup>st</sup> at two true leaf stage and the 2<sup>nd</sup> at four true leaf stage. The observed data were analyzed using Gen stat and Duncan's Multiple Range Test (DMRT) to find out the significant differences between the mean values at 5% level of significance. Among various concentration of ethephon, the most potent doses of ethephon to increase female flower were 100 ppm, 300 ppm and 400 ppm. The treatment with 300 ppm ethephon result early emergence of female flower, higher number of female flower and lower sex ratio followed by 400 ppm. Yield of cucumber was found higher with 300 ppm ethephon comparison to other treatment. The benefit-cost ratio (B:C ratio) was found highest at 300 ppm ethephon treatment and lowest in the control group. Considering various impacts of different doses of ethephon, treatment with 300 ppm ethephon is recommended.

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### Introduction

The cucumber (*Cucumis sativus* L.) (2n = 14) is vegetable that grows on a trailing vine, an annual member of the Cucurbitaceae family. It has numerous uses as vegetables and salad along with superb flavor, texture, and therapeutic potential (Sebastian et al., 2010). Cucumbers help avoid dehydration because they contain essential electrolytes. Cucumbers are a warm-season crop, which can be harmed by cold and frost and requires ideally 75<sup>0</sup> to 90<sup>0</sup> F for plantation in summer. Extremely high temperatures can result in bitterness and a pale green fruit color as well water-logged soil can disrupt the growth of cucumber (Brandenberger, 2021). It contains Cucurbitacins which inhibit the growth of cancer cells. Tyrosinase inhibition has been used as a moisturizer and skin toner (Ugwu & Suru, 2021). The cucumber vine is a creeper that produces cruciform fruits and grows on frames and trellises by twirling tendrils around them. Its plant can grow up to 2-3 meters in height, producing radial and tubular flower. The economic part is fruit which is generally cylindrical in shape and called as pepo in

pomology (Vidhi, 2016). Cucurbit plants have distinct male and female flowers in different parts of the plant, and only the female flowers ripen into fruit. Male flowers are at base of plant whereas females are at base of stem which appearance is controlled by genetics and environmental condition (Dhakal et al., 2019).

Agriculture is the main source of food, income, and employment for most people, with 65.6% of economically active people working in agriculture where 9.71% is normally contributed by vegetables. (MoALD, 2021/22). Due to limited area coverage and unawareness of farmer about its flowering, the production and productivity of cucumber in Nepal is almost less than half of the world's average productivity (Agriculture Guide, 2023). To enhance cucumber production, PGRS (plant growth regulators) and pruning are ideal options. Plant production can be improved in a geometric ratio by 3G cutting in conjunction with ideal pollination condition on branches to increase the number of female flowers on plants (Bhandari, 2020). Growth regulators greatly influence vegetative

traits, sex expression, and flowering. Cucumber's sex expression is influenced by its internal growth hormone levels, but can also be induced by applying external growth regulators (Singh and Singh, 1988). They are phytohormones which are often active at very low doses for development of plants (Singh et al., 2021).

Ethephon suits best for the growth of cucumber and its tremendous development and increase in yield. It was found superior @ 400 ppm for increasing the number of female flowers and inhibiting male flowers, and consequently increase the yield (Pandey et al., 2019). Exogenous application of PGRs effects on endogenous hormones of plant which alter the physiological processes of plants and reduces the harvesting time of the fruit (Gosai et al., 2020). The action of ethephon during the early stages of vine growth was anticipated because it releases ethylene, which has been linked to a decrease in auxin levels and an inhibitory influence on shoot growth (Lieberman & Knecht, 1977). The anti-gibberellic feature of ethylene stops mitotic processes in the meristem of roots and shoots causes plants' decreased height (Hayashi, Cameron, & Carlson, 2001). Fruits dipped in Ethephon 1000 ppm (Kriphone 39%, 2.56 ml per liter of water) for five minutes were effective for banana ripening, so there is no need to use a higher dose. Fruits started to be soften in three days and became ready to consume in five days (KC et al., 2009).

Certain growth regulators cause plants to grow taller and their shoots to grow longer, while other growth regulators function as retardants, causing plants to lose their vegetative and reproductive characteristics. Precision agricultural production benefits from the use of growth regulators that promote growth as well as those that function as retardants (Waqas et al., 2019).

For making low male to female flower ratio and for increasing fruit set, exogenous ethephon spraying is must today. Moreover, Lamjung is area with suitable climate and soil for commercialization of cucumber vegetable. The right way and right dose of application of ethephon is yet to be precisely determined for the optimum production in our country. Therefore, this experiment was carried out to assess the effectiveness of different ethylene concentrations on the performance of cucumber in Lamjung, Nepal.

## Method and Methodology

### Site Description

The experiment was conducted at Rainas Municipality-8, Lamjung district, Gandaki province, Nepal (Figure 1). This region lies in Subtropical mid hill of Nepal situated within 28° 48' North latitude to 84° 28' east longitude with an altitude of 600 masl. The study was carried out from 1st week of March 2022 to June 2022. The weather condition of the experiment site for the research plot was taken from secondary source (Department of Hydrology and Meteorology, Ministry of energy, Water resource and Irrigation, Babarmahal, Kathmandu, Nepal, 2022). During the research period, the mean temperature and precipitation recorded on the field was 23.54°C and 1.2-15.4 mm respectively (Figure 2).

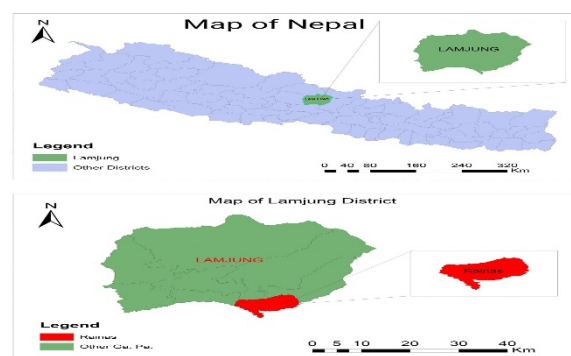


Figure 1. Map showing Lamjung district and research site

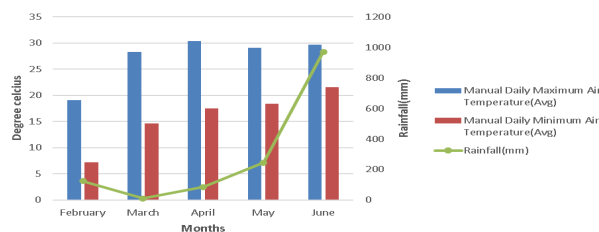


Figure 2. Weather condition of the research site at Lamjung from February to June, 2022

### Experimental Design

The experiment was conducted in Randomized Complete Block Design (RCBD) with 5 treatments and each treatment was replicated 4 times. Individual plot was 5.5 m in length and 3m in breadth. Spacing was 2×1.5 m<sup>2</sup>, in which plot will consist of two rows with four plants each. Net experimental plot area will be 16.5 m<sup>2</sup>, space between replication was 1m and 50 cm in border, thus total area 535.5 m<sup>2</sup>.

### Seedling Preparation and Transplantation

The cucumber seeds were planted on the tray for germination on 4th March 2022. The medium was prepared by mixing vermiculite, coco-peat, soil and perlite at a ratio of 3:1:1:1; the amount was as set as required. The tray was kept under seedling tunnel inside which temperature was 23°-25°C where it was protected and regular watered. The seedlings were ready for transplantation after 20 days as 2-3 true leaves appeared on all the seedlings and attained a height of about 3-4 inches. The seedlings were transplanted in the well-prepared main field on 26<sup>th</sup> March 2022 in given spacing. Manual weeding was done three times at 20 DAP (Days after planting), 30 DAP and 40 DAP. Irrigation was done through surface irrigation system as per plant requirement.

### Experimental Materials

The experimental materials which was used for the investigation comprised of Bhaktapur local variety of Cucumber. Different concentration of ethephon (2-chloroethyl phosphonic acid) was applied to study its effect on vegetative character, flowering, yield and quality traits of cucumber.

Stages of plant with two and four true leaf were given different doses of ethephon that were prepared in the lab. Only the freshly prepared solutions have ethephon 39% S.L- a commercial form of ethephon. First, the weighted amount of chemicals was dissolved and the necessary amount of distilled water was added to create the stock

solution of 1000 ppm for ethephon. It was diluted to 125 ppm, 250 ppm, 375 ppm, and 500 ppm using distilled water at the time of foliar application for solution of various concentration. Sticker (surfactant) was used at concentration of 0.25% in volume to increase the effectiveness of application of ethephon. A sufficient amount of ethephon was sprayed on the shoot until runoff.

**Treatment of Experiment**

There were total of 5 treatments of different doses of ethephon replicated four times.

T1	Check
T2	100 ppm dose of ethephon
T3	300 ppm dose of ethephon
T4	400 ppm dose of ethephon
T5	500 ppm dose of ethephon

**Manuring and Fertilizer**

The recommended dose of nitrogen, phosphorus and potassium is 140:40:100 kg/ha, was applied along with well-decomposed FYM (farm yard manure). Full dose of FYM, DAP (di-ammonium phosphate) and MOP (murate of potash) and half of recommended urea was applied as basal dose and remaining urea was top-dressed in split application at different vegetative stages. Staking was arranged in; single row trellis type such that a rope was run along the plant line and remain held by poles fixed at the ends.

**Data Collection and Analysis**

Among 8 plants, randomly selected 4 sample plants in each plot were observed for data collection to record vegetative characteristics, flowering behaviors and yield of each plot. Vegetative observation was done by measuring length of main stem (cm), number of primary branches per vine and number of nodes on main stem and calculating their average. This was recorded five times in the interval of 15 days beginning from 30 DAT (days after transplanting). For observation of sex expression, we recorded days to first female and male flower emergence after sowing, node number for emergence of first female and male flower, number of female and male flower per plant, sex ratio and calculated their average. For yield observation, Fruit length(cm), fruit circumference (cm),

number of fruit per plant, fruit weight per vine (cm), fruit yield per hectare (mt/ha) was measured and average was calculated.

Data was entered using MS Excel and one-way ANOVA of the treatment was done by using G-stat. The significance of the difference among the means was evaluated by Duncan’s Multiple Range Test (DMRT) by (Gomez and Gomez, 1984) for interpretation of the result at a 5% level of probability.

To assess the economic feasibility of the research, benefit cost analysis (BCA) was applied encompassing research activities. It involves identifying, quantifying and comparing the expected benefits and costs associated with research which supports in decision-making, resource allocation and proper justification. In this experiment, BCA of using different dose of ethephon was carried out by using formula:

$$B/C \text{ ratio} = (\text{Gross return} / \text{Variable cost})$$

**Results and Discussion**

**Vegetative Characters**

At 90 DAS (days after sowing), maximum plant height was observed with control (279.5 cm) while the minimum height with ethephon @500 ppm (256.1 cm) (Table 1). Similar results were found at 30 DAS, 45 DAS, 60 DAS and 75 DAS. Duncan’s Multiple Range Test (DMRT) at p<0.05 shows similarities among ethephon concentration @400 ppm and @500 ppm while ethephon @100 ppm and @300 ppm are being statistically par at 90 DAS. For branching, at 90 DAS, the maximum number of branches were seen in case of 300 ppm (5.34) followed by 100 ppm (4.83), 400 ppm (5.42), 500 ppm (4.34) and least number of branches were found in control (3.92) (Table 2). Duncan’s Multiple Range Test (DMRT) at p<0.05 shows similarities among ethephon concentration @400 ppm and @500 ppm at 90 DAS. Then for node number, at 90 DAS, the maximum number of nodes were seen in case of 300 ppm (44.33) followed by 400 ppm (44.25), 100 ppm (43.00), 500 ppm (42.33) and least number of nodes were found in control (40.42) (Table 3). Similar trend was seen on 30 DAS, 45 DAS, 60 DAS and 75 DAS. DMRT at p<0.05 shows that 300 ppm was statistically at par with 400 ppm.

Table 1. Plant height at various days after Sowing

Concentration of ethephon	Plant height at various days after Sowing in cm				
	30 DAS	45DAS	60DAS	75DAS	90 DAS
Control	38.500 <sup>a</sup>	85.50 <sup>a</sup>	186.2 <sup>a</sup>	266.8 <sup>a</sup>	279.5 <sup>a</sup>
100 ppm	34.67 <sup>b</sup>	78.92 <sup>ab</sup>	181.8 <sup>ab</sup>	259.7 <sup>ab</sup>	271.5 <sup>ab</sup>
300 ppm	34.67 <sup>b</sup>	75.75 <sup>bc</sup>	171.1 <sup>bc</sup>	253.4 <sup>abc</sup>	265.2 <sup>ab</sup>
400 ppm	34.75 <sup>b</sup>	76.25 <sup>bc</sup>	169.2 <sup>c</sup>	247.3 <sup>bc</sup>	262.1 <sup>ab</sup>
500 ppm	34.08 <sup>b</sup>	71.33 <sup>c</sup>	163.1 <sup>c</sup>	244.2 <sup>c</sup>	256.1 <sup>b</sup>
GM	35.43	77.55	174.3	255.37	266.9
SEM	1.011	3.051	3.63	4.25	5.39
LSD	3.1157	6.647	11.19	13.10	16.60
CV%	5.70	5.60	4.20	3.3	4.0
F test	*	**	**	**	*

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance.



Table 2. Effect of different dose of ethephon on number of primary branches/ vine

Concentration of ethephon	Effect of different dose of ethephon on number of primary branches/ vine				
	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
Control	0.25 <sup>a</sup>	1.333 <sup>b</sup>	2.583 <sup>bc</sup>	3.833 <sup>d</sup>	3.917 <sup>c</sup>
100 ppm	0.1667 <sup>a</sup>	1.750 <sup>a</sup>	2.750 <sup>abc</sup>	4.417 <sup>b</sup>	4.833 <sup>b</sup>
300 ppm	0.333 <sup>a</sup>	1.833 <sup>a</sup>	3.250 <sup>a</sup>	5.167 <sup>a</sup>	5.333 <sup>a</sup>
400 ppm	0.5 <sup>a</sup>	1.583 <sup>ab</sup>	3.000 <sup>ab</sup>	4.167 <sup>bc</sup>	4.417 <sup>bc</sup>
500 ppm	0.25 <sup>a</sup>	1.417 <sup>b</sup>	2.417 <sup>c</sup>	3.917 <sup>cd</sup>	4.333 <sup>bc</sup>
Grand mean	0.300	1.583	2.800	4.300	4.567
SEM	0.1498	0.0998	0.2453	0.0900	0.1574
LSD	0.4617	0.3074	0.5345	0.2773	0.4849
CV%	99.9	12.6	12.4	4.2	6.9
F test	NS	**	**	***	***

Means followed by common letter(s) within column are non-significantly different based on Duncan's Multiple Range Test (DMRT) at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

Table 3. Effect of different dose of ethephon on number of node

Concentration of ethephon	Effect of different dose of ethephon on number of node				
	30 DAS	45DAS	60 DAS	75DAS	90 DAS
Control	5.250 <sup>a</sup>	14.83 <sup>a</sup>	23.50 <sup>b</sup>	40.00 <sup>c</sup>	40.42 <sup>c</sup>
100 ppm	5.917 <sup>a</sup>	15.08 <sup>a</sup>	23.92 <sup>ab</sup>	42.42 <sup>ab</sup>	43.00 <sup>ab</sup>
300 ppm	6.583 <sup>a</sup>	15.92 <sup>a</sup>	24.17 <sup>ab</sup>	43.58 <sup>a</sup>	44.33 <sup>a</sup>
400 ppm	7.417 <sup>a</sup>	17.08 <sup>a</sup>	25.42 <sup>a</sup>	43.50 <sup>a</sup>	44.25 <sup>a</sup>
500 ppm	6.167 <sup>a</sup>	15.25 <sup>a</sup>	23.75 <sup>ab</sup>	41.83 <sup>b</sup>	42.33 <sup>b</sup>
Grand mean	6.27	15.63	24.15	42.27	42.87
SEM	0.812	0.773	0.533	0.466	0.480
LSD	2.502	2.380	1.641	1.437	1.479
CV%	25.9	9.9	4.4	2.2	2.2
F test	NS	NS	*	**	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

Table 4. First Flowering Date

Concentration of ethephon	First Flowering Date (DAS)	
	Male	Female
Control	31.17 <sup>c</sup>	45.92 <sup>a</sup>
100 ppm	34.42 <sup>b</sup>	43.67 <sup>b</sup>
300 ppm	35.92 <sup>ab</sup>	42.50 <sup>c</sup>
400 ppm	35.92 <sup>ab</sup>	42.33 <sup>c</sup>
500 ppm	37.17 <sup>a</sup>	43.83 <sup>b</sup>
Grand mean	34.92	43.65
SEM	0.756	0.255
LSD	2.329	0.787
CV%	4.3	1.2
F test	***	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

**Sex Expression**

Earliest male flowering (31.17 DAS) was seen in control followed by 100 ppm (34.42 DAS) and later male flowering (37.17 DAS) was seen in 500 ppm which is statistically at par with 400 ppm and 300 ppm (Table 4). Likewise, earliest female flowering (42.33 DAS) was seen in 400 ppm which is statistically similar with 300 ppm (42.50 DAS). The later female flowering (45.92 DAS) was seen on control. Application of ethephon @ 500 ppm produced male flower at upper nodes (4.84) compared to other treatments being at par with 400 ppm (4.67) (Table 5). Control produced male flower at lower nodes (3.25) followed by 100 ppm (3.50). In case of

female flowers 500 ppm produced female flowers in lowest node (5.58) being at par with 400 ppm (6.17) and control produced female flowers in uppermost nodes (8.58) being at par with 100 ppm (8.08). The highest number of male flower (38.75) was obtained in control. And least number of male flower was obtained in 400 ppm (24.00) (Table 5). Plant treated with 300 ppm produced highest number of female flowers (22.25) per plant compared with higher and lower concentrations. Similarly, lowest number of female flowers were found in control (12.50). Highest sex ratio (3.106) was found in control while lowest sex ratio was found in 400 ppm (1.100) (Table 6).

Table 5. First Flowering node

Concentration of ethephon	First Flowering node	
	Male	Female
Control	3.25 <sup>d</sup>	8.583 <sup>a</sup>
100 ppm	3.5 <sup>c</sup>	8.083 <sup>a</sup>
300 ppm	4.25 <sup>b</sup>	6.667 <sup>b</sup>
400 ppm	4.667 <sup>a</sup>	6.167 <sup>bc</sup>
500 ppm	4.833 <sup>a</sup>	5.583 <sup>c</sup>
Grand mean	4.1	7.02
SEM	0.0761	0.260
LSD	0.2344	0.800
CV%	3.7	7.4
F test	***	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

Table 6. Total male flower, female flower and sex ratio

Concentration of ethephon	Total male flower, female flower and sex ratio		
	Male flower	Female flower	Sex ratio
Control	38.75 <sup>a</sup>	12.50 <sup>c</sup>	3.106 <sup>a</sup>
100 ppm	29.83 <sup>b</sup>	19.25 <sup>b</sup>	1.568 <sup>b</sup>
300 ppm	25.33 <sup>c</sup>	22.25 <sup>a</sup>	1.148 <sup>c</sup>
400 ppm	24.00 <sup>c</sup>	21.83 <sup>a</sup>	1.100 <sup>c</sup>
500 ppm	24.08 <sup>c</sup>	20.08 <sup>ab</sup>	1.199 <sup>bc</sup>
Grand mean	28.40	19.18	1.624
SED	0.761	0.737	0.0695
LSD	2.346	2.270	0.2140
CV%	5.4	7.7	8.6
F test	***	***	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

Table 7. Average fruit length and Circumference

Concentration of ethephon	Average fruit length and Circumference in cm	
	Circumference	Length
Control	22.77 <sup>d</sup>	24.34 <sup>a</sup>
100 ppm	23.93 <sup>c</sup>	24.04 <sup>a</sup>
300 ppm	25.91 <sup>a</sup>	22.99 <sup>b</sup>
400 ppm	26.51 <sup>a</sup>	22.92 <sup>b</sup>
500 ppm	24.90 <sup>b</sup>	22.13 <sup>c</sup>
Grand mean	24.80	23.29
SEM	0.225	0.232
LSD	0.693	0.716
CV%	1.8	2.0
F test	***	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance.

### Yield

The highest fruit length was obtained in control (24.34 cm) being at par with 100 ppm (24.04 cm) followed by 300 ppm (22.99 cm) and 400 ppm (22.92 cm) while lowest fruit length was obtained in 500 ppm (22.13 cm) (Table 7). Fruit weight in 400 ppm (511.90 g) was found superior to other treatment being statistically similar with 300 ppm (510.80 g) followed by 500 ppm (496.80 g) (Table 8). Control had least fruit weight (477.70 g) being at par with 100 ppm (482.0). Similarly, Highest fruit number was seen in 300 ppm (12.17) followed by 400 ppm (12.08) and higher or lower concentrations had decreasing fruit number and least number of fruits was seen on control (9.25).

All application of ethephon and water spray are profitable. The BC ratio was affected by application of ethephon as with increase in level of ethephon there was increase in BC ratio upto 300 ppm (4.604) and beyond 300 ppm there was decrease in bc ratio. The minimum BC ratio was found in control (3.272).

All doses of ethephon sprayed on plant showed significant differences in the vegetative development of the plants as compared to control treatment. Although ethephon @300 ppm was found to be most effective for maximum branching and node number, control or no application of ethephon was found to be effective for maximum height.

Table 8. Individual fruit weight and number of fruit per plant

Concentration of ethephon	Individual fruit weight and number of fruit per plant		
	Weight(gm)	Number	Yield(mt/ha)
Control	477.70 <sup>b</sup>	9.25 <sup>c</sup>	14.73 <sup>c</sup>
100 ppm	482.0 <sup>b</sup>	11.08 <sup>b</sup>	17.81 <sup>b</sup>
300 ppm	510.8 <sup>a</sup>	12.17 <sup>a</sup>	20.72 <sup>a</sup>
400 ppm	511.9 <sup>a</sup>	12.08 <sup>a</sup>	20.61 <sup>a</sup>
500 ppm	496.8 <sup>ab</sup>	11.33 <sup>ab</sup>	18.80 <sup>b</sup>
Grand mean	495.9	11.18	18.53
SEM	6.52	0.283	0.549
LSD	20.08	0.873	1.692
CV%	2.6	5.1	5.9
F test	**	***	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

Table 9. Total cost (NRS lakh/ha), total revenue (NRS lakh/ha), net revenue (NRS lakh/ha) and b: c ratio

Concentration of ethephon	Total cost (NRS lakh/ha), total revenue (NRS lakh/ha), net revenue (NRS lakh/ha) and b:c ratio			
	Cost of cultivation	Total revenue	Net revenue	B:C ratio
Control	1.8	5.890 <sup>c</sup>	4.090 <sup>c</sup>	3.272 <sup>c</sup>
100 ppm	1.8	7.122 <sup>b</sup>	5.323 <sup>b</sup>	3.957 <sup>b</sup>
300 ppm	1.8	8.288 <sup>a</sup>	6.488 <sup>a</sup>	4.604 <sup>a</sup>
400 ppm	1.8	8.242 <sup>a</sup>	6.443 <sup>a</sup>	4.579 <sup>a</sup>
500 ppm	1.8	7.519 <sup>b</sup>	5.719 <sup>b</sup>	4.177 <sup>b</sup>
GM		7.412	5.613	4.118
SEM		0.2196	0.2196	0.1220
LSD		0.6768	0.6768	0.3760
CV%		5.9	7.8	5.9
F test		***	***	***

Means followed by common letter(s) within column are non-significantly different based on DMRT at P=0.05; LSD, Least Significant Difference; SEM, Standard Error of Mean; CV, Coefficient of Variation; DAS, Days After Sowing; NS, Non-Significant, \* significant at 5% level of significance, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance

This result is found to support the research conducted in *Cucumis sativus* by Dhakal et al. (2019). The reduced height of plants may be due to ethylene's anti gibberellic property, which stops mitotic processes in the root and shoot's meristem, impacting plant length (Hayashi et al., 2001). During the initial stage of vine development, the anticipated impact of ethephon was based on its ability to release ethylene, as the inhibitory effect of ethylene on shoot growth has been linked to the decrease in levels of auxin (Lieberman and Knecht, 1977). Ethephon at 300-400 ppm proved superior to its higher levels and control for increasing number of branches and nodes. This may be related to fact that ethephon at 300 and 400 ppm reduced plant height to a greater extent as compared to higher concentrations. A significant alteration in the physical characteristics of plants, characterized by increased branching, which could be attributed to modifications in the nuclear genomes, was documented by Selga and Selga (1993) and Rafeekhar et al. (2001). The increased number of nodes due to ethephon treatment may be attributed to reduction in inter nodal distance by suppressing cell division (Dhakal et al., 2019).

With increasing level of ethephon first male flower appearance delayed while female flowering was enhanced. It showed similar result of earliness to female flowering within ethephon application is in agreement with Pandey, (2019). According to Singh & Singh (1984), reason for earliest production of female may be attributed to the maximum increase in starch and carbohydrate with

ethephon treatments. On other side, with an increase in ethephon dosage, it demonstrated a trend of rising nodes for first male and falling nodes for first female. Early appearance of female flowers on lower nodes and delayed induction of male flowers on upper nodes with different concentrations of ethephon is in confirmation with the result of Nikumbh and Musmade (2006) with 100 ppm in cucumber and Ranjit and Satya (2006) with 250 ppm in pumpkin.

Total number of male flowers decreased with increasing dose and vice versa for total number of female flowers. Sex ratio was highest in no application of ethephon and lowest at high dose of its application. This result was found similar with decrease in number of male flowers with narrow sex ratio due to application of ethephon given by Bhandary (1974). Little et al. (2007) proved the dual role of ethylene in both sex determination and subsequent carpel maturation in melon. PGR application at the crucial stages of two and four leaf stages, which determine whether to promote or suppress both sexes, is essential for the variations of the sex ratio (Hossain et al., 2006).

With application increasing ethephon concentration fruit length decreased and fruit circumference increased significantly. The decrease in fruit length and increase in fruit circumference with ethephon treatment is in agreement with Dhakal et al. (2019). However, highest fruit number, weight and yield was obtained from plant applied with @300 and @400 ppm dose of ethephon and

lowest in control. The increase in yield was predominantly due to increased number of pistillate flowers, fruit number and was also linked to increase in fruit diameter and average fruit weight (Patrick, 1982). Yield in 300 ppm was found almost twice compared to control according to Dhakal et al. (2019).

## Conclusion

The research findings show that among different concentrations of ethephon; 300 ppm and 400 ppm were found effective. As the concentration of ethephon increased, the length of the main stem decreased. The treatment with 300 ppm ethephon resulted in the highest number of primary branches per vine and the highest number of nodes on the main stem. Furthermore, the application of 300 ppm ethephon led to the early emergence of female flowers, an increased number of female flowers, and a lower sex ratio. In terms of cucumber yield, it was higher with the 300 ppm ethephon treatment compared to others. The benefit-cost ratio (B: C ratio) was the highest with the 300 ppm ethephon treatment and lowest in the control group. This study recommends 300 ppm ethephon as the best alternative for inducing female flowers and increasing yield.

## Declarations

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## Weed Problems Faced by Hazelnut Growers in Düzce, Türkiye

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 11.05.2024 Accepted : 14.07.2024</p> <p>Keywords: Hazelnut Weeds Questionnaire Düzce Growers</p>	<p>This study was aimed to determine the weed problem, the knowledge, experience and competence of the growers in solving the issues of controlling weeds in Düzce, which is an important region of Türkiye for hazelnut production. Within the scope of the study, 30 questions were asked to a total of 100 hazelnut growers in Düzce in 2021-2022. According to the survey results, it has been understood that 53% of the growers have an education level of high school or above, 52% of them produce hazelnuts because it is a habit inherited from family, and 34% of them do it to earn their living. It was determined that 84% of growers consider weeds to be an important problem; 80% of them used cutting and 9% used chemical methods for weed control. Apart from hazelnut cultivation, 40% of the growers declared that they earned additional income by working in industry and commerce, 17% in public institutions, and the majority of them (65%) declared that they did not grow any agricultural product other than hazelnuts. Growers have reported 45 different weed species that are trouble in the hazelnut orchards. Among these weeds; downy brome (<i>Bromus tectorum</i> L.), annual meadow grass (<i>Poa annua</i> L.), common self-heal (<i>Prunella vulgaris</i> L.), alfalfa (<i>Medicago sativa</i> L.), common nipplewort (<i>Lapsana communis</i> L.), ribwort plantain (<i>Plantago lanceolata</i> L.), red sorrel (<i>Rumex acetosella</i> L.), white clover (<i>Trifolium repens</i> L.), holy bramble (<i>Rubus sanctus</i> Schreb), field bindweed (<i>Convolvulus arvensis</i> L.), wild strawberry (<i>Fragaria vesca</i> L.), and meadow soft grass (<i>Holcus lanatus</i> L.) were stated the most common species. In addition, growers stated that they solved the weed problem to a certain extent with the chemical control they applied against the existing weeds, but they could not achieve sufficient success in the controlling of holy bramble, ferns, field bindweed, nettles and Bermuda grass.</p>

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 12(s1): 1988-1996, 2024

## Düzce’de Fındık Üreticilerinin Yabancı Otlar Konusunda Karşılaştıkları Sorunlar

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 11.05.2024 Kabul : 14.07.2024</p> <p>Anahtar Kelimeler: Fındık Yabancı ot Anket Düzce Üretici</p>	<p>Bu çalışma, Türkiye’nin önemli bir fındık bölgesi olan Düzce ilinde yabancı ot sorunu ve mücadelesinde üreticinin bilgi, deneyim ve sorunları çözmedeki yetkinliğini tespit etmek amacıyla planlanmıştır. Çalışma kapsamında, Düzce’de 2021-2022 yıllarında toplam 100 fındık üreticisine 30 soru yöneltilmiştir. Anket sonuçlarına göre; üreticilerin %53’ünün eğitim seviyesinin lise ve üzeri olduğu, %52’sinin fındık yetiştiriciliğini aile varlığı olduğu için ve %34’ünün ise geçimlerini sağlamak amacıyla yaptığı anlaşılmıştır. Üreticilerin %84’ünün yabancı otları önemli dercede sorun gördüğü; mücadele için %80’inin biçme, %9’unun ise kimyasal mücadele uyguladığı tespit edilmiştir. Üreticilerin %40’ı fındık yetiştiriciliği dışında sanayi ve ticaret alanında, %17’si kamu kurumlarında çalışarak ek gelir elde ettiklerini; yine %65 gibi büyük bir çoğunluğu ise fındık dışında herhangi bir ürün yetiştirmediklerini beyan etmişlerdir. Üreticiler fındık bahçelerinde sorun olan 45 farklı yabancı ot türü bildirmişlerdir. Bu yabancı ot türlerinden; dam bromu (<i>Bromus tectorum</i> L.), tek yıllık salkımotu (<i>Poa annua</i> L.), yara otu (<i>Prunella vulgaris</i> L.), yonca (<i>Medicago sativa</i> L.), şebrek (<i>Lapsana communis</i> L.), bataklık sınırotu (<i>Plantago lanceolata</i> L.), kuzukulağı (<i>Rumex acetosella</i> L.), ak üçgül (<i>Trifolium repens</i> L.), böğürtlen (<i>Rubus sanctus</i> Schreb), tarla sarmaşığı (<i>Convolvulus arvensis</i> L.), yabani çilek (<i>Fragaria vesca</i> L.) ve kadife otu (<i>Holcus lanatus</i> L.) türlerinin yaygın olduğu bildirilmiştir. Ayrıca, üreticiler mevcut yabancı otlara karşı uyguladıkları kimyasal mücadele ile yabancı ot sorununu belli bir ölçüde çözdüklerini ancak böğürtlen, eğrelti otu, tarla sarmaşığı, ısırgan otu ve köpek dişi ayrığı ile mücadelede yeterli başarı elde edemediklerini belirtmişlerdir.</p>

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## Giriş

Ülkemizin ihraç ürünlerinden biri olan fındık, bitkisel üretimde ekonomik açıdan önemli bir yere sahiptir. Fındık üreten ülkeler arasında dünyada birinci sırada yer alan Türkiye'yi İtalya, İspanya, ABD ve Yunanistan takip etmektedir. Türkiye, dünya fındık üretiminin yaklaşık %70'ini, ihracatın ise %82'sini gerçekleştirmektedir (TOB, 2022). Türkiye'de fındık 765 bin ton üretim hacmiyle kabuklu kuruyemişler arasında lider konumdadır. Fındık üretimini 396 bin tonla badem, 335 bin tonla ceviz ve 80 bin tonla antepfıstığı takip etmektedir (TÜİK, 2022).

Fındık, nemli ve ılıman iklimlerde iyi yetişerek yüksek verim sağlayan bir bitkidir (Karadeniz ve ark., 2009). Bu sebeple, Karadeniz Bölgesi'nin önemli ürünlerinden bir olup bölgede yaklaşık beş bin yıldır yetiştirilmektedir. Doğu Karadeniz Bölgesi'nde Ordu ve Giresun başta olmak üzere Trabzon, Rize, Artvin, Samsun, Sinop illerinde ve Batı Karadeniz Bölgesi'nde Düzce, Sakarya, Zonguldak ve Kocaeli illerinde yetiştiriciliği yoğun olarak yapılmaktadır. Bu illerde yaklaşık 500 bin üretici, yedi milyon hektarlık alanda üretim yaparak geçimlerini sağlamaktadır (İslam, 2018). Fındık bu bölgeler için ekonomik ve sosyal açıdan stratejik öneme sahiptir (Tanrıvermiş ve ark., 2006).

Daha önce yürütülen çalışmalarda, Karadeniz Bölgesi fındık üretim alanlarında tek ve çok yıllık çok sayıda yabancı ot türünün sorun olduğu belirlenmiştir (Başaran & Adıgüzel, 2001; Mennan ve ark., 1999). İlman iklimle sahip Batı Karadeniz Bölgesi'nde yağmurlu gün sayısının fazla olması nedeniyle üretim sezonu boyunca farklı yabancı otlar çimlenmekte ve gelişmektedir. Fındık bahçelerinde yabancı otlar verim kayıplarına sebep olmalarının yanı sıra, hasat döneminde ciddi zorluklara da neden olurlar.

Stratejik bir öneme sahip olan fındık üretiminde verimi ve kaliteyi olumsuz etkileyen pek çok bitki koruma etmeni bulunmaktadır. Bu etmenler içerisinde yabancı otlar, doğrudan ve dolaylı olarak verdikleri zararlar nedeniyle verim ve kaliteyi kısıtlayan en önemli faktördür. Bu çalışmanın amacı, Düzce ili fındık üreticilerinin yabancı otlar ve mücadelesi konusunda yaşadıkları sorunları anlamak ve başarılı mücadele stratejilerinin geliştirilmesine katkı sağlamaktır.

## Materyal ve Yöntem

Çalışma, Düzce ili fındık (*Corylus avellana* L.) yetiştiricileri ile 2021 ve 2022 yıllarında yürütülmüştür. Çalışma kapsamında Düzce ilinin Akçakoca, Cumayeri, Çilimli, Gölyaka, Gümüşova, Kaynaşlı, Merkez ve Yığılca ilçelerine gidilmiş, fındık üreticileri ile yüz yüze görüşülerek anketler yapılmıştır (Şekil 1). Anketlerin fındık yetiştiriciliği yapılan ilçelerde üretim alanı büyüklüğüne bağlı olarak Bora ve Karaca (1970)'nin bölümlü örnekleme yöntemi esas alınarak homojen dağılmasına dikkat edilmiştir (Çizelge 1). Anket formundaki sorular, teorik bilgiler ve daha önce literatürde yapılan ampirik çalışmalar çerçevesinde oluşturulmuştur. Üreticilere toplam 30 soru yöneltilmiş, anketler üreticilerin toplu olarak bulunduğu alanlarda, iş yerlerinde, evlerde veya bahçelerde üreticilerle yüz yüze görüşülerek yapılmıştır. Bu anket sorularının 10 tanesi beşli Likert ölçeğine (1: hiç etkilemez, 2: az, 3: ne az ne çok, 4: çok fazla, 5: tamamiyle etkiler) tabi tutulmuş ve soruların Likert ölçeği

ortalaması 3'ün üzerinde ise olumlu, 3'ün altında ise olumsuz olarak kabul edilmiştir (Likert, 1932). Diğer sorulardan 5 tanesi genel bilgi içerikli, 15 tanesi ise ucu açık sorulardan oluşmaktadır. Likert ölçeğine göre hazırlanmamış diğer sorulardan elde edilen veriler ise uygun istatistik ve grafiksel yöntemlerle değerlendirilmiştir.



Şekil 1. Düzce ili haritası.

Figure 1. Düzce province map

Çizelge 1. Düzce ilinde yapılan anketlerin ilçelere göre dağılımı

Table 1. Distribution of surveys conducted in Düzce province by districts

İlçeler	Üretim alanı (dekar)*	Anket sayısı
Akçakoca	218.670	34
Cumayeri	54.000	9
Çilimli	35.250	6
Gölyaka	42.290	7
Gümüşova	34.760	6
Kaynaşlı	23.150	4
Merkez	129.030	19
Yığılca	94.500	15
Toplam	631.650	100

\* 2019 yılı kayıtlarından alınmıştır (TÜİK, 2020).

## Bulgular ve Tartışma

Anket sonuçları; üreticilerin genel profili, üreticilerin fındık yetiştiriciliği faaliyetleri ile yabancı ot sorunu ve mücadelesi olmak üzere üç başlık altında incelenmiştir.

### Fındık Üreticilerinin Genel Profili

Yapılan anketler sonucunda Düzce ili fındık üreticilerinin 35 ile 60 yaş arası orta yaş grubunda olduğu, %53'ünün lise ve üzeri eğitim aldığı, %52'sinin fındık yetiştiriciliğini aile mirası olması nedeniyle yaparken %34'ünün ise geçimlerini sağlamak amacıyla yaptığı anlaşılmıştır. Fındık üreticilerinin Dünya ve Türkiye gündemini yakından takip ettikleri, tarım teşkilatı/üniversite personeli uzman kişiler ile sık görüşmeyi önemsedikleri, uzman kişiler tarafından düzenlenen eğitimlere katıldıkları ve %40'ının televizyon programlarını sık sık takip ettiği belirlenmiştir (Çizelge 2).



Çizelge 2. Üreticileri tanıtmaya yönelik sorular ve Likert değerleri

Table 2. Questions to get to know the growers, and their Likert values

Anket sorusu	Likert ölçek değerleri (%)					Anket sayısı	Likert Ölçeği Ortalaması
	1	2	3	4	5		
Üreticilerin tarım teşkilatı / uzmanlar tarafından düzenlenen herhangi bir eğitim toplantısına katılma durumu	22,0	10,0	33,0	24,0	11,0	100	2,92
Üreticilerin medyadaki çiftçi eğitim programlarını takip etme durumu	8,0	19,0	33,0	28,0	12,0	100	3,17
Üreticilerin uzman elemanlarla görüşme sıklığı	13,0	13,0	20,0	39,0	15,0	100	3,30

Likert ölçek değerleri: 1. Hiç, 2. Çok nadir, 3. Bazen, 4. Sık-sık, 5. Sürekli olarak veya tamamen

Üreticilerin mezuniyet durumları ile ilgili olarak %53'ünün lise ve üzeri eğitim almış oldukları belirlenmiştir. TEDMEM (2022) tarafından yürütülen anket çalışması sonucunda 25-64 yaş aralığındaki üreticilerin dörtte birinin yüksek öğretim mezunu olduğu, Uşak ilinde yapılan bir çalışmaya göre üreticilerin %50'sinden fazlasının ilkökul mezunu olduğu (Lökü ve ark., 2020), Mersin ilinde yapılan diğer bir çalışmada ise üreticilerin yaklaşık %30'unun herhangi bir eğitim kurumundan mezun olmadığı görülmüştür (Torun, 2022). Antalya ili Korkuteli ilçesinde yapılan bir çalışmada üreticilerin sadece %25'inin lise veya üniversite mezunu, %60'ının ise ilkökul mezunu olduğu belirlenmiştir (Ay ve ark., 2006). Ordu ili fındık üreticileri ile yapılan benzer bir çalışmaya katılan üreticilerin eğitim düzeylerinin yüksek olmadığı, sadece %37'sinin lise ve üzeri eğitim aldığı ortaya çıkmıştır (Yonat & Kolören, 2023). Benzer şekilde, Giresun ili fındık üreticilerinin %4'ünün hiç tahsil görmemişken %36'sının ilkökul, %23'ünün ortaokul, %28'inin lise ve %9'unun üniversite mezunu olduğu belirlenmiştir (Turan ve ark., 2023). Çukurova'da yapılan bir çalışmada ankete katılan üreticilerin %46'sının ilkökul, %5,3'ünün ise yüksekokul mezunu olduğu tespit edilmiştir (Üremiş ve ark., 1996). Diğer bir çalışma sonucunda, Diyarbakır ili mercimek üreticilerinin çoğunun (%45) eğitim düzeylerinin de ilkökul mezunu olduğu tespit edilmiştir (Uruç & Bozdoğan, 2024). Yürütülen çalışma bulguları diğer çalışmalar ile kıyaslandığında, Düzce fındık üreticilerin eğitim durumlarının daha iyi olduğu ortaya çıkmıştır.

Üreticilerin %34'ü geçimini sağlamak için fındık yetiştiriciliği yaptığını belirtmiştir. Fındık Düzce ekonomisi açısından önemli bir gelir kaynağı olmasının yanı sıra, ihracattaki payı ile ülke ekonomisine de katkı sunmaktadır. Düzce fındık üreticilerinin %32'sinin yapılan çiftçi eğitim toplantılarına katılmayı önemsemez iken, %73'ünün televizyondan veya internetten çiftçi eğitim programları izleyerek Dünya ve Türkiye gündemini yakından takip ettikleri anlaşılmıştır. Ayrıca, üreticilerin yarısından fazlasının tarım teşkilatındaki teknik elemanlar/serbest danışmanlar ve üniversite öğretim üyeleri ile görüşmeyi önemsedikleri ortaya çıkmıştır. Hatay ili zeytin üreticileriyle 2017 yılında yapılan çalışmada üreticilerin %55'lik kısmının hiçbir tarımsal eğitim faaliyetine katılmadığını, %43'ünün ise ileride yapılabilecek herhangi bir eğitime katılım isteklerinin olmadığını belirlemiştir (Demirtaş, 2017), Batı Karadeniz Bölgesi'nde yürütülen bir çalışmada, fındık üreticilerinin %40'ının yazılı ve görsel basını takip ettiği, görsel basını takip edenlerin ise %75'inin TV, %15'inin broşür ve

%10'unun dergi yayınlarını takip ettikleri belirlenmiştir (Siray ve ark., 2012).

Düzce fındık üreticilerinin %82 gibi büyük bir çoğunluğu aile geleneğinden geldiği için fındık yetiştirdiğini, %11'inin kârlı olduğu ve iklim koşulları uygun olduğu için, geriye kalan %7'sinin ise üretimi kolay ve yöresel ürün olduğu için yetiştirdiğini bildirmiştir. Hiçbir üretici devletin verdiği ürün desteğinden dolayı fındık yetiştirdiğini söylememiştir. Ayrıca, üreticiler fındığın önemli bir ihracat ürünü olduğunu, katma değerinin yüksek olduğunu ve pazar sorununun olmadığını belirtmişlerdir. Fındık yetiştiriciliği dışında üreticilerin; %40'ı sanayi ve ticaret alanında, %17'si kamu kurumlarında çalışarak; %16'sı hayvancılık/arıcılık yaparak, %9'u diğer tarım ürünlerini yetiştirerek ek gelir elde ettiklerini ve %18'i ise fındık dışında hiçbir ürün yetiştirmediklerini belirtmişlerdir. Mardin ili kiraz üreticileri ile yapılan anket çalışmasında üreticilerin %83'ünün tarıma bağlı bir sosyal güvenliğe sahip olduğu, geri kalan %17'lik kısmın ise tarım dışındaki bir alandan sosyal güvenliğinin ve gelirinin olduğu belirlenmiştir (Kaplan & Ayaz, 2023). Yine patates üreticilerine yönelik Nevşehir'de yürütülen bir çalışmada üreticilerin %74'ünün sosyal güvencesi ve %32'sinin çiftçilik dışında başka gelire sahip oldukları bildirilmiştir (Erdoğan & Gökdoğan, 2017).

#### Üreticilerin Fındık Yetiştiriciliği Faaliyetleri

Üreticilere toplam ne kadar alanda fındık yetiştiriciliği yaptıkları sorulduğunda; %39'u 20-50 dekar arası, %26'sı 10-20 dekar arası, %22'si 50 dekar üzeri ve %11'i 5-10 dekar arası alanda ve sadece %2'si 5 dekardan daha küçük alanda fındık yetiştiriciliği yaptıklarını belirtmişlerdir. Benzer şekilde, Batı Karadeniz Bölgesi'nde yürütülen benzer bir çalışmada fındık bahçelerinin büyüklüğünün ortalama 40 da olduğu bildirilmiştir (Siray ve ark., 2012). Benzer bir çalışma sonucunda, Ordu ili fındık bahçelerinin büyük kısmının 5 dekardan küçük bahçelerden oluştuğu (Yonat & Kolören, 2023), Samsun ilinde Ova kesiminde arazi büyüklüğü 37,8 dekar iken, yüksek kesimde 31,5 dekar olduğu belirlenmiştir (Alkan, 2006). Diğer bir çalışma sonucunda, Giresun ili fındık bahçelerinin sadece %16,9'unun 30 dekardan büyük, %75,6'sının 6 ile 30 dekar arasında ve %7,6'sının 5 dekardan daha küçük olduğu tespit edilmiştir (Turan ve ark., 2023). Karadeniz Bölgesi'nden Düzce, Sakarya, Samsun, Ordu, Giresun ve Trabzon illerinde fındık üretimi yapan çiftçilerin %67'sinin 0-25 dekar, %33'ünün 31 ve daha fazla dekar alana sahip olduğu ve çiftçilerin küçük işletme tanımına (ortalama 3 dekar) uygun alanlarda tarım yaptıkları belirlenmiştir (Taylan, 2020). Kocaeli fındık üreticilerin

arazi varlıkları konusunda %29'unun 0-5 da, %50'sinin 5-20 da ve %21'inin ise 20-50 da arasında olduğu belirtilmiştir (Uzun, 2021). Üreticilere hangi gübreleri kullandıkları sorulduğunda, %33'ü kompoze taban gübresi (15-15-15), %32'si çiftlik gübresi, %15'i üre + nitrat (%26 + %33) gübre kullandığını, %10'u organo-mineral gübre kullandığını, %7'si diğer gübreler ile birlikte suni gübre kullandığını ve %3'ü ise hiç gübre kullanmadığını ifade etmiştir.

Üreticilere, kullandıkları bitki koruma ürünlerinin seçiminde kimlere danıştıkları sorulduğunda; %56'sı danışmanlık şirketlerine, %18'i ilaç bayisi veya ilaç şirketlerine danıştıklarını bildirirken, Tarım Bakanlığı veya Ziraat Fakülteleri gibi resmi kurumlara danışmadıklarını belirtmişlerdir. Elde edilen bulgunun aksine, Giresun ili fındık üreticilerinin %66'sının tarımsal ilaç seçimini bayilere danışarak, %18'inin danışmanı olan Ziraat Mühendisleri'ne sorarak, %7'sinin teknik teşkilata sorarak, %5'inin kendi deneyimine göre, %3'ünün Ziraat Mühendisi'ne sorarak ve %1'inin ise komşularına danışarak kullandıkları saptanmıştır (Kılıç ve ark., 2018). Ay ve ark. (2006), Antalya ili meyve üreticilerinin karşılaştıkları bitki koruma sorunları ile ilgili olarak %82'sinin uzman kişi veya kuruluşlar yerine tarım ilacı satan bayilere danıştıklarını belirlemişlerdir. Bununla birlikte, Kaplan ve Ayaz (2023) Mardin ili kiraz üreticilerine yönelik 2023 yılında yürütmüş oldukları çalışma sonucunda, üreticilerin kimyasal ilaçlamalarda kullanacakları ürünleri seçerken %55'inin Tarım İl ve İlçe Müdürlüklerine, %25'inin zirai ilaç bayilerine, %20'sinin ise kendi tecrübe ve çevredeki üreticilerin kullandıkları ürünlere göre tercih yaptıklarını belirtmişlerdir. Yine Erdoğan ve Gökdoğan (2017), Nevşehir'de patates üreticilerine yönelik yürüttükleri bir araştırmada üreticilerin %89'unun bitki koruma bayilerinden, %8'inin kendi tecrübelerinden, %2'sinin tarımsal kurumlardan, %0,5'inin komşularından ve %0,5'inin ise danışman mühendislerden tavsiye alarak tercihlerini yaptıklarını bildirmiştir. Turunçgil üreticilerine yönelik Antalya'da yürütülen bir çalışmada ise bitki koruma ürünü tercihlerinin %50 oranında tecrübeye göre, %43 oranında bayi tavsiyelerine göre, %4 oranında tarım teşkilatlarının tavsiyelerine göre, %3 oranında ise diğer üreticilerin kullandıkları ürünlere göre gerçekleştiği rapor edilmiştir (Özkan ve ark., 2003). Çukurova Bölgesi'nde

yapılan bir çalışmada; işletmenizde zirai mücadele işlerini kim yürütüyor sorusuna, üreticilerin %29'u yalnız kendisinin, %14'ü ziraat mühendisi veya teknisyenin, %44'ü hem kendisinin hem de ziraat mühendisi veya teknisyenin, %9'u ilaç bayisinin, %3'ü kendisi ile beraber ziraat mühendisi veya teknisyenin ve ilaç bayisinin, %0,5'i de çiftlik kâhyasının ilgilendiğini belirtmiştir (Üremiş ve ark., 1996). Üreticilere bitki koruma ürünlerini nereden aldıkları sorulduğunda; %73'ü kadar büyük bir çoğunluğunun zirai ilaç bayilerinden, %21'inin ise Tarım Kredi Kooperatifleri'nden aldıklarını bildirmişlerdir. Elde edilen veriler kıyaslandığında, Düzce fındık üreticilerinin sadece %18'inin ilaç bayisi ve ilaç şirketlerine danıştıkları anlaşılmıştır.

Üreticilere fındık yetiştiriciliğinde karşılaştıkları sorunların önem sırası sorulduğunda, %36'sı yetiştiricilik sorunları (toprak işleme, gübreleme, hasat), %25'i bitki koruma sorunları, %25'i fiyat sorunu ve %13'ü çeşit uyumu ve iklim faktörleri sorunlarının önemli olduğunu belirtmişlerdir (Şekil 2). Ayrıca üreticiler, pazarlama ile ilgili sorunlarının olmadığını da beyan etmişlerdir. Türkiye'de fındık yetiştiriciliğinde yeni üretim tekniklerinin araştırılması ve bazı uygulamaların başlaması ile gelecekte yetiştiricilik konusunda yaşanan sorunların azalması, dolayısıyla fındık verim ve kalitesinin artması söz konusu olacaktır. Ülkemizde bu konuda her ocak için tek fidan kullanmak, ocaklardaki yaşlı köklerin sökülerek yerine yeni fidanlar ile gençleştirme yapılması şeklinde bazı uygulamalar daha iyi sonuçlar vermektedir (TAGEM, 2023). Çukurova Bölgesi'nde üreticilerin karşılaştıkları zorlukları belirlemek amacıyla yapılan bir anket çalışmasında; üreticilerin %37'si bitki koruma (hastalık, zararlı ve yabancı ot) sorunlarını, %28'i pazarlama sorununu, %19'si işçilik problemini, %16'si ise sulama, maliyet ve iklimsel kaynaklı faktörlerin önemli olduğunu bildirmiştir (Üremiş ve ark., 1996).

Üreticilere karşılaştıkları sorunlar ile ilgili bilgi danıştıkları kurum ve kişilerden aldıkları çözüm önerileri sorulduğunda, %30'u memnun olmadığını, %24'ü orta düzeyde memnun olduğunu, %46'sı ise memnun olduğunu bildirmişlerdir. Bu sorunun Likert ölçeği ortalaması 3,09 olup bu değere göre üreticilerin genel olarak danıştıkları yer, kurum ve kişilerden memnun oldukları anlaşılmaktadır (Çizelge 3).

Çizelge 3. Fındık yetiştiriciliği ile ilgili sorular ve Likert değerleri

Table 3. Questions about hazelnut cultivation, and Likert values

Anket sorusu	Likert ölçek değerleri (%)					Anket sayısı	Likert Ölçeği Ortalaması
	1	2	3	4	5		
Üreticilerin fındık veriminden memnuniyeti	5,0	15,0	21,0	53,0	6,0	100	3,40
Üreticinin karşılaştığı sorunlarla ilgili bilgi danıştığı yerin çözüm önerilerinden memnuniyeti	14,0	16,0	24,0	39,0	7,0	100	3,09

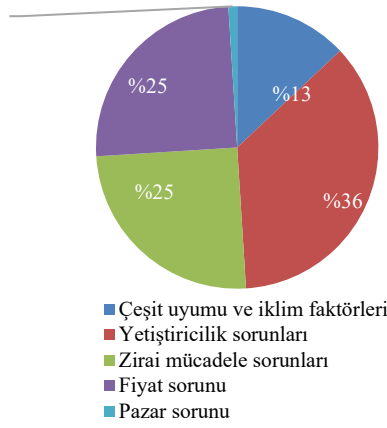
Likert ölçek değerleri: 1: Hiç memnun değilim, 2: Biraz memnunuz, 3: Orta düzeyde memnunuz, 4: Memnunuz, 5: Çok memnunuz.

Çizelge 4. Fındık yetiştiriciliğinde yabancı ot sorunu ve Likert değerleri

Table 4. Weed problem in hazelnut cultivation, and Likert values

Anket sorusu	Likert ölçek değerleri (%)					Anket sayısı	Likert Ölçeği Ortalaması
	1	2	3	4	5		
Fındık bahçelerindeki yabancı ot sorununun önem derecesi	5,0	11,0	16,0	32,0	36,0	100	3,78
Fındık bahçelerindeki yabancı ot yoğunluğu	2,0	5,0	27,0	30,0	36,0	100	3,93

Likert ölçek değerleri: 1: Hiç yok, 2: Az önemli veya az yoğun, 3: Orta, 4: Önemli veya yoğun, 5: Çok önemli veya çok yoğun



Şekil 2. Düzce'de fındık üreticilerinin en önemli tarımsal sorunları

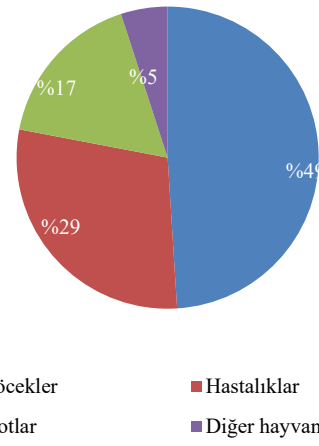
Figure 2. The main agricultural problems of hazelnut growers in Düzce

### Fındık Yetiştiriciliğinde Yabancı Ot Sorunu ve Mücadelesi

Fındık üreticilerine bahçelerindeki bitki koruma sorunlarının önem sıralaması sorulduğunda; en önemli sorunun %49 ile zararlı böcekler, %29 ile hastalıklar, %17 ile yabancı otlar ve %5 ile diğer hayvansal zararlılar olduğunu belirtmişlerdir (Şekil 3).

Üreticilerin yabancı otların önemi konusundaki soruya verdiği cevaplara bakıldığında %36'sı çok önemli, %32'si önemli, %16'sı orta derecede önemli olduğunu belirtmiştir. Bu soruya verilen cevapların Likert ölçeği ortalaması 3,78 olarak hesaplanmıştır; bu değere göre üreticiler için yabancı ot sorununun çok önemli olduğu kanısına varılmıştır. Ayrıca üreticilere bahçelerindeki yabancı ot yoğunluğu sorulduğunda; %36'sı çok yoğun, %30'u yoğun, %27'si orta derecede yoğun olduğunu belirtmiştir. Verilen cevapların Likert ölçeği ortalaması 3,93'tür ve bu değere bakıldığında da üreticiler açısından yabancı otların bahçelerde çok yoğun olarak değerlendirildiği anlaşılmıştır (Çizelge 4).

Anket yapılan üreticilerin beyanlarına göre fındık bahçelerinde sorun olan toplam 45 adet yabancı ot türü olduğu anlaşılmıştır (Çizelge 5). Bu yabancı ot türlerinden; dam bromu (*Bromus tectorum* L.), tek yıllık salkımotu (*Poa annua* L.), yara otu (*Prunella vulgaris* L.), yonca (*Medicago sativa* L.), şebrek (*Lapsana communis* L.), bataklık sinirotu (*Plantago lanceolata* L.), kuzukulağı (*Rumex acetosella* L.), ak üçgül (*Trifolium repens* L.), böğürtlen (*Rubus sanctus* Schreb), tarla sarmaşığı (*Convolvulus arvensis* L.), yabani çilek (*Fragaria vesca* L.) ve kadife otu (*Holcus lanatus* L.) türlerinin bahçelerde daha yaygın ve yoğun olduğu kanısına varılmıştır. Düzce ili Batı Karadeniz Bölgesi iklim kuşağında olması ve sürekli yağış alması nedeniyle, bahçelerde üretim sezonu boyunca yabancı ot çıkışları devam etmektedir. Bölgede çok sayıda dar ve geniş yapraklı yabancı otların olduğu bildirilmiştir (Ermeç, 2022). Düzce ili fındık bahçelerinde yürütülen bir çalışmada bahçelerde çok sayıda dar ve geniş yapraklı yabancı ot türü belirlemiştir (Sizer, 2024). Ordu ilinde yapılan benzer bir anket çalışması (Yonat & Kolören, 2023) sonucunda üreticilerin fındık bahçelerinde en fazla sorun yaşadıkları ve mücadelesinde sorun yaşadıkları 10 yabancı ot türü belirlenmiştir. Bunlar



Şekil 3. Düzce'de fındık üreticilerinin yaşadığı bitki koruma sorunlarının önem düzeyi.

Figure 3. The importance of plant protection problems experienced by hazelnut growers in Düzce.

sırasıyla; *Urtica dioica* L. (%89), *Rubus canescens* DC. (%71), *Pteridium aquilinum* (L.) Kuhn (%63), *Helleborus orientalis* Lam. (%61), *Smilax excelsa* L. (%57), *Rumex crispus* L. (%47), *Convolvulus arvensis* L. (%24), *Artemisia vulgaris* L. (%23), *Euphorbia helioscopia* L. (%17) ve *Oenanthe pimpinelloides* L. (%11) türleridir. Bu çalışma sonucunda özellikle çok yıllık, sarmaşık, dikenli ve boylanabilen yabancı ot türleri sorun olarak görülmüş olup yürütülen çalışma ile kıyaslandığında, sadece *C. arvensis* türü mücadelesinde zorluk yaşanan ortak tür olmuştur.

Üreticilerin yabancı otlar ile mücadele durumuna bakıldığında; %72'si sürekli olarak mücadele yaptıklarını, %22'si ise genellikle mücadele yaptıklarını belirtmiştir. Likert ölçeği ortalaması 4,64 olarak hesaplanmış ve bu değere göre üreticiler açısından yabancı otlarla mücadele yapılmasının çok önemli olduğu anlaşılmıştır (Çizelge 7). Sert çekirdekli meyve bahçelerinde yapılan bir çalışmada, üreticilerin %93'ü bahçesindeki yabancı otlar ile mücadele yaptıklarını, %7'si ise yapmadıklarını bildirmiştir (Ay ve ark., 2006).

Üreticilere kullandıkları herbisitlerin yabancı otlara karşı ne kadar etkili olduğu sorulduğunda; %75'i çok etkili, %19,6'sı orta düzeyde etkili, %3,6'sı çok az etkili ve sadece %1,8'i etkisiz olduğunu belirtmiştir. Üreticilerin bu soruya verdikleri cevapta Likert ölçeği ortalaması 3,9 olarak hesaplanmış olup üreticilerin kullandıkları herbisitlerin etkisinin yüksek olduğu kanısına varılmıştır (Çizelge 7).

Ayrıca üreticilerin %62,5'i kullandıkları herbisitlerin büyük oranda yabancı ot sorununu çözdüğünü, %28,6'sı orta derecede çözdüğünü ve %5'i ise düşük oranda çözdüğünü belirtmiştir. Bu sorudaki Likert ölçeği ortalaması 3,73 olarak hesaplanmış olup üreticilerin yaptığı kimyasal mücadelenin yabancı ot sorununu önemli oranda çözdüğü anlaşılmıştır (Çizelge 6).

Üreticilerin yabancı otlarla mücadele kararı verirken büyük çoğunlukla (%83) bahçedeki yabancı otların durumuna bakarak mücadeleye kendilerinin karar verdiğini, geriye kalan üreticilerin (%17) ise ilaç bayisine, Tarım ve Orman Bakanlığı'na, özel danışmanlara ve komşularına danışarak karar verdiklerini belirtmişlerdir.

Çizelge 5. Düzce’de üreticiler tarafından fındık bahçelerinde sorun olduğu bildirilen yabancı otlar

Table 5. Troublesome weeds in hazelnut orchards reported by growers in Düzce

Bilimsel ismi	Türkçesi
<i>Amaranthus retroflexus</i> L.	Kırmızı köklü horozibiği
<i>Anthemis cotula</i> L.	Pis kokulu köpek papatyası
<i>Bromus hordeaceus</i> L.	Arpamsı brom
<i>Bromus tectorum</i> L.	Dam bromu, Püsküllü çayır
<i>Chenopodium album</i> L.	Sirken
<i>Convolvulus arvensis</i> L.	Tarla sarmaşığı
<i>Cynodon dactylon</i> (L.) Pers.	Köpekdişi ayrığı
<i>Cyperus rotundus</i> L.	Topalak
<i>Digitaria sanguinalis</i> (L.) Scop.	Çatal otu
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Darican
<i>Elymus repens</i> (L.) Gould	Tarla ayrığı
<i>Conyza canadensis</i> (L.) Cronquist	Şifa otu, pire otu
<i>Euphorbia rigida</i> L.	Çift bezeli sütleğen, Sert sütleğen
<i>Fragaria vesca</i> L.	Yabani çilek
<i>Galium aperine</i> L.	Yapışkan ot, Yoğurt otu
<i>Geranium pyrenaicum</i> Burm.f.	Turna gagası, Gelin çarşafı
<i>Hedera helix</i> L.	Duvar sarmaşığı
<i>Holcus lanatus</i> L.	Kadife otu
<i>Hordeum bulbosum</i> L.	Yumruku arpa
<i>Lamium purpureum</i> L.	Kırmızı çiçekli ballıbaba
<i>Lapsana communis</i> L.	Şebrek
<i>Lolium rigidum</i> L.	İnce delice, Sert delice
<i>Medicago sativa</i> L.	Yonca
<i>Plantago lanceolata</i> L.	Bataklık sinir otu
<i>Poa annua</i> L.	Yıllık salkım otu, tavşanbıyığı
<i>Poa trivialis</i> L.	Adi salkım otu
<i>Polygonum aviculare</i> L.	Çoban değneği
<i>Portulaca oleracea</i> L.	Semizotu
<i>Potentilla reptans</i> L.	Beşpençe, Sürünücü beşparmak otu
<i>Prunella vulgaris</i> L.	Yara otu
<i>Pteridium aquilinum</i> (L.) Kuhn	Eğrelti otu
<i>Rubus sanctus</i> Schreb.	Böğürtlen
<i>Rumex acetosella</i> L.	Kuzukulağı
<i>Secale cereale</i> L.	Çavdar
<i>Setaria viridis</i> (L.) P. Beauv.	Yeşil kirpi darı
<i>Sinapis arvensis</i> L.	Yabani hardal
<i>Solanum nigrum</i> L.	İt üzümü, köpek üzümü
<i>Sonchus oleraceus</i> L.	Eşek marulu
<i>Stellaria media</i> (L.) Vill.	Serçe dili
<i>Trifolium pratense</i> L.	Çayır üçgülü, çayır tırfılı
<i>Trifolium repens</i> L.	Ak üçgül
<i>Urtica dioica</i> L.	Büyük ısırgan
<i>Urtica urens</i> L.	Bahçe ısırganı
<i>Veronica persica</i> Poir.	İran yavşan otu
<i>Vicia sativa</i> L.	Fiğ

Çizelge 6. Fındık bahçelerinde yabancı otlarla mücadele durumu

Table 6. Weed control situation in hazelnut orchards

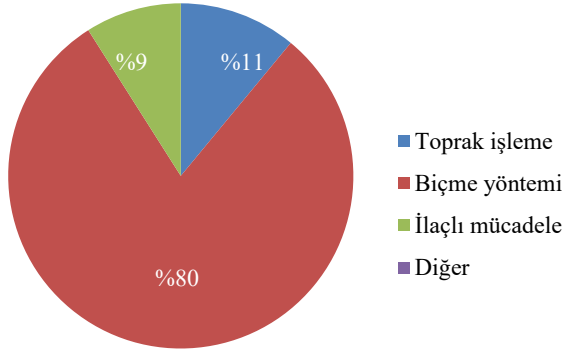
Anket sorusu	Likert ölçek değerleri (%)					Anket sayısı	Likert Ölçeği Ortalaması
	1	2	3	4	5		
Üreticinin yabancı otlarla mücadele edip etmediği	1,0	0,0	5,0	22,0	72,0	100	4,64
Üreticilerin kullandığı herbisitleri ne kadar etkili bulduğu	1,8	3,6	19,6	64,3	10,7	56	3,79
Yapılan kimyasal mücadelenin yabancı ot sorununu ne ölçüde çözdüğü	0,0	8,9	28,6	42,9	19,6	56	3,73

Likert ölçek değerleri; 1: Hiç, 2: Az veya nadir, 3: Orta düzeyde veya bazen, 4: Önemli, çok veya sıklıkla, 5: Her zaman veya tamamen

Çizelge 7. Düzce fındık üreticilerinin uyguladıkları herbisitler

Table 7. Herbicides applied by hazelnut growers in Düzce

Aktif maddesi	Ticari adı	Uygulama dozu
Glifosat potasyum tuzu 441 g/l	Raoundup Star	600 ml/da
Glifosat isopropylamin tuzu 480 g/l	Knock-Out 48 SL	600 ml/da
Diquat (Kurutucu) 200 g/l	Reglone	400 ml/da
Carfentrazone-ethyl 60 g/l	Egnit	100 ml/da



Şekil 4. Düzce’de fındık üreticilerinin yabancı otlarla mücadele yöntemleri

Figure 4. Weed control methods applied by hazelnut growers in Düzce.

Oysa, Tokat ili bağ üreticilerinin yaklaşık %40’ının ilaçlamayı bitki koruma bayilerinden aldıkları tavsiyelere göre yaptıklarını belirlenmiştir (Cangi & Topçu, 2010). Yine, Manisa bağ üreticilerinin %55’inin Tokat ilinde olduğu gibi bitki koruma ürünleri bayisinin görüşlerini dikkate alarak hareket ettikleri tespit edilmiştir (Erdil & Tiryaki, 2020) Şanlıurfa ilinde yapılan bir başka çalışmada ise durum biraz daha farklı bulunmuş; çiftçilerin %81’inin zirai mücadele kararı verirken komşularını ve çevreyi takip ettikleri, sadece %2’sinin tarım kuruluşlarına danışarak mücadeleye karar verdikleri rapor edilmiştir (Çıkman & Yarba, 2008).

Fındık üreticilerine yabancı otlarla mücadelede hangi yöntemleri kullandıkları sorulduğunda üreticilerin %80’i biçme, %11’i toprak işleme ve %9’u kimyasal mücadeleye yöntemlerini tercih ettikleri anlaşılmıştır (Şekil 4). Üreticilerin çoğunun nisan-mayıs ve ağustos ayları olmak üzere yılda iki kez biçim yaptıkları; toprak işleyerek mücadele edenlerin bu amaçla ilkbahar aylarında rotatiller kullandıkları ve herbisit kullananların bazılarının hasattan önce bir kez, bazılarının ise ilkbahar ve hasat öncesinde olmak üzere yılda iki kez uygulama yaptıkları belirlenmiştir. Kocaeli fındık üreticilerinin bitki koruma etmenleri ile mücadelede kimyasal mücadeleyi diğer mücadele yöntemlerinden daha fazla tercih ettiği, kimyasal mücadelede herbisit kullanımının ilk sırada geldiği tespit edilmiştir (Uzun, 2021). Üreticilerin tamamına yakını herbisitleri; “kimyasal, ot ilacı, yabancı ot kurutucu” ve çok az bir kısmı “total herbisit” olarak tanımlamıştır. Üreticilere bahçelerinde hangi herbisitleri kullandıkları sorulduğunda büyük çoğunluğu kullandığı herbisitlerin ticari ismini ve/veya aktif maddesini hemen hatırlamadıklarını söylemiştir. Ancak üreticilerin kullandıkları herbisitlerin isimleri ve uygulama dozları ilaç ambalajlarına bakılarak, danışmanlarına veya ilaç bayilerine sorularak belirlenmiş ve Çizelge 7’de verilmiştir.

Üreticiler kullandıkları herbisitlerin tarla sarmaşığı (*C. arvensis*), köpekdişi ayrığı (*C. dactylon*), eğrelti otu (*P. aquilinum*), böğürtlen (*R. sanctus*) ve büyük ısırgan otu (*U. dioica*)’na etkisinin düşük olduğunu veya hiç etmediğini de bildirmiştir. Benzer şekilde, Ordu ili fındık üreticilerinin de *U. dioica*, *R. canescens*, *P. aquilinum* ve

*C. arvensis* türlerinin mücadelesinde sorun yaşadıkları belirlenmiştir (Yonat ve Kolören, 2023).

Üreticilere fındık bahçelerindeki bitkisel atıkları (budanan dallar, dip sürgünleri, fındık zürufu) nasıl değerlendirdikleri sorulduğunda %52’sinin yakacak olarak kullandığı, %34’ünün parçaladıktan sonra kompost gübre olarak toprağa karıştırdığı, %8’inin malç olarak bahçeye serdiği, %5’inin hiçbir şekilde değerlendirmedeği ve %1’inin ise bitkisel atıklarını satarak gelir elde ettiği anlaşılmıştır.

## Sonuç

Bilindiği üzere Düzce ilinde yapılan fındık üretimi, ülkesel üretimde önemli bir paya sahiptir. Bu çalışma sonucunda, Düzce fındık üreticilerinin yetiştiricilik hakkındaki bilgi düzeylerinin iyi olduğu, Dünya ve Türkiye gündemini yakından takip ettikleri, tarım teşkilatı veya üniversite personeli gibi uzman kişiler ile sık görüşmeyi önemsedikleri, uzman kişiler tarafından düzenlenen eğitimlere katıldıkları anlaşılmıştır. Üreticilerin elde ettikleri verimden memnun oldukları, pazar sorunlarının olmadığı ancak bazı yetiştiricilik ve zirai mücadele sorunlarından yüksek oranda şikâyetçi oldukları görülmüştür. Fındık üreticilerinin bahçelerinde yabancı otları önemli derecede sorun olarak gördüğü, çoğunlukla bahçedeki duruma bakarak yabancı otlarla mücadeleye kendilerinin karar verdiği, en yaygın olarak biçme yöntemiyle ve düşük oranda ise herbisit kullanarak mücadele ettikleri anlaşılmıştır. Üreticilerin çoğunun nisan-mayıs ve ağustos aylarında olmak üzere yılda iki kez biçme yöntemi ile yabancı ot mücadelesi yaptığı belirlenmiştir. Kimyasal mücadele yapan üreticilerin tarım ilacı satın aldıkları bayinin ziraat mühendisi unvanına sahip olmasına önem verdiği ve genellikle bayilerin önerileri doğrultusunda herbisitleri kullandıkları tespit edilmiştir. En çok glifosat aktif madde içerikli herbisitlerin kullanıldığı, bu herbisitlerin yabancı ot sorununu önemli ölçüde çözdüğü, ancak tarla sarmaşığı (*C. arvensis*), köpekdişi ayrığı (*C. dactylon*), eğrelti otu (*P. aquilinum*), böğürtlen (*R. sanctus*) ve büyük ısırgan otu (*U. dioica*) gibi bazı yabancı otlarla mücadelede yeterli başarının elde edilemediği anlaşılmıştır. Bu sorunların aşılabilmesi için farklı mücadele yöntemlerinin araştırılmasına ihtiyaç duyulmaktadır. Ayrıca, fındık özelinde kullanılan aktif maddelerin alternatiflerinin artırılması ve bunun için farklı etki mekanizmalarına sahip olanların belirlenmesi ve ruhsatlandırılması gerekmektedir. Sonuç olarak, bilimsel çalışmaların desteği ile daha etkili mücadele yöntemlerinin ve entegre edilmiş yaklaşımların geliştirilmesinin gerekli olduğu kanısına varılmıştır.

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## Weed Management Effects on Weed Dynamics, Yield and Economics of Spring Maize at Dang, Nepal

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### ABSTRACT

Weeds pose a significant challenge in maize fields in the Dang district, leading to a substantial 52% reduction in yield. Hence, this study was conducted in Satbariya village of Dang, Nepal, in 2023 to evaluate the impact of various weed management practices on weed dynamics, growth, and yield of spring maize. The experiment included seven treatments: a weedy check, weed-free plot, pre-emergence application of atrazine at 1.25 a.i. kg/ha (AtPrE), post-emergence application of atrazine at 1.25 a.i. kg/ha (AtPoE), manual weeding at 30 DAS, mini-tiller at 30 DAS, and LaPoE (tembotrione 42% SC + atrazine 50% WP) applied as post-emergence. The Subarna variety of maize was chosen for the study. Fifteen weed species from seven different families were identified in the experimental area. Specific morphological and phenological parameters, such as plant height and days to tasseling and silking, were not significantly influenced by the weed control methods. However, significantly lower weed density and biomass were observed in the weed-free plot and LaPoE. Similarly, weed-free plots and LaPoE exhibited significantly higher weed control efficiency (WCE) and weed control index at both 45 and 60 DAS, leading to a lower weed index (0.00–16.71%) and more effective weed control. Concerning the yield parameters, cob length, number of kernels per row, and 1000-grain weight were significantly higher in weed-free plots, followed by LaPoE, and the highest grain and biological yield were observed in weed-free plot (6.14–15.18 tons/ha) and LaPoE (5.12–13.32 tons/ha). Moreover, the benefit-cost ratio and net return were observed to be highest with LaPoE. This study suggests that LaPoE can be an effective and economical weed management strategy for increasing maize yield and profitability. Further research could explore the long-term effects of using LaPoE on weed control and crop productivity.

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### Introduction

Maize (*Zea mays* L.) is a significant cereal crop with a high photosynthetic potential, making it adaptable to a wide range of environments (Thakur et al., 2020). It is a member of the grass family, Poaceae, and is the third most important cereal crop globally, after wheat and rice (Swaroop & David, 2021). It has 66.3% carbohydrates, 11.1% protein, 3.6% fat, 2.7% fiber, 1.5% minerals (calcium, phosphorus, iron), and 1.5% vitamins (A, B, and E). It continues to be a staple in the diet of many because of its nutritional value, and the rapid growth of Nepal's poultry and feed industries is contributing to the increasing demand (Timsina et al., 2019).

In Nepal, 985,565 hectares were planted with maize, producing 3,106,397 metric tons at a productivity rate of 3.15 metric tons per hectare (MoALD, 2022). This productivity is significantly lower than the achievable yield of 5.7 metric tons per hectare, as reported by Timsina et al. (2019). Despite the vast marketing potential, the accelerated rate of demand growth outpaces the increase in maize output due to factors such as inadequate production capacity and distribution inefficiencies, leading to a substantial disparity between maize output and demand (Dhakal et al., 2022).

Several factors contribute to the gap between maize demand and the supply chain, including weed infestation, soil fertility decline, climatic uncertainty, and the subsistence farming approach. Among these, weed infestation is a major concern. Wide spacing between maize rows, frequent irrigation, and heavy use of chemical fertilizers create favorable conditions for weed development and establishment, leading to increased yield loss (Shrestha et al., 2021). This situation is further exacerbated in Sadbariya, Dang, due to inappropriate weed management practices adopted by farmers.

Farmers often remain unaware that understanding the critical weed infestation period is essential for successful maize cultivation. They typically prefer manual weeding methods over other techniques, which leaves them unaware of alternative methods that could be more cost-effective and productive. Studies have identified the critical weed infestation period to be between 2 to 8 weeks after sowing, specifically from the third to fifth leaf tip stages of development (Duwadi et al., 2021; Cerrudo et al., 2012). During this period, weed interference can lead to increased plant-to-plant variability, reduced grain yield, and a higher incidence of bareness (Cerrudo et al., 2012). Employing non-chemical weed management strategies, including mechanical, biological, and cultural methods, during this critical period can effectively suppress weed populations and improve economic returns (Duwadi et al., 2021).

Jain (2022) observed that maintaining weed-free conditions significantly enhances maize growth and yield. Similarly, Khan et al. (2013) and Abimbola (2019) documented the effectiveness of various weed control methods, such as pre-emergence herbicides, hoe-weeding, and mulching, in suppressing weed growth and enhancing maize yield. Singh et al. (2021), BB et al. (2021) and O.S. et al. (2021) reported a significant reduction in weed density and dry matter, as well as a superior maize yield with the use of atrazine.

Idziak & Woznica (2014) found that the post-emergence application of tembotrione, particularly when combined with a surfactant, effectively controlled weeds and increased grain yield. Sharma et al. (2023) reported that the combined application of tembotrione and atrazine was more effective in controlling weeds and increasing maize yield compared to the sole application of tembotrione. A combination of hoeing and herbicides has been found to increase maize yield and its components significantly, while also effectively controlling weeds (Khatam et al., 2013; Soliman & Hamz, 2014).

The objective of this study is to address weed infestation in maize farming, which significantly negatively impacts crop productivity. The study aims to examine the prevalent weed population and how different weed management practices affect weed dynamics, growth, phenology, yield attributes, and the economic aspects of maize cultivation. Understanding the prevalence and effects of various weeds on the maize crop is crucial for informed decision-making and effective cultivation strategies. Furthermore, the evaluation of various weed management practices will help to identify the most cost-effective and productive approaches. Therefore, farmers will be empowered to improve their current practices.

## Materials and methods

### Location and Site Weather and Soil Properties

A field experiment was conducted in Lamahi Municipality-8, Satbariya, Dang, Nepal, which falls under the PMAMP superzone of Maize, Lamahi. The experimental site is situated at an altitude of 629 meters above sea level. Figures 1 and 2 present information on the climatic conditions during the study's duration and the location of the experimental area, respectively.

### Plant Material and Experimental Design

The seeds of the Subarna variety were utilized for this experimental study. The field was thoroughly plowed and leveled one week prior to planting. Seeds were sown on February 26, 2023, with a row-to-row spacing of 60 cm and a plant-to-plant spacing of 20 cm. During field preparation, well-decomposed farmyard manure (20 tons ha<sup>-1</sup>) was mixed with the soil. A full dose of DAP (60 kg ha<sup>-1</sup>) and MOP (40 kg ha<sup>-1</sup>) was applied as a basal dose at planting. Half of the urea dose (180 kg ha<sup>-1</sup>) was incorporated during field preparation, with the remaining half applied in split doses at the knee-high and tasseling stages. The first irrigation was carried out immediately after planting, followed by subsequent irrigations every 6–7 days, adjusted based on rainfall and soil moisture levels.

The experiment was conducted using a Randomized Block Design (RCBD) with ten treatments, each replicated three times. Each experimental plot measured 12 m<sup>2</sup>, with dimensions of 4 m by 3 m, and consisted of five rows with 20 plants per row. The total research area covered was 362.25 m<sup>2</sup>, with inter-plot spacing of 60 cm, block-to-block spacing of 75 cm, and a border width of 30 cm. Details of the treatments used in the experiment are provided in Table 1.

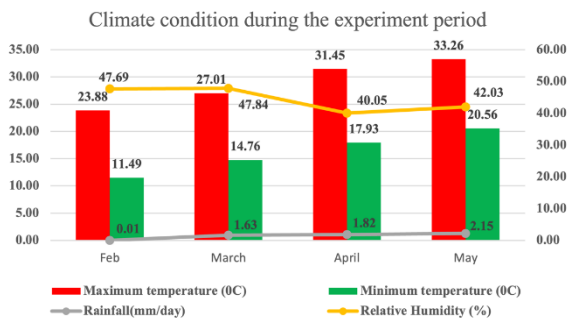


Figure 1. The climatic condition of the experimental area  
Data Source: (POWERNASA, 2023)

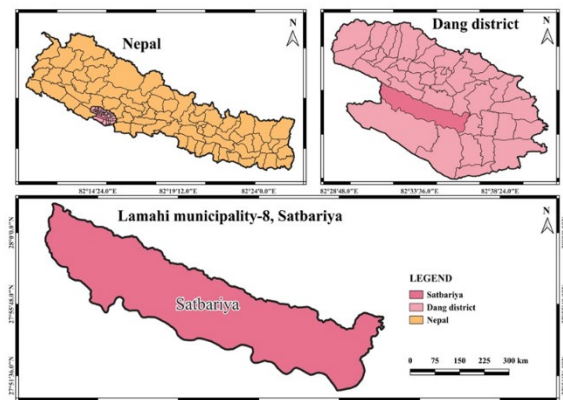


Figure 2. Location of the experimental field.  
(Created by using QGIS 10.8 software)

Table 1. Treatment details

Treatments	Trade name	Chemical name	Dose	Application time
T1: Weedy check	-	-	-	No weed control
T2: Weed-free	-	-	-	Weeding at 7-10 days interval
T3: Atrazine as pre-emergence (AtPrE)	Foost	Atrazine 50% WP	1.25 a.i. kg/ha (Nadiger et al., 2013)	Applied at 2 DAS
T4: Atrazine as post-emergence (AtPoE)	Foost	Atrazine 50% WP	1.25 a.i. kg/ha (Nadiger et al., 2013)	Applied at 30 DAS
T5: Tembotrione as post-emergence (LaPoE)	Laudis	(Tembotrione 42% SC + surfactant)	120gm a.i/ha Tembotrione + 1000ml/ha surfactant (Horowitz et al., 1990) + 0.6 a.i. kg/ha atrazine 50% WP (Sharma et al., 2018)	Applied at 30 DAS
T6: Mini tiller	-	-	-	Applied at 30 DAS
T7: Manual Weeding	-	-	-	Applied at 30 DAS

Note: WP: wettable powder; SC: soluble concentrate; DAS: days after sowing; a.i.: active ingredient; kg/ha: Kilograms per hectare, ml/ha: milli liters/hectare, gm/ha: grams per hectare

### Data Collection and Observation

#### Weed dynamics parameters

To assess the impact of weed management practices on weed dynamics, various observations including weed flora, density, dry weight, weed control efficiency (WCE) (equation 1), and weed control index (WCI) (equation 2) were conducted. Quadrants measuring 0.5 m<sup>2</sup> (0.5 m × 1 m) were established at four distinct locations within each plot to monitor weed parameters. The weed flora within each quadrant was identified with the help of online resources and experts, and categorized into three groups: sedge, broadleaf, and narrow-leaf weeds, based on their morphological characteristics. The total weed count for each quadrant was recorded and expressed as the number of weeds per square meter. For dry weight measurement, the weeds were sun-dried and subsequently oven-dried at 72°C for 3 days. Dry weight and weed count data were collected separately for sedges, broadleaf, and narrow-leaf weeds at 30, 45, and 60 days after sowing (DAS). Weed control efficiency was calculated as the percentage reduction in weed population due to weed management practices compared to the control (weedy check). The weed control index was determined by comparing the dry weight of weeds from different treatments to that from the control plot.

$$WCE = \frac{(WPWC - WPTP)}{WPWC} \times 100 \quad (1)$$

WCE : Weed control efficiency (%)  
 WPWC : Weed population(no/m<sup>2</sup>) in weedy check  
 WPTP : weed population(no/m<sup>2</sup>) in treated plot  
 (Mishra et al., 2020)

$$WCI = \frac{(DMPWC - DMPTP)}{DMPWC} \times 100 \quad (2)$$

WCI : Weed control index (%)  
 DMPWC : Dry matter production (g) in weedy check  
 DMPTP : Dry matter production (g) in treated plot  
 (Mishra et al., 2020)

$$WI = \frac{(TYWFC - TYST)}{TYWFC} \times 100 \quad (3)$$

WI : Weed index  
 TYWFC : Total yield from weed free check  
 TYST : Total yield from specific treatment  
 (Mishra et al., 2020)

#### Biometrical and Phenological Parameters of Maize

Plant height was measured from 10 randomly selected plants in each plot at 30, 45, 60, 75, and 90 days after sowing (DAS) at 15-day intervals. Phenological observations were recorded at the tasseling and silking stages, corresponding to 50% and 100% completion, respectively, and noted as Days After Sowing (DAS).

#### Yield Attributing Parameters of Maize

The maize crops were harvested on June 14, 2023. To evaluate yield parameters, measurements were taken for the number of kernels per ear, cob length (cm), cob girth (mm) (equation 4), 1000-grain weight (g), shelling percentage (equation 5), sterility percentage (equation 6), grain yield (kg/ha) (equation 7), and harvest index (equation 8) from ten maize plants per plot.

$$\text{Cob girth (mm)} = \frac{\text{Circumference of the girth}}{3.14} \quad (4)$$

$$\text{Shelling Percentage (\%)} = \frac{\text{Grain yield}}{\text{Cob yield}} \times 100 \quad (5)$$

(Adhikari et al., 2023)

$$\text{Sterility Percentage} = \frac{\text{LUGC}}{\text{TLC}} \times 100 \quad (6)$$

LUGC : Length of unfilled grain in cob  
 TLC : Total length of the cob  
 (Adhikari et al., 2023)

$$GY = \frac{(100-MC) \times PY \times 1000}{84 \times \text{net plot area (m}^2\text{)}} \quad (7)$$

GY : Grain yield (kg/ha)

MC : Moisture content

PY : Plot yield(kg)

(Adhikari et al., 2023)

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} * 100 \quad (8)$$

(Adhikari et al., 2023)

### Economic Analysis

Gross monetary return (NPR) is the entire monetary worth of the economic products and byproducts produced from the crop grown under the different treatments (equation 9); this value was determined using local market prices. Cost of cultivation (NPR) included fixed costs (land lease) and variable costs (labor, a hired miner, the cost of seeds, manures, fertilizers, pesticides, and herbicides, as well as the charges for irrigation, harvesting, drying, and transportation) incurred during maize cultivation. It was estimated using the local charges. Net return (NPR) was determined by deducting cultivation costs from gross returns (equation 10). The benefit-cost ratio (BCR) measures the relationship between the gross return and the cost of cultivation for a specific treatment (equation 11). This index calculates the advantage a farmer receives in exchange for the cost of adopting a specific treatment.

$$GR = TMY \times SPM \quad (9)$$

GR : Gross return

TMY: Total marketable yield

SPM: Selling price of maize

$$NR = \text{Gross return} - \text{total cost of production} \quad (10)$$

NR: Net returns

$$\text{Benefit - cost ratio} = \frac{\text{Net return}}{\text{Total cost of production}} \quad (11)$$

### Data Analysis

The data was systematically organized, with treatments applied across four replicates and various parameters measured. Statistical analysis was conducted using MS Excel and R-Studio. Variances were analyzed, and mean values of individual parameters were compared using Duncan's Multiple Range Test (DMRT) at a significance level of  $p < 0.05$ . The results were thoroughly analyzed and discussed in relation to existing literature.

### Results

#### Weed Species

Table 2 revealed that fifteen different weed species belonging to seven different families were found in the experimental field. *Cyperus rotundus*, *Cyperus esculentus*, and *Argemone Mexicana* were major weed species identified in the field. The population of sedges was significantly higher than that of dicots and monocots. According to Shrestha et al. (2021), sixteen distinct weed species from six different families were also reported at Dhading Besi, Nepal. The most common weed species found in the study area were *Digitaria spp.*, *Fimbristylis spp.*, *Cynodon dactylon*, *Ageratum conyzoides*, *Oryza sativa*, *Digitaria album*, *Cyperus rotundus*, and *Stellaria media*, according to Shrestha et al. (2021).

#### Weed Density

Weed control methods significantly influenced weed density, which was consistently observed to be lower in weed-free plots and greater under weedy check at all observations (Table 3). After weed-free, post-emergence application of AtPrE resulted in the lowest weed density across all three weed categories at 30 DAS. Conversely, the post-emergence application of Tembotrione resulted in the lowest weed density across all three weed categories at 45 and 60 DAS following the weed-free. A similar trend was observed in total weed density, with the lowest value recorded after the post-emergence application of atrazine at 30 DAS and the post-emergence application of Tembotrione at 45 and 60 DAS.

Table 2. Weed species observed in the experimental plots of spring maize at Dang, Nepal

Common name	Scientific name	Family
Grasses		
Bermuda grass	<i>Cynodon dactylon</i>	Poaceae
Cockspur	<i>Echinochloa crusgalli</i>	Poaceae
Signal grass	<i>Brachiaria repens</i>	Poaceae
Fountain grass	<i>Pennisetum cenchroides</i>	Poaceae
Egyptian crowfoot grass	<i>Dactyloctenium aegyptium</i>	Poaceae
Hairy crabgrass	<i>Digitaria sanguinalis</i>	Poaceae
Dicots		
Parthenium	<i>Parthenium hysterophorus</i>	Asteraceae
Indian nettle	<i>Acalypha indica</i>	Euphorbiaceae
Mexican prickly poppy	<i>Argemone Mexicana</i>	Papaveraceae
Hemp	<i>Cannabis sativa</i>	Cannabaceae
Billygoat weed	<i>Ageratum conyzoides</i>	Asteraceae
Ballon cherry	<i>Physalis angulate</i>	Solanaceae
Sedges		
Purple nutsedge	<i>Cyperus rotundus</i>	Cyperaceae
Yellow nutsedge	<i>Cyperus esculentus</i>	Cyperaceae
Rice flat sedge	<i>Cyperus iria</i>	Cyperaceae

Table 3. Weed density as influenced by different weed management practices at Dang, Nepal in 2023

Weed management practices	Weed Density (no/m <sup>2</sup> )			
	30 DAS			
	SW	GW	BW	Total
Weed free	0.71 <sup>c</sup> (0.00)	0.71 <sup>d</sup> (0.00)	0.71 <sup>b</sup> (0.00)	0.71 <sup>c</sup> (0.00)
Manual weeding	16.17 <sup>a</sup> (272.00)	6.38 <sup>a</sup> (41.33)	7.51 <sup>a</sup> (56.00)	18.99 <sup>a</sup> (369.33)
AtPrE	9.52 <sup>b</sup> (94.00)	2.59 <sup>cd</sup> (8.00)	2.59 <sup>b</sup> (8.00)	10.34 <sup>b</sup> (110.00)
AtPoE	17.44 <sup>a</sup> (306.67)	5.53 <sup>ab</sup> (32.67)	5.57 <sup>a</sup> (30.67)	19.19 <sup>a</sup> (370.00)
LaPoE	20.04 <sup>a</sup> (450.00)	3.89 <sup>abc</sup> (15.33)	7.11 <sup>a</sup> (54.00)	22.17 <sup>a</sup> (519.33)
Mini Tiller	17.98 <sup>a</sup> (332.67)	3.15 <sup>bc</sup> (14.67)	6.27 <sup>a</sup> (40.00)	19.47 <sup>a</sup> (387.33)
Weedy check	17.55 <sup>a</sup> (309.33)	4.84 <sup>abc</sup> (25.33)	6.74 <sup>a</sup> (46.00)	19.48 <sup>a</sup> (380.67)
F-test ( $\alpha=0.05$ )	S***	S**	S***	S***
SEM ( $\pm$ )	2.07	0.75	0.73	1.71
LSD ( $\alpha=0.05$ )	6.39	2.33	2.24	5.27
CV (%)	25.30	33.81	24.12	17.79
Weed management practices	Weed Density (no/m <sup>2</sup> )			
	45 DAS			
	SW	GW	BW	Total
Weed free	0.71 <sup>d</sup> (0.00)	0.71 <sup>d</sup> (0.00)	0.71 <sup>c</sup> (0.00)	0.71 <sup>d</sup> (0.00)
Manual weeding	14.27 <sup>b</sup> (204.00)	3.97 <sup>ab</sup> (16.00)	5.63 <sup>b</sup> (31.33)	15.84 <sup>b</sup> (251.33)
AtPrE	13.78 <sup>bc</sup> (197.33)	2.50 <sup>c</sup> (6.67)	2.95 <sup>cd</sup> (10.67)	14.49 <sup>b</sup> (214.67)
AtPoE	16.00 <sup>b</sup> (256.00)	4.98 <sup>a</sup> (26.67)	4.72 <sup>bc</sup> (22.00)	17.44 <sup>b</sup> (304.67)
LaPoE	10.71 <sup>c</sup> (115.33)	1.00 <sup>d</sup> (0.67)	2.45 <sup>dc</sup> (6.00)	11.03 <sup>c</sup> (122.00)
Mini Tiller	14.76 <sup>b</sup> (222.67)	2.68 <sup>bc</sup> (7.33)	5.07 <sup>b</sup> (25.33)	15.83 <sup>b</sup> (255.33)
Weedy check	21.55 <sup>a</sup> (466.00)	4.70 <sup>a</sup> (22.00)	8.25 <sup>a</sup> (68.67)	23.57 <sup>a</sup> (556.67)
F-test ( $\alpha=0.05$ )	S***	S***	S***	S***
SEM ( $\pm$ )	1.09	0.43	0.58	1.01
LSD ( $\alpha=0.05$ )	3.38	1.32	1.78	3.11
CV (%)	14.52	25.21	23.52	12.36
Weed management practices	Weed Density (no/m <sup>2</sup> )			
	60 DAS			
	SW	GW	BW	Total
Weed free	0.71 <sup>a</sup> (0.00)	0.71 <sup>c</sup> (0.00)	0.71 <sup>d</sup> (0.00)	0.71 <sup>c</sup> (0.00)
Manual weeding	20.49 <sup>ab</sup> (429.33)	3.72 <sup>abc</sup> (15.33)	5.47 <sup>bc</sup> (30.00)	21.56 <sup>abc</sup> (474.67)
AtPrE	16.63 <sup>bc</sup> (293.33)	3.28 <sup>bc</sup> (15.33)	6.43 <sup>bc</sup> (42.67)	18.17 <sup>c</sup> (351.33)
AtPoE	17.53 <sup>ab</sup> (311.33)	5.59 <sup>ab</sup> (31.33)	7.79 <sup>b</sup> (60.67)	20.02 <sup>bc</sup> (403.33)
LaPoE	12.74 <sup>c</sup> (170.00)	0.71 <sup>c</sup> (0.00)	3.63 <sup>cd</sup> (11.33)	13.17 <sup>d</sup> (181.33)
Mini Tiller	21.16 <sup>a</sup> (458.00)	6.39 <sup>ab</sup> (47.33)	4.99 <sup>bc</sup> (31.33)	23.07 <sup>ab</sup> (536.67)
Weedy check	21.77 <sup>a</sup> (476.00)	6.96 <sup>a</sup> (53.33)	10.84 <sup>a</sup> (118.00)	25.42 <sup>a</sup> (647.33)
F-test ( $\alpha=0.05$ )	S***	S**	S***	S***
SEM ( $\pm$ )	1.31	1.08	0.94	1.28
LSD ( $\alpha=0.05$ )	4.03	3.32	2.89	3.94
CV (%)	14.27	47.84	28.77	12.69

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; data are subjected to square root transformation  $\sqrt{x + 0.5}$  and data on parentheses are original values; DAS: days after sowing; S\*:significant at 5% probability level; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; SW: sedge weight; GW: grass weight; BW: broadleaf weight; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence; no/m<sup>2</sup>: number per square meter

### Weed Dry Weight

Weed dry weight was significantly influenced by weed control methods (Table 4). Weed-free plots consistently resulted in the lowest sedge, broadleaf, and grassy weed dry weight at all stages of observation. At 30 DAS, LaPoE exhibited the highest sedge and broadleaf weed dry weight, while the lowest was observed in AtPoE after weed-free treatment. Conversely, LaPoE resulted in the lowest dry weight across all three weed categories after weed-free at 45 and 60 DAS and was statistically similar to AtPrE. The lowest dry weight in all three weed categories was observed in weedy check plots at 45 and 60 DAS. A similar trend was observed in total weed dry weight. The highest dry weight was observed in the weedy check at all stages of observation, statistically similar to manual tillering at 30

and 60 DAS. Conversely, the lowest total weed dry weight was observed under AtPrE at 30 DAS and under LaPoE at 45 and 60 DAS after weed-free.

### Weed Control Efficiency, Weed Control Index, and Weed Index

Weed control methods significantly influenced weed control efficiency, weed control index, and weed index (Table 5). Weed-free consistently resulted in the highest weed control efficiency and weed control index at 45 and 60 DAS, in contrast to the lowest values observed under the weedy check. After the weed-free method, LaPoE resulted in higher weed control efficiency at 45 and 60 DAS. A similar trend was observed in the weed control index with LaPoE which was statistically similar to AtPrE at 60 DAS. The lowest value for



weed index was observed in weed-free check (0.00), which was statistically similar to LaPoE (16.7). In contrast, the highest value for weed index was reported in the weedy check (51.61), which was statistically similar to mini tiller (35.88) and manual weeding (33.84).

### Plant Height

There was no significant effect of the weed control method on plant height (Table 6). However, the weed-free plot exhibited maximum plant height at all stages of observation except at 60 DAS, when LaPoE resulted in maximum plant height. Conversely, a weedy check resulted in the minimum plant height, followed by a mini tiller at all stages of observation.

### Days to Tasselling and Silking

There was no significant influence of the weed control method on phenological attributes such as days to tasseling and silking (Table 7). However, the weed-free plot exhibited the shortest duration to reach 50% tasseling and 100% tasseling, followed by the LaPoE treatment. Conversely, the longest duration to achieve 50% tasseling and 100% tasseling was observed in the manual weeding treatment, followed by the weedy check treatment. A similar pattern was noted for the duration to achieve 50% silking and 100% silking, with the shortest duration in the weed-free treatment and the longest duration in the manual weeding treatment.

Table 4. Weed dry weight as influenced by different weed management practices at Dang, Nepal in 2023

Weed management practices	Weed Dry Weight (g/m <sup>2</sup> )			
	30 DAS			
	SW	GW	BW	Total
Weed free	0.71 <sup>c</sup> (0.00)	0.71(0.00)	0.71 <sup>d</sup> (0.00)	0.71 <sup>d</sup> (0.00)
Manual weeding	5.52 <sup>a</sup> (30.67)	3.63(13.00)	2.91 <sup>bc</sup> (8.67)	7.26 <sup>b</sup> (52.33)
AtPrE	3.16 <sup>b</sup> (10.67)	2.22(5.67)	1.92 <sup>cd</sup> (4.00)	4.44 <sup>c</sup> (20.33)
AtPoE	6.24 <sup>a</sup> (38.67)	2.94(8.33)	3.41 <sup>abc</sup> (11.67)	7.65 <sup>b</sup> (58.67)
LaPoE	6.79 <sup>a</sup> (47.67)	2.77(8.00)	4.95 <sup>a</sup> (25.33)	9.01 <sup>a</sup> (81.00)
Mini Tiller	6.42 <sup>a</sup> (42.33)	2.18(5.33)	4.38 <sup>ab</sup> (20.67)	8.29 <sup>ab</sup> (68.33)
Weedy check	6.36 <sup>a</sup> (40.33)	3.31(12.00)	4.50 <sup>ab</sup> (21.00)	8.56 <sup>ab</sup> (73.33)
F-test ( $\alpha=0.05$ )	S***	NS	S**	S***
SEM ( $\pm$ )	0.59	0.58	0.59	0.41
LSD ( $\alpha=0.05$ )	1.82	1.79	1.83	1.25
CV (%)	20.31	39.72	31.68	10.73
Weed management practices	Weed Dry Weight (g/m <sup>2</sup> )			
	45 DAS			
	SW	GW	BW	Total
Weed free	0.71 <sup>d</sup> (0.00)	0.71 <sup>d</sup> (0.00)	0.71 <sup>c</sup> (0.00)	0.71 <sup>c</sup> (0.00)
Manual weeding	4.62 <sup>bc</sup> (21.00)	2.8 <sup>a</sup> (7.33)	2.41 <sup>b</sup> (5.67)	5.87 <sup>bc</sup> (34.00)
AtPrE	4.77 <sup>bc</sup> (23.67)	1.59 <sup>bc</sup> (2.33)	2.53 <sup>b</sup> (6.00)	5.61 <sup>cd</sup> (32.00)
AtPoE	5.51 <sup>ab</sup> (30.00)	3.11 <sup>a</sup> (10.00)	3.15 <sup>b</sup> (10.00)	7.09 <sup>b</sup> (50.00)
LaPoE	3.74 <sup>c</sup> (14.00)	0.81 <sup>cd</sup> (0.17)	2.21 <sup>b</sup> (4.67)	4.31 <sup>d</sup> (18.83)
Mini Tiller	4.78 <sup>bc</sup> (22.33)	1.76 <sup>b</sup> (2.67)	3.42 <sup>b</sup> (11.33)	6.06 <sup>bc</sup> (36.33)
Weedy check	6.56 <sup>a</sup> (42.67)	2.61 <sup>a</sup> (6.33)	5.55 <sup>a</sup> (31.33)	8.97 <sup>a</sup> (80.33)
F-test ( $\alpha=0.05$ )	S***	S***	S***	S***
SEM ( $\pm$ )	0.40	0.27	0.39	0.43
LSD ( $\alpha=0.05$ )	1.24	0.80	1.19	1.33
CV (%)	15.88	24.71	23.47	13.52
Weed management practices	Weed Dry Weight (g/m <sup>2</sup> )			
	60 DAS			
	SW	GW	BW	Total
Weed free	0.71 <sup>c</sup> (0.00)	0.71 <sup>c</sup> (0.00)	0.71 <sup>d</sup> (0.00)	0.71 <sup>c</sup> (0.00)
Manual weeding	7.92 <sup>a</sup> (65.33)	2.50 <sup>abc</sup> (6.67)	3.95 <sup>bc</sup> (15.67)	9.19 <sup>bc</sup> (87.67)
AtPrE	5.43 <sup>b</sup> (31.67)	1.97 <sup>bc</sup> (4.33)	3.75 <sup>bc</sup> (13.67)	6.91 <sup>cd</sup> (49.67)
AtPoE	6.55 <sup>ab</sup> (44.00)	4.37 <sup>a</sup> (18.67)	3.36 <sup>bc</sup> (11.00)	8.51 <sup>bc</sup> (73.67)
LaPoE	4.19 <sup>b</sup> (17.33)	0.71 <sup>c</sup> (0.00)	2.16 <sup>cd</sup> (4.67)	4.68 <sup>d</sup> (22.00)
Mini Tiller	8.45 <sup>a</sup> (73.67)	3.78 <sup>ab</sup> (16.33)	5.26 <sup>b</sup> (29.00)	10.91 <sup>ab</sup> (119.00)
Weedy check	8.02 <sup>a</sup> (64.00)	3.97 <sup>ab</sup> (17.00)	8.11 <sup>a</sup> (67.00)	12.16 <sup>a</sup> (148.00)
F-test ( $\alpha=0.05$ )	S***	S**	S***	S***
SEM ( $\pm$ )	0.76	0.62	0.58	0.77
LSD ( $\alpha=0.05$ )	2.33	1.92	1.80	2.38
CV (%)	22.23	42.00	25.96	17.64

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; data are subjected to square root transformation  $\sqrt{(x + 0.5)}$  and data on parentheses are original values; DAS: days after sowing; NS: non-significant; S\*: significant at 5% probability level; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; SW: sedge weight; GW: grass weight; BW: broadleaf weight; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence; g/m<sup>2</sup>: grams per square meter

Table 5. Weed control efficiency, weed control index, and weed index as influenced by different weed management practices of spring maize at Dang, Nepal in 2023

Weed Management Practices	Weed Control Efficiency (WCE)		Weed Control Index (WCI)		Weed Index (WI)
	45 DAS	60 DAS	45 DAS	60 DAS	
Weed free	96.98 <sup>a</sup>	97.20 <sup>a</sup>	92.04 <sup>a</sup>	94.14 <sup>a</sup>	0.00 <sup>c</sup>
Manual weeding	32.58 <sup>c</sup>	15.58 <sup>cde</sup>	34.21 <sup>c</sup>	24.65 <sup>cd</sup>	33.84 <sup>ab</sup>
AtPrE	38.75 <sup>c</sup>	29.26 <sup>c</sup>	36.63 <sup>c</sup>	43.16 <sup>bc</sup>	26.99 <sup>b</sup>
AtPoE	25.78 <sup>c</sup>	21.33 <sup>cd</sup>	20.9 <sup>c</sup>	29.16 <sup>cd</sup>	30.2 <sup>ab</sup>
LaPoE	52.90 <sup>b</sup>	48.65 <sup>b</sup>	52.41 <sup>b</sup>	61.01 <sup>b</sup>	16.7 <sup>bc</sup>
Mini tiller	32.78 <sup>c</sup>	9.29 <sup>de</sup>	31.96 <sup>c</sup>	10.04 <sup>de</sup>	35.88 <sup>ab</sup>
Weedy Check	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	51.61 <sup>a</sup>
F-test ( $\alpha=0.05$ )	S***	S***	S***	S***	S**
SEM ( $\pm$ )	4.23	4.92	4.81	6.18	6.79
LSD ( $\alpha=0.05$ )	13.03	15.15	14.84	19.04	20.93
CV (%)	18.33	26.95	21.77	28.57	42.20

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; S\*:significant at 5% probability level; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence

Table 6. Plant height as influenced by different weed management practices of spring maize at Dang, Nepal in 2023

Weed Management Practices	Plant height (cm)			
	30 DAS	45 DAS	60 DAS	90 DAS
Weed free	23.77	45.55	98.55	195.79
Manual weeding	22.42	40.81	91.38	177.57
AtPrE	23.42	40.86	97.45	187.69
AtPoE	22.75	40.83	90.35	177.58
LaPoE	22.47	44.63	101.24	191.71
Mini tiller	21.54	40.09	84.18	176.38
Weedy Check	21.29	36.71	83.52	170.89
F-test ( $\alpha=0.05$ )	NS	NS	NS	NS
SEM ( $\pm$ )	1.56	2.66	8.14	6.38
LSD ( $\alpha=0.05$ )	4.81	8.19	25.08	19.67
CV (%)	11.99	11.14	15.26	6.06

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; DAS: days after sowing; NS: non-significant; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; NS: Non-significant; S\*:significant at 5% probability level; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence; cm: centimeters

Table 7. Days to tasselling and silking as influenced by different weed management practices of spring maize at Dang, Nepal in 2023

Weed Management Practices	Days to 50% Tasselling	Days to 100% Tasselling	Days to 50% Silking	Days to 100% Silking
	Weed free	76.67	79.33	80.67
Manual weeding	78.67	81	83	86
AtPrE	77.67	80.33	82	84
AtPoE	77.33	80	81.67	84
LaPoE	77	79.67	80.67	83.33
Mini tiller	77.67	80.67	82	84.33
Weedy Check	78	80.67	82.33	84.67
F-test ( $\alpha=0.05$ )	NS	NS	NS	NS
SEM ( $\pm$ )	0.89	0.96	0.84	0.94
LSD ( $\alpha=0.05$ )	2.75	2.95	2.59	2.90
CV (%)	1.99	2.06	1.78	1.93

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; NS: non-significant; SEM: standard error of the mean; LSD: Least significant difference; CV: coefficient of variation; S\*:significant at 5% probability level; NS: Non-significant; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence

### Yield Attributing Characters

There was no statistically significant influence of weed control methods on the number of cobs harvested per m<sup>2</sup>, cob girth, and number of rows/cobs. However, maximum values for these parameters were observed at weed-free plots, followed by AtPrE for cob harvested/ m<sup>2</sup>, and LaPoE

for cob girth and the number of rows/cobs. Conversely, the lowest number of cobs harvested per m<sup>2</sup> was observed at the mini-tiller plots. followed by AtPoE. The minimum values for cob girth and the number of rows per cob were observed at the weedy check plots, followed by the manual weeding plots.

Cob length, number of kernels per row, and thousand-grain weight were significantly influenced by weed control methods (Table 8). The highest cob length (15.83 cm) was observed at the weed-free plot, followed by LaPoE (14.79cm). In contrast, the lowest cob length (13.05 cm) was noted at the weedy check plot, followed by mini tiller (13.35 cm). The highest value for the number of kernels per row was noted at the weed-free plot (32.07) followed by LaPoE (29.92 cm). Conversely, the lowest value for the same parameter was recorded at the weedy check plot (23.47), followed by the mini tiller plot (26.13). The highest value for thousand-grain weight was found to be 248.33g and 234.33g at LaPoE and weed-free, respectively. Conversely, the lowest values for thousand-grain weight were found to be 171.67g and 187.33g at weedy check and mini-tiller plots, respectively.

#### Grain Yield, Harvest Index, Shelling Percentage, and Sterility Percentage

Weed control methods significantly influenced the yield parameters of maize except for the harvest index (Table 9). Weed-free plots and LaPoE plots achieved

maximum grain yield (6.14-5.12 tons/ha) and biological yield (15.18–13.32 tons/ha). Conversely, weedy check and mini tiller plots reported minimum grain yield (2.96-3.89 tons/ha) and biological yield (9.13-10.82 tons/ha).

The maximum shelling percentage was observed at weed-free (80.77%) and LaPoE (79.14%), whereas the minimum shelling percentage was recorded at weedy check (77.55%) and manual weeding (77.732%). In terms of sterility percentage, higher values were noted at the weedy check plot (20.69%) and mini tiller plot (18.33%), while lower values were observed at weed-free (14.27%) and LaPoE (16.52%).

#### Economic Analysis of Maize Cultivation

The economic parameters of maize cultivation significantly varied with weed control methods (Table 10). LaPoE plot had the highest B: C ratio and gross return, indicating a strong financial performance. Although the gross return is higher with weed-free management, the net return and B: C ratio were lower due to the increased costs associated with this method.

Table 8. Yield attributing characters as influenced by different weed management practices of spring maize at Dang, Nepal in 2023

Weed Management Practices	No. of cob harvested/m <sup>2</sup>	Cob Length	Cob Girth	No. of rows/cob	No. of kernels/row	Thousand Grain Weight
Weed free	7.92	15.83 <sup>a</sup>	14.09	14.53	32.07 <sup>a</sup>	234.33 <sup>ab</sup>
Manual weeding	7.73	13.99 <sup>bcd</sup>	13.48	13.87	27.73 <sup>b</sup>	200.67 <sup>cd</sup>
AtPrE	7.78	14.72 <sup>b</sup>	13.8	14.13	28.20 <sup>ab</sup>	208.00 <sup>bc</sup>
AtPoE	7.55	14.09 <sup>bc</sup>	13.59	14.13	26.67 <sup>bc</sup>	203.33 <sup>bcd</sup>
LaPoE	7.73	14.79 <sup>b</sup>	13.83	14.67	29.2 <sup>ab</sup>	248.33 <sup>a</sup>
Mini tiller	7.36	13.35 <sup>cd</sup>	13.63	13.87	26.13 <sup>bc</sup>	187.33 <sup>cd</sup>
Weedy Check	7.69	13.05 <sup>d</sup>	13.32	13.33	23.47 <sup>c</sup>	171.67 <sup>d</sup>
F-test ( $\alpha=0.05$ )	NS	S***	NS	NS	S*	S**
SEM ( $\pm$ )	0.27	0.30	0.33	0.51	1.28	9.92
LSD ( $\alpha=0.05$ )	0.83	0.94	1.03	1.59	3.95	30.58
CV (%)	6.10	3.71	4.24	6.34	8.04	8.28

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; NS: non-significant; SEM: standard error of the mean; LSD: Least significant difference; CV: coefficient of variation; S\*:significant at 5% probability level; NS: Non-significant; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence; m<sup>2</sup>: square meters

Table 9. Grain yield, harvest index, shelling percentage, and sterility percentage as influenced by different weed management practices of spring maize at Dang, Nepal in 2023

Weed Management Practices	Grain yield (t ha <sup>-1</sup> )	Biological yield (t ha <sup>-1</sup> )	Harvest index	Shelling percentage	Sterility percentage
Weed free	6.14 <sup>a</sup>	15.18 <sup>a</sup>	40.42	80.77	14.27 <sup>c</sup>
Manual weeding	4.05 <sup>bc</sup>	11.14 <sup>c</sup>	36.98	77.73	18.18 <sup>ab</sup>
AtPrE	4.50 <sup>b</sup>	12.11 <sup>bc</sup>	37.14	78.56	17.10 <sup>abc</sup>
AtPoE	4.27 <sup>bc</sup>	11.4 <sup>c</sup>	37.62	78.12	17.88 <sup>abc</sup>
LaPoE	5.12 <sup>ab</sup>	13.32 <sup>b</sup>	38.34	79.14	16.52 <sup>bc</sup>
Mini tiller	3.89 <sup>bc</sup>	10.82 <sup>cd</sup>	35.47	77.99	18.33 <sup>ab</sup>
Weedy Check	2.96 <sup>c</sup>	9.13 <sup>d</sup>	32.95	77.55	20.69 <sup>a</sup>
F-test ( $\alpha=0.05$ )	S**	S***	NS	NS	S*
SEM ( $\pm$ )	0.42	0.59	3.22	1.30	1.09
LSD ( $\alpha=0.05$ )	1.29	1.83	9.92	4.02	3.37
CV (%)	16.52	8.66	15.08	2.87	10.79

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; NS: non-significant; SEM: standard error of the mean; LSD: Least significant difference; CV: coefficient of variation; S\*:significant at 5% probability level; NS: Non-significant; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence; t ha<sup>-1</sup>: tons per hectare

Table 10. Economic analysis of the treatments

Weed Management Practices	Total Cost of Cultivation (NPR ha <sup>-1</sup> )	Gross Return (NPR ha <sup>-1</sup> )	Net Return (NPR ha <sup>-1</sup> )	Benefit to Cost Ratio (B: C)
Weed free	174855	316633.30 <sup>a</sup>	141778.33 <sup>ab</sup>	1.81 <sup>cd</sup>
Manual weeding	103665	212833.30 <sup>bc</sup>	109168.33 <sup>abc</sup>	2.06 <sup>bcd</sup>
AtPrE	94530	235933.30 <sup>b</sup>	141403.33 <sup>ab</sup>	2.50 <sup>ab</sup>
AtPoE	94530	223183.30 <sup>b</sup>	128653.33 <sup>ab</sup>	2.36 <sup>abc</sup>
LaPoE	97995	266783.30 <sup>ab</sup>	168788.33 <sup>a</sup>	2.72 <sup>a</sup>
Mini tiller	100515	205083.30 <sup>bc</sup>	104568.33 <sup>bc</sup>	2.04 <sup>bcd</sup>
Weedy Check	92955	159183.30 <sup>c</sup>	66228.33 <sup>c</sup>	1.71 <sup>d</sup>
F-test ( $\alpha=0.05$ )	-	S**	S*	S*
SEM ( $\pm$ )	-	18478.03	18478.03	0.19
LSD ( $\alpha=0.05$ )	-	56936.49	56936.49	0.58
CV (%)	-	13.83	26.03	15.05

Note: Means followed by the same letter(s) in a column are not significantly different by DMRT at 5% level of significance; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; S\*: significant at 5% probability level; S\*\*: significant at 1% probability level; S\*\*\*: significant at 0.1% probability level; AtPrE: Atrazine as pre-emergence; AtPoE: Atrazine as post-emergence; LaPoE: Tembotrione as post-emergence; NPR ha<sup>-1</sup>: Nepalese rupee per hectare; price of maize grain: NPR 45/kg

## Discussion

### *Morphological and Phenological Traits*

This study examines the effect of different weed control methods on the morphological and phenological traits of maize plants. There was no evidence of a statistically significant effect of weed control methods on plant height. Nonetheless, weed-free plots and LaPoE plots consistently resulted in higher plant heights at all stages of observation. Studies conducted by Safdar et al. (2011) and Shrestha et al. (2021) also reported no significant effect of weed control methods on plant height. The use of the same variety might be an explanation for this result. Plant height is primarily influenced by genotype rather than its environment; agronomic practices, such as weed control methods alone, do not impose a significant effect (Safdar et al., 2011). However, Alptekin et al. (2023) and Ikhajiagbe et al. (2023) reported a significant effect of weed management practices on the height of maize plants. These varying outcomes underscore the intricate relationship between weed control methods and plant height, which can vary based on specific plant genotypes, environmental conditions, and weed control strategies.

Similarly, there was no significant effect of weed control methods on the days to tasseling and silking. Nonetheless, there was a pronounced trend toward fewer days to tasseling and silking with weed-free plots and Tembotrione plots. Our results align with the studies conducted by Acharya et al. (2022) and Bhutto (2019) who reported no significant effect of weed control methods on the days to tasseling and silking. While individual weed control methods did not exert a statistically significant impact on plant height or phenological traits, the sustained effectiveness observed in both the weed-free and Tembotrione treatments, resulting in enhanced plant height and accelerated phenological growth, underscores the efficacy of weed-free environments.

### *Weed Parameters*

This study examines the effect of weed control methods on weed dynamics and their efficiency in controlling weed populations in the field. It is crucial to control weed populations to maximize maize yields because weeds compete vigorously for essential resources like light, water, and nutrients. Weed control methods significantly

affected both weed density and weed dry weight. The weed-free plot consistently showed lower weed density and dry weight at all observation stages compared to the higher values seen in the weedy check. After the weed-free plot, AtPrE resulted in lower values at 30 DAS, while LaPoE exhibited lower values at 45 and 60 DAS. This result aligns with the findings of Arivukkarasu et al. (2020) and Sharma et al. (2018) who documented the lowest weed density and total dry weight with the combined application of tembotrione and atrazine. Kaur et al. (2019) also reported the lowest total weed density and weed biomass in weed-free plots, followed by the application of tembotrione at 120 g/ha along with surfactant relative to other control methods, including atrazine.

The study also found that weed control methods had a significant impact on weed control efficiency, weed control index, and weed index. Weed-free plots consistently demonstrated the highest levels of weed control efficiency and weed control index, whereas weedy check plots exhibited the lowest values. After the weed-free plot, LaPoE exhibited higher efficiency in controlling the weed, which was statistically similar to AtPrE at 60 DAS. This result aligns with the findings of Arivukkarasu et al. (2020) and Sharma et al. (2018) who reported higher weed control efficiency and weed control index with the combined application of tembotrione and atrazine. Kaur et al. (2019) also reported the lowest weed index in the application of tembotrione at 120 g/ha along with surfactant. Frequent removal of weeds under weed-free plots is the most probable reason for its superiority in weed management. LaPoE's superior effectiveness in controlling weed after weed-free plot can be attributed to its post-emergence application, allowing for targeted and timely weed control (Kaur et al., 2019, Sharma et al., 2023). LaPoE also combines two herbicides, tembotrione and atrazine, with different modes of action, which leads to better weed control because of the synergistic effect achieved by targeting multiple biochemical pathways in the weed (Bagale, 2023). In summary, tembotrione showed higher efficiency in weed control and management after weed-free plot which can be attributed to its post-emergence application and its dual herbicide composition targeting multiple biochemical pathways in weeds.

### **Yield and Yield Parameters**

Weeds significantly impact yield-attributing parameters in maize plants by competing for essential resources like light, water, and nutrients, leading to reduced growth and yield (Alptekin et al., 2023). Effective weed management practices are crucial for reducing weed competition, minimizing detrimental effects, and optimizing resource utilization by maize plants, ultimately leading to increased crop yield and productivity. The study found a statistically significant impact of weed control methods on cob length, number of kernels per row, and thousand-grain weight, with no significant variation observed in cob girth, number of rows per cob, and cob harvested per m<sup>2</sup>.

Compared to other treatments, weed-free plots, and those treated with LaPoE showed superior cob length, number of kernels per row, and thousand-grain weight. Previous studies by Kaur et al. (2019), Sharma et al. (2018) and Shrestha et al. (2018) also reported increased cob length and thousand-grain weight in response to weed-free plots followed by the application of tembotrione and atrazine, aligning with our findings. Rana et al. (2018) observed that various weed control methods significantly impact the number of kernels per row, with the highest kernel count per row recorded in plots treated with tembotrione combined with a surfactant at a dosage of 150 g/ha. The improved yield-attributing characteristics in weed-free plots and LaPoE-treated plots can be attributed to the higher efficiency of weed management practices in these treatments. This is supported by the lower weed density, reduced dry weight, and higher weed control index and efficiency observed under these treatments, which minimized weed-crop competition and enhanced nutrient utilization by maize plants. Improvements in certain yield attributing parameters, including cob length, number of kernels per row, and thousand-grain weight, were observed because of this effect. This aligns with the findings of Alptekin et al. (2023), Ramesha et al. (2019) and Sarma & Gautam (2006) who reported better yield attributing parameters in those treatments that efficiently limited weed growth.

Weed control methods significantly influenced the yield parameters of maize, except for the harvest index (Table 9). The weed-free plot resulted in the highest grain yield and biological yield, which was statistically similar to LaPoE. Conversely, weedy check resulted in minimum grain yield and biological yield, which were statistically at par with mini tiller treatment. Studies conducted by Kaur et al. (2019), Rana et al. (2018), Sharma et al. (2023) and Sharma et al. (2018) reported higher grain yields under the application of tembotrione and atrazine, which is consistent with our finding. Arivukkarasu et al. (2020) also observed higher biological yield in response to tembotrione and atrazine and a minimum under the weedy check. Improved grain yield and biological yield in response to weed-free and LaPoE can be attributed to limited weed growth and improved yield attributing parameters under them. Studies by Alptekin et al. (2023), D et al. (2001), and Safdar et al. (2011) demonstrated a negative correlation between grain yield and dry biomass accumulation of weed. Similarly, Tahir et al. (2009) reported that all weed control methods led to an increase in grain yield compared to the weedy check, with improvements ranging from 29% to 78%, attributed to a higher number of grains per cob and

a greater thousand-grain weight compared to the weedy check. These are in line with our findings that showed higher grain yield under weed-free and LaPoE that exhibited significantly lower weed dry weight and higher number of kernels per row and thousand-grain weight. Likewise, diminished grain yield and biological yield observed in the weedy check treatment can be ascribed to intense competition for vital resources such as nutrients, water, and space encountered by maize plants from weeds throughout their growth cycle, including critical stages.

In conclusion, effective weed management practices, as demonstrated by weed-free plots and LaPoE treatment, not only minimize weed competition but also optimize resource utilization by maize plants, leading to improved yield attributing parameters and ultimately higher crop yield and productivity.

### **Economic Analysis**

LaPoE resulted in the highest net return and benefit-cost ratio, which was statistically on par with the application of AtPrE. Shrestha et al. (2018) also reported the highest net return and benefit-cost ratio in tembotrione and atrazine-treated plots. Weed free plot had the highest gross return but a lower net return and benefit: cost ratio. This can be attributed to the higher cost of cultivation associated with routine weed clearance. Weedy check had the lowest cost of production but the lowest returns and benefit-cost ratio because of the yield reduction caused by weed competition. In line with this, AtPrE treatment in our study emerged as the next best option in terms of returns and benefit-to-cost ratio. Shrestha et al. (2021) also reported similar findings, in which atrazine as PE was stated as the second-best alternative for the control of weeds.

### **Conclusion**

In conclusion, this study underscores the significance of weed control methods in shaping various aspects of maize cultivation, from morphological and phenological traits to yield parameters and economic viability. Fifteen different weed species belonging to seven different families were observed in the experimental field. Major weed species identified in the field include *Cyperus rotundus*, *Cyperus esculentus*, and *Argemone Mexicana*. Weed-free plots and LaPoE stood out and showed superior performance in terms of both better weed management and higher yield, highlighting the importance of effective weed management strategies in maximizing maize production. Similarly, the results of economic analysis highlight the importance of considering both yield outcomes and the cost-effectiveness of weed control methods. LaPoE treatment resulted in superior economic returns, highlighting the economic viability of this strategy.

### **Limitations of the Study**

Though this study provides detailed insights regarding the effects of different weed management techniques on spring maize at Dang, Nepal, it has some limitations. Our study was conducted in a single location in a single season due to which its applicability and generalization are somehow narrowed. That's why future research must be performed in multiple locations within multiple seasons to finally achieve the insights of the study on a large scale.

Despite the fact this study is focused on the economics of production for each treatment so that an economical treatment can be recommended to the farmers, the variation in the price of grain as well as the herbicides applied, has imposed some limitations in the study.

### Recommendations

Thus, our study suggests that farmers can effectively manage weeds, increase their crop productivity, and achieve a higher economic return with the application of tembotrione 42% (SC + surfactant) plus Atrazine 50% WP i.e. LaPoE. Future research in maize weed management could explore alternative methods, such as integrated pest management or biological control agents to minimize environmental impact while maintaining crop productivity. Long-term studies on the sustainability of weed control methods, including their effects on soil health and weed seedbanks, could provide valuable insights for developing more sustainable weed management practices. Furthermore, mechanistic studies exploring the molecular and physiological interactions between weeds and maize plants could offer valuable insights for the development of targeted weed management strategies. Overall, continued exploration in these areas could advance weed management practices and significantly contribute to the development of more sustainable maize production systems.

### Declarations

#### Author Contribution Statement

*Manjul Devkota:* Performed and designed the experiments experimental setup, data collection, analyzed and interpreted the data, literature review, wrote the paper, proofreading, and revision.

*Rijwan Sai:* Data collection, analyzed and interpreted the data, methodology design, literature review, wrote the paper, proofreading and revision.

*Aavash Shrestha:* Data analysis, softwares, results and discussion, proofreading and review

*Shiva Chaudhary:* Data collection, proofreading, review, revision, wrote the paper

*Prajwal Koirala:* Data interpretation, generated figures, software, proofreading and review

*Mohan Mahato:* Supervision, conceptualization, methodology, review and proofreading

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#### Conflict of Interest

The authors declare no conflict of interest

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## Mean Performance of Field pea (*Pisum sativum* L) Advanced Genotypes for Yield and Yield-related Traits in Arsi Zone, Ethiopia

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### ABSTRACT

Pea (*Pisum sativum* L.) is the second most important cool-season food legume in Ethiopia after faba bean, both in terms of production area and annual yield. The study comprises 13 advanced field pea genotypes that were evaluated in a randomized complete block design with four replications across four different environments during two consecutive main cropping seasons (2020–2021). The primary objectives were to identify a field pea genotype with a reliable, high grain yield that could be subsequently released as a new cultivar for farmers in specific areas of Ethiopia. A combined analysis of field pea grain yield revealed a significant difference ( $P < 0.01$ ) between genotypes and environments, suggesting that the genetic composition of the genotypes varied and the environments were distinct. The average grain yield ranged from 1614 kg/ha to 2412 kg/ha, with a mean of 2032.69 kg/ha. Genotype G13 had the highest average grain yield (2412 kg/ha) compared to the standard check varieties Bilalo (2190 kg/ha) and Bursa (2100 kg/ha), indicating its potential for developing adaptable varieties suited to specific environments. This outcome may aid breeders in choosing the most appropriate cultivars for particular environments, resulting in higher field pea yields and productivity. Nonetheless, the research also indicates that to create broadly adaptable and climate-resilient varieties, it is crucial to carry out trials in various locations and across multiple years.

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## Introduction

Field pea (*Pisum sativum* L.) is a diploid ( $2n=14$ ) annual plant that undergoes self-pollination (Gurmu et al., 2022) and (Kindie et al., 2019). It is considered one of the most significant cool season pulse crops and is highly considered for its high protein content. This crop is extensively cultivated in cooler temperate zones as well as in the highlands of tropical regions across the globe. While it can thrive in various soil types, ranging from light sandy loams to heavy clays, it is unable to withstand saline and waterlogged soil conditions. The crop covers 219,927.59 ha, ranking fourth in pulse crops production in Ethiopia after faba bean, haricot bean, and chickpea. The average yield productivity is 1.71 t/ha (Kebede et al., 2023).

The yield of field pea production varies from location to location due to the differences in the environment and the lack of suitable field pea varieties that can adapt to a broader range of environments (Yihunie and Gessese, 2018). Farmers produce various types of varieties that differ from one place to another. They have also developed local cultivars and somewhat improved ones that show

instability in their performance. (Tadele et al., 2017). Therefore, it is necessary to test selected materials across various sites and years to ensure that new varieties exhibit consistent performance in different environments. The traditional approach of dividing the total variation into different components such as genotype, environment, and GEI provides limited insight into the specific response patterns of individuals (Tadele et al., 2017). Additionally, using stability measures can help to identify varieties that are adaptable to a wide range of conditions or specific environments, which is important for large-scale production.

The term stability in agriculture is commonly used to evaluate how consistent certain characteristics are over time or in different locations. Specifically, it refers to the capacity of agricultural outputs, such as yield, to remain consistent over a long period or in diverse spatial settings. In recent years, the importance of yield stability analysis has increased due to the effect of climate variability on crop yield stability.

Selection progress can be limited by significant G x E interaction for quantitative traits like grain yield due to climate change and ecological variations. According to predictions, by 2050 we can expect significant impacts such as higher temperatures, more frequent droughts caused by increased evaporation and changes in rainfall patterns, and increased levels of CO<sub>2</sub> due to greenhouse and agricultural gas emissions (Andrews and Hodge, 2010). It is predicted that current levels of agricultural production and field crop productivity in different ecologies and regions will be significantly impacted. It is predicted that people in developing countries who rely mostly on vegetarian diets will face a major challenge in accessing legumes by the year 2050. To address this issue, it is important to develop an efficient agronomic production system, introduce widely adopted resistant and high-yielding cultivars, and utilize diverse genetic sources to improve new varieties that can thrive in different ecologies and regions.

## Materials and Methods

### Descriptions of Experimental Locations

The field experiment was carried out in four Kulumsa Agricultural Research sub-stations, each situated in the South-Eastern region of Ethiopia and characterized by a unique set of climatic and soil conditions that influence the growth of cool-season legumes, including field peas. The experiment, which spanned over two cropping years, from 2020 to 2021, was meticulously designed to investigate the impact of various environmental factors on the yield of these legumes.

### Design and Experimental Materials

Thirteen advanced field pea genotypes were tested against two standard checks (Bursa and Bilalo) in the main growing season of 2020-2021, under rain-fed conditions, across four environments (Table 2). A randomized complete block design was utilized across all testing sites, with four replications. The plots were all the same size, measuring 4 meters in length and 0.8 meters in width. The distances between the replications, plots, rows, and plants were carefully maintained at 1.5 meters, 1 meter, and 20 centimeters between rows, respectively. The recommended amount of bulked fertilizer (NPS) used was 100 kg/ha, and hand weeding was carried out three to four weeks after emergence.

### Data Collection

Data on yield and yield component traits were collected from each experimental unit on both plot and plant basis. Phenology data, such as the date of flowering and maturity, were recorded when each plot attained 50% of the plant starting to flower and 90% of the pod changed to black or physiological maturity, respectively in plot bases. Thousand seed weight was determined by measuring the weight of 1000 randomly selected seeds from each plot. Plant height (in cm), number of pods per plant, and number of seeds per pod were measured by randomly selecting five sample plants from each plot. The average measurements of these five sample plants were then used for analysis. The grain yields in grams of each plot were measured on clean, dried seed, and the measured grain yield was adjusted to 10% grain moisture content and converted to kg ha<sup>-1</sup> for analysis.

The severity of *Ascochyta* blight and powdery mildew diseases was measured using a scale ranging from 1 to 9. A score of 1 meant there were no visible disease symptoms, indicating immunity. A score of 3 indicated a few disease symptoms, meaning resistance. A score of 5 meant there were some coalesced lesions with some defoliation, indicating moderate resistance. A score of 7 meant large coalesced sporulation lesions, and 50% defoliation of some dead plants, indicating susceptibility. A score of 9 meant extensive, heavy sporulation, stem girdling, blackening, and death of more than 80% of plants, indicating heavy susceptibility (Tadele et al., 2021).

### Data Analyses

The collected data were analyzed using the R software version 4.3.2 with Metan and other appropriate packages. The analysis of variance was performed on data from both individuals and combined environments. The significance of the main effect and interactions related to the measured parameters were determined using a randomized complete block design (RCBD) for each environment and across four environments. A combined ANOVA was conducted using combined ANOVA a model in which the genotype was fixed, environment and interactions were random. The year factor was considered a separate environment.

The model:  $R_{ijk} = M + G_i + L_j + r(L) + (G \times L)_{ij} + e_{ijk}$ . Where M = grand mean; G = genotype; L = location; and (G×L)<sub>ij</sub> = genotype by location interaction; r(L) = replication within the location; and e<sub>ijk</sub> = random error. A statistical analysis was performed using Duncan's Multiple Range Test (DMRT) to determine the significant differences between the means of various genotypes and environments.

Table 1 Description of the experiment area soil physical with chemical property and climate data

Location	Altitude (m)	Latitude	Longitude	%Clay	%Silt	% Sand	Soil type	Soil pH	%TN	%OC	Av.P (ppm)
Assasa	2372	07007'04.3"	039011'50.4"	39.375	23.125	37.5	Clay-loam	6.25	0.12	2.26	34.22
Bekoji	2811	07032'32.7"	039015'18.6"	61.875	30.625	7.5	Clay-soil	5.48	0.19	2.44	4.72
climate data											
	Year- 2020			Year-2021							
	Temperature			Temperature							
Location	MAX	MIN	Rainfall	MAX	MIN	Representing Agro-ecology					
Assasa	19.3	8.1	539.6	21.9	5.7	Terminal drought prone					
Bekoji	20.7	4.8	876.1	22	4.6	Highland and high rainfall					

Note: Av.ppm = available phosphors in parts per million, %OC = Organic carbon in

Table 2. Descriptions of Plant Materials

Code	Genotypes	Sources
G1	EH 012014-4	Hybrid
G2	EH0120206-6	Hybrid
G3	Bursa	Released variety
G4	EH012026-3	Hybrid
G5	EH012002-1	Hybrid
G6	EH012020-5	Hybrid
G7	EH012002-2	Hybrid
V2	EH012024-5	Hybrid
G9	Bilalo	Released variety
G10	EH012026-6	Hybrid
G11	EH012024-3	Hybrid
G12	EH012003-3	Hybrid
G13	EH012026-7	Hybrid

Note: G1, G2, G3...G13 are Genotype one up to Genotype thirteen and V1 and V2 variety one, variety two

Table 3. Combined Analysis of Variance (ANOVA) for nine traits of 13 Field pea Genotypes evaluated at 4 environments (2020-2021)

TR	Source of variations					
	Genotype (df = 12)			Location (df =3)		
	S	M	P	S	M	P
DF	987.36	82.28	0.000	7086.63	2362.21	0.000
DM	118.77	9.90	0.001	41861.17	13953.72	0.000
PH	4941.55	411.80	0.482	281554.77	93851.59	0.000
PPL	119.73	9.98	0.298	2484.36	828.12	0.000
SPP	7.14	0.60	0.260	238.96	79.65	0.000
TSW	26885.62	2240.47	0.000	95532.99	31844.33	0.000
AB	7.48	0.62	0.076	38.36	12.79	0.000
PM	22.89	1.91	0.000	42.48	14.16	0.000
YLD	14936615.7	1244718.0	0.000	414548046.0	138182682.0	0.000

TR	Source of variations							
	Genotype × Loc (df= 36)			Rep(Loc) df= 12			Error (df=144)	
	S	M	P	S	M	P	S	M
DF	459.18	12.76	0.000	58.52	4.88	0.000	218.23	1.52
DM	193.08	5.36	0.038	108.04	9.00	0.004	498.46	3.46
PH	14198.61	394.41	0.591	5162.23	430.19	0.441	61242.77	425.30
PPL	290.51	8.07	0.540	178.56	14.88	0.058	1210.44	8.41
SPP	10.74	0.30	0.951	11.38	0.95	0.030	68.96	0.48
TSW	12891.13	358.09	0.361	7662.45	638.54	0.035	47633.72	330.79
AB	12.33	0.34	0.594	18.98	1.58	0.000	53.27	0.37
PM	25.14	0.70	0.008	39.19	3.27	0.000	55.81	0.39
YLD	11739331.2	326092.5	0.263	2178966.0	181580.5	0.8	40345726.0	280178.7

Note: TR: Traits; S: Sum sq.; M: Mean sq.; P: P<0.05.; DF = Number of days to 50% flower, DM = Number of days to Mature, PH = Plant height, PPL = Number of pods per plant, SPP = Number of seeds per pod, TSW = 1000 seeds weight, AB = Ascochyta blight, PM = Powdery mildew and YLD = grain yield

## Results and Discussion

### Analysis of Variance for Yield and Other Component Traits

The study found significant differences ( $P < 0.01$ ) in grain yield and seed weight among different genotypes and locations. However, the genotype by environmental interactions showed non-significance ( $P < 0.05$ ) for both traits (Table 3). The results of this study align with the findings of (Argaye et al., 2023), (Kindle et al., 2019) and (Girma Mangistu et al., 2011) significant variations were reported. indicating that the yield component traits, namely Number of pods per plant (PPL), number of seeds per pod (SPP), and plant height (PLH) did not exhibit significant differences ( $P < 0.05$ ) between genotypes or genotype by environment interactions. These results suggest that the thirteen genotypes studied demonstrated uniform

responses for yield-related traits across varied locations, as evidenced by the data presented in Table 3.

The study found significant differences ( $P < 0.01$ ) among genotypes, location, and genotype by location interaction in the phenological traits of days to 50% flower and days to mature. The results suggest that the genotypes were genetically diverse and that location had varying effects on these traits. Moreover, the significant effects of genotype by location interaction indicate that different genotypes exhibited different flowering and maturing patterns in different locations (Table 3).

The study investigated the reactions of different genotypes to the major field pea disease; Ascochyta blight (AB) and Powdery mildew (PM).

Table 4. Combined Mean performance of 13 field pea genotypes for the nine traits conducted in 2020 - 2021 main cropping seasons

Genotype	Entry	DF	DM	PH	PPP	SPP	TSW	Yldkg/ha	AB	PM
EH 012014-4	G1	74 <sup>ef</sup>	140 <sup>b-d</sup>	172 <sup>abc</sup>	10 <sup>abc</sup>	4 <sup>bc</sup>	223 <sup>a</sup>	2269 <sup>abc</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH0120206-6	G2	77 <sup>b</sup>	140 <sup>b-d</sup>	165 <sup>abc</sup>	10 <sup>abc</sup>	4 <sup>abc</sup>	218 <sup>a</sup>	2360 <sup>ab</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
Bursa	G3	71 <sup>g</sup>	140 <sup>b-d</sup>	164 <sup>bc</sup>	11 <sup>a</sup>	4 <sup>abc</sup>	179 <sup>d</sup>	2100 <sup>a-d</sup>	5 <sup>a</sup>	5 <sup>a</sup>
EH012026-3	G4	74 <sup>ef</sup>	139 <sup>de</sup>	171 <sup>abc</sup>	10 <sup>abc</sup>	4 <sup>c</sup>	216 <sup>a</sup>	1642 <sup>f</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012002-1	G5	74 <sup>de</sup>	140 <sup>b-d</sup>	178 <sup>ab</sup>	10 <sup>abc</sup>	4 <sup>abc</sup>	202 <sup>bc</sup>	1820 <sup>def</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012020-5	G6	76 <sup>bc</sup>	140 <sup>abc</sup>	169 <sup>abc</sup>	10 <sup>abc</sup>	4 <sup>c</sup>	195 <sup>c</sup>	2038 <sup>b-e</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012002-2	G7	76 <sup>c</sup>	140 <sup>bcd</sup>	174 <sup>abc</sup>	9 <sup>bc</sup>	4 <sup>abc</sup>	203 <sup>bc</sup>	1614 <sup>f</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012024-5	G8	78 <sup>a</sup>	141 <sup>a</sup>	178 <sup>a</sup>	9 <sup>abc</sup>	4 <sup>abc</sup>	201 <sup>bc</sup>	1945 <sup>c-f</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
Bilalo	G9	76 <sup>b</sup>	141 <sup>ab</sup>	169 <sup>abc</sup>	8 <sup>c</sup>	5 <sup>a</sup>	213 <sup>ab</sup>	2190 <sup>abc</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012026-6	G10	75 <sup>cd</sup>	139 <sup>cde</sup>	165 <sup>abc</sup>	10 <sup>abc</sup>	4 <sup>abc</sup>	203 <sup>bc</sup>	1722 <sup>ef</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012024-3	G11	77 <sup>b</sup>	140 <sup>abc</sup>	163 <sup>c</sup>	9 <sup>abc</sup>	4 <sup>abc</sup>	201 <sup>bc</sup>	1946 <sup>c-f</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012003-3	G12	70 <sup>g</sup>	140 <sup>abc</sup>	169 <sup>abc</sup>	11 <sup>a</sup>	5 <sup>ab</sup>	194 <sup>c</sup>	2369 <sup>ab</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
EH012026-7	G13	73 <sup>f</sup>	138 <sup>e</sup>	174 <sup>abc</sup>	11 <sup>ab</sup>	4 <sup>abc</sup>	195 <sup>c</sup>	2412 <sup>a</sup>	5 <sup>a</sup>	4 <sup>ab</sup>
	Mean	74.70	139.80	170.02	9.85	4.31	203.41	2032.69	5	3.95
	CV	1.65	1.33	12.13	29.43	16.07	8.94	26.04	12.04	15.75
	LSD	0.86	1.30	14.41	2.03	0.48	12.71	369.90	0.43	0.44

Table 5. Within Environment mean grain yield (kg/ha<sup>-1</sup>) performance of 13 field pea genotypes conducted across two years (2020 - 2021)

Genotypes	Code	E 1	E2	E3	E4
EH 012014-4	G-1	3232.03 <sup>ab</sup>	4166.87 <sup>ab</sup>	1098.04 <sup>abc</sup>	577.52 <sup>ab</sup>
EH0120206-6	G-2	3336.34 <sup>a</sup>	4520.91 <sup>ab</sup>	941.4 <sup>bcde</sup>	640.45 <sup>ab</sup>
Bursa	G-3	2933.59 <sup>abcd</sup>	4321.32 <sup>ab</sup>	722.79 <sup>def</sup>	421.48 <sup>b</sup>
EH012026-3	G-4	1901.55 <sup>cd</sup>	3753.67 <sup>abc</sup>	662.93 <sup>ef</sup>	251.16 <sup>b</sup>
EH012002-1	G-5	2188.86 <sup>bcd</sup>	3985.36 <sup>ab</sup>	899.61 <sup>bcdef</sup>	270.44 <sup>b</sup>
EH012020-5	G-6	2742.5 <sup>abcd</sup>	4148.09 <sup>ab</sup>	642.85 <sup>f</sup>	616.72 <sup>ab</sup>
EH012002-2	G-7	2094.21 <sup>cd</sup>	3091.85 <sup>c</sup>	834.96 <sup>cdef</sup>	435.08 <sup>b</sup>
EH012024-5	G-8	2803.62 <sup>abcd</sup>	3698.85 <sup>bc</sup>	858.95 <sup>cdef</sup>	481.94 <sup>b</sup>
Bilalo	G-9	2511.41 <sup>abcd</sup>	4559.91 <sup>a</sup>	1174.69 <sup>ab</sup>	513.05 <sup>b</sup>
EH012026-6	G-10	1883.24 <sup>d</sup>	3682.54 <sup>bc</sup>	735.99 <sup>def</sup>	585.68 <sup>ab</sup>
EH012024-3	G-11	2870.85 <sup>abcd</sup>	3686.74 <sup>bc</sup>	682.3 <sup>ef</sup>	543.5 <sup>b</sup>
EH012003-3	G-12	3023.27 <sup>abc</sup>	4437.73 <sup>ab</sup>	1006.93 <sup>abcd</sup>	1006.48 <sup>a</sup>
EH012026-7	G-13	3541.65 <sup>a</sup>	4209.53 <sup>ab</sup>	1240.79 <sup>a</sup>	656.45 <sup>ab</sup>
	Mean	2697.2	4020.3	884.8	538.5
	CV	29.2	14.2	23.2	28.8
	LSD	1133.1	845	295.5	454.8

Note: G1, G2, up to G13 Number of Genotypes, E1, E2, E3, E4 Number of Environments

The study found that there was no significant difference (P<0.05) between genotypes and the genotype by location interaction for Aschochyta blight disease. However, Powdery mildew disease showed a highly significant difference among genotypes, location, and genotype by location interaction. Interestingly, none of the tested genotypes showed different reactions to Aschochyta blight disease. However, the study revealed that different genotypes showed distinct reactions to the powdery mildew disease at different locations and genotype-by-location interaction. These findings are important for plant breeders and growers as they help them select the best genotypes that can resist the powdery mildew disease, which is a major threat to field pea production in Ethiopia.

**Mean Performance of Grain Yield and Other Related Traits**

The mean grain yield of 13 different field pea genotypes was analyzed, and it ranged from 1614kg/ha<sup>-1</sup> to 2412kg/ha<sup>-1</sup>, with a grand mean of 2032.69 kg/ha<sup>-1</sup>. Five of the genotypes produced a higher yield than the control varieties, Bursa and Bilalo, as shown in (Table 4). The

findings of similar advanced field pea research have been documented by previous researchers (Haile and Tesfaye, 2024; Zeleke et al., 2024; Tolessa et al., 2013). Genotype G13 had the highest mean grain yield amongst all the genotypes, relative to the two standard check varieties. This suggests that there is potential for better genetic gain from the field pea breeding program. This finding is consistent with the study conducted by (Yang et al. 2023) and (Vasileva et al., 2021) which also reported significant differences among the advanced field pea genotypes compared to the control varieties.

There were genetic differences among the field pea genotypes for days to 50% flowering. The genotype G12 had the shortest time to reach 50% flowering compared to other genotypes. On the other hand, the genotype G8 was delayed in flowering. The findings of (Tadele et al., 2021) support this result, demonstrating significant variations in both yield and yield component traits in faba bean mean performance trials. When it comes to days to maturity, genotype G13 matured the earliest, while G8 was the latest. G13 was a relatively early-maturing genotype that had a

high number of pods per plant and produced the highest yield. The mean disease score for AB was found to be consistently similar across all tested genotypes, while PM showed no significant differences, except for the control variety Bursa i.e. susceptible. These results strongly suggest that breeding strategies for field peas must prioritize disease resistance to ensure better crop yield and robustness.

The experiment evaluated the performance of 13 different genotypes of field peas in different environments. The data collected from the study showed that the highest mean yield was obtained from genotype G-13, followed by G-2, at Environment E1. In contrast, the lowest yield was recorded from G-10, indicating significant differences in the performance of the genotypes under varying environmental conditions. Further analysis of the data showed that at Environment E2, the highest mean yield was obtained from genotype G-9, with an average grain yield of 4209.53 kg/ha. Considering the environmental mean performance, it was observed that the highest yield was obtained from Environment E-2, while the lowest yield was recorded from Environment E4.

Upon closer examination, it was found that the low performance of the genotypes at Environment E4 could be attributed to the terminal moisture deficit during the podding and seed seating stages, as well as the irregular rainfall distribution during that particular growing season. These factors limited the growth and development of the plants, leading to reduced yield. In conclusion, the study highlights the importance of selecting the appropriate genotype for different environments to maximize yield. It also underscores the need for adequate moisture management to ensure optimal growth and development of plants, especially during critical growth stages.

Several genotypes exhibited consistent incremental or decremented performance across the target population environments. Notably, genotype G-13 demonstrated superiority in environment E1 and exhibited a consistent increase in yield across all other environments, as indicated in (Table 5). Although there were differences in yield across environments, G-13 did not experience any rank changes or cross-over interactions, but only magnitude changes. This result is supported by the previous researchers According to (Atlin et al., 2011) trial-to-trial variation within the target population environment exists at all times, even in highly uniform regions due to annual variations in rainfall, disease pressure, planting dates, and so on.

Consequently, both genotypes G-13 and G-2 exhibited superior mean grain yield performance, as illustrated in Table 3, and were relatively stable across all environments. These results suggest that the novel hybrid field pea genotypes exhibit superior performance for grain yield when compared to the two control varieties, Bursa and Bilalo. Therefore, identifying and releasing better-adapted field pea varieties for commercial production in our target environment is highly probable.

## Conclusion

This study has provided valuable insights into the performance of different field pea genotypes across various environments, which can guide breeders in selecting

cultivars for specific target environments. While there were differences in the performance of genotypes compared to standard check varieties, the study found that genotype by environment interaction did not significantly impact the grain yield performance of the 13 field pea genotypes tested. This means that breeders can confidently select cultivars based on their mean yield and adaptation without considering the influence of genotype by environment interactions.

However, the study also suggests that to develop widely adaptable varieties, it is important to conduct experiments in multiple locations and over several years. By doing so, breeders can identify genotypes that perform consistently well across different environments using appropriate stability analysis tools. Overall, the study found that the hybrid field pea genotypes G-13 and G12 performed exceptionally well in terms of mean grain yield across different environments and these could be strong candidates for developing environment-specific adaptable varieties. This information can assist breeders in selecting the most suitable cultivars for specific environments, ultimately leading to increased field pea yields and productivity.

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## Sowing Dates Effects and Varieties Comparison and Their Interaction on Yield and Yield Components of Wheat (*Triticum aestivum* L.)

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### ABSTRACT

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Wheat (*Triticum aestivum* L.) growth is directly affected by sowing dates. However, the yield of wheat in Afghanistan is significantly lower than the global standard. Several factors including fertilizers, sowing dates, seeds and cultivation methods, contribute to this low yield. The objective of this research was to address this critical issue by comparison of the effects of different sowing dates on wheat yield and its components under Kabul climatic conditions. The same experiment was conducted at two sites in 2020 using a randomized complete block design (RCBD) with three replications and twelve treatments. The varieties used were Darolaman-07 (V<sub>1</sub>), Mazar- 99 (V<sub>2</sub>), and Chunta-1 (V<sub>3</sub>) as factor one, and sowing dates of November 10<sup>th</sup> (S<sub>1</sub>), November 18<sup>th</sup> (S<sub>2</sub>), November 26<sup>th</sup> (S<sub>3</sub>), and December 4<sup>th</sup> (S<sub>4</sub>) as factor two. Growth and yield parameters measured and analyzed included plant height, number of leaves plant<sup>-1</sup>, total number of tillers plant<sup>-1</sup>, leaf area index (LAI), leaf nitrogen content (N), spike length, number of spikelets spike<sup>-1</sup>, number of grains spike<sup>-1</sup>, grain weight spike<sup>-1</sup>, thousand- grain-weight, days to maturity, number of spikes plant<sup>-1</sup>, biological yield, grain yield, straw yield, and harvest index. Sowing dates had significant effects on some wheat growth and yield parameters. Sowing on November 10<sup>th</sup>, 2020, resulted in the highest plant height and leaves number per plant compared to later sowing dates. There was a significant interaction between sowing date and variety at (P<0.01) in both growth and yield parameters. ANOVA analysis highlighted significant differences among wheat varieties in spike length, grain weight spike<sup>-1</sup>, thousand grain-weight, and harvest index, with notable variations observed among different varieties. Based on the results, the longest duration to maturity and the highest grain yield were observed on sowing date of November 10, 2020.

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### Introduction

Wheat (*Triticum sativum* L.) is one of the economically important crops in Afghanistan, which is used as bread, cake, cokes, and consumed with so many different varieties of food. Wheat is cultivated as rain-fed and irrigated in Afghanistan but due to the dry climate and low precipitation; most farmers cultivate it in irrigated land. Wheat is planted in broadcast and line system followed by technologies and animal power. Wheat is a staple crop in Afghanistan, it estimated the 70 percent of the production in Afghanistan (FAO, 2003). Wheat cultivation area was estimated at 2.55 million hectares produced 4.26 million tons in 2023 (Habibi, 2023).

The annual need for wheat in Afghanistan estimated at about seven –million-ton to achieve self-sufficiency (Waziri et al., 2013; Sharma and Nang, 2018). A multipronged strategy utilizing more widespread use of improved seed and fertilizer, date of cultivation, better crop management and an effective research and extension system is needed to achieve self-sufficiency in wheat production (Waziri et al., 2013).

The wheat possess very low yield in Afghanistan than the world countries like the USA (3539), China (5409) and India-(3093) kg-ha<sup>-1</sup>, respectively (FAOSTAT, 2016). Causes of low yield in Afghanistan are the use of poor-quality seed, improper sowing time, pest infestation,

scarcity of irrigation water and less use of fertilizers, especially phosphorus. Increasing temperatures are also adding to this reduction (Aslam, 2016). Therefore, this study seeks to fill this critical gap by evaluating the effects of different sowing dates on wheat yield. Uncertainty of wheat cultivation date is one of the problems of farmers in Afghanistan. Low yield of wheat per unit area regarding lack of awareness and information is the biggest problem of wheat production.

Wheat growth is directly affected by sowing time and climate, which act as critical components of crop production. The optimum time of wheat sowing results in a maximum number of secondary branches (tillers), the height of the plant, races development, thousand grains weight, grain yield, and total dry matter that gradually decrease with late sowing time (Baloch et al., 2010).

Cultivation date and climatic conditions can adversely affect wheat yield by effecting dry matter, leaf index, 1000-grain weight as well as harvest index. All the components involving in crop production are very sensitive to climatic factors and all the processes in crop production from germination to harvesting will have a direct effect on any alteration in weather change (Asseng et al., 2017).

Crop sown on November\*15, had better produce than early and late planting time (Subhan et al., 2004). Therefore, it is very valuable to describe the ideal date of sowing, due to the variation in weather parameters of environments (Kristo et al., 2007). Different stages of wheat improvement are impacted by genetic and climate factors (Sial et al., 2005). The crop sown at an ideal time is the best practice to enable the crops growing under prevailing climatic conditions (El-Gizawy, 2009). Higher yield can be achieved by given long maturation period to crop due to early sowing than late sowing. Late sowing of crop increases chance to face the hot weather conditions during critical stages such as grain formation stage that is important parameter in low yield of wheat (Turk and Tawaha, 2002). Climatic conditions of November favor the seed germination, dense crop stand and ultimately reduce the weed infestation (Khan and Salim, 1986).

The observed data shows that it is economically unfavorable to increase the seed rate at an optimum sowing time, but it is favorable in respect to delay sowing to overcome the yield losses increasing the seed rate (Pan et al., 1994). High temperature (up to 30°C) can limit the crop growth period as it limits days to booting, an-thesis, physiological maturity and ultimately total maturation period (Ishibashi et al., 2008). The high temperature then its normal range at grain filling stage can reduce the crop yield (Maças et al., 2000). The net-grain weight decreased due to rise in temperature which ultimately reduces the total grain yield (Humphreys et al., 2001). Poor stand establishment also reduced the total grain yield (Farooq et al., 2008).

The produce and output components of wheat are influenced by various sowing times among varieties (Tahir et al., 2009).

Best time of sowing shows the satisfactory length of growing season, but the growth rate may be slower than the crop sown at the optimum time. Cultivation time is one of the important factors among of all agricultural practices (El-Gizawy, 2009).

Both soil and climate are required for germination and emergence rate as well as for the development of the crop. The suitable sowing time and temperature contribute plant protection (Pavlova et al., 2014).

Wheat is becoming susceptible to climate variability all over the world (Hossain and Da Silva, 2012). They concluded their results from two sowing dates and three cultivars for growth, phenology and yield.

Sowing date meaningfully influenced on all the parameters of crop except harvest index [HI (Lak et al., 2013)].

Late sowing reduces LAI during the early season. The different thermal times will cause differences in LAI development among sowing dates (Milford et al., 1985).

Conducted a field experiment having three wheat varieties (Chakwal-50, Wafaq-2001, and an advance line NR-268) and four sowing dates from 20<sup>th</sup> of October, 5<sup>th</sup> November, 20<sup>th</sup> of November and 5<sup>th</sup> of December in winter season of 2008-2009 in a (RCBD) using factorial arrangements (Nawaz et al., 2013).

Tools for crop growth modeling are used to extend agriculture research by supporting in planning and decision making in agriculture (Fakhar and Khaid, 2023).

The plant growth modeling is used to assess CGR, in the developmental stages and the allocation of dry mass into various growing components of the plant. Due to the vibrant nature of all these processes, they are affected by cultivar-specific and environmental factors. The description of all these vital processes provides an approach to figure out the cultivars differences and a system of simulation and grain yield forecasting by means of crop models.

Most of the climate associated factors like daily sunshine hours, maximum-minimum temperature ranges, rainfall, the water holding capacity of the soil and various factors which are related to crops (White et al., 2011).

For 60% of the cultivated area, there was a variation of less than one month between observed and simulated sowing times (Waha et al., 2012). Five months variation was observed in a tropical climate where climatic conditions stay favorable all over the year.

The overall objectives of this study were to study the effects of sowing dates on growth and yield of three varieties of wheat, to determine the yield potential in these wheat varieties under various sowing dates and to find out the suitable dates for planting of wheat in Kabul climatic conditions. Research Hypothesis was as following:

H<sub>0</sub>: Different dates of sowing don't have effects on yield and yield component of wheat.

H<sub>1</sub>: Different dates of sowing have significant effects on yield and yield components of wheat.

## Materials and Methods

Two cultivars of wheat were sown in a factorial design (Factorial RCBD) with three replications and 12 treatments, which three wheat cultivars such as Darolaman-07, Mazar- 99 and Chunta-1, and for dates of cultivation such as 10<sup>th</sup>, 18<sup>th</sup>, 26<sup>th</sup>, of November and 4<sup>th</sup> of December.

This experiment was conducted into two sites at the Experimental Research Farm of Agriculture Faculty of Kabul University in 2020.

Seedbed was prepared by one application of rotator followed by two plowing and planking. The distances were 20 cm row to row and 120 kg ha<sup>-1</sup> seed rate. Fertilizers (N: DAP) were applied at 250: 125 kg.ha<sup>-1</sup>. respectively. The whole recommended amount of DAP and half of the nitrogen was applied at the time of sowing. The remaining nitrogen was applied into two equal splits at the time of 1<sup>st</sup> and 2<sup>nd</sup> irrigations.

Vernier Clipper was used to measure the seed length and width. A chlorophyll meter (SPAD-502) made in Japan was used to indicate the nitrogen level in the leaves.

Growth parameters such as plant height, number of leaves plant<sup>-1</sup>, number of tillers plant<sup>-1</sup>, number of spike plant<sup>-1</sup>, spike length, leaf area index, and leaf nitrogen content as well as and yield parameters including number of grain spike<sup>-1</sup>, number of spikelet spike<sup>-1</sup>, grain weight spike<sup>-1</sup>, thousand grain-weight, grain yield, straw yield and biological yield were measured by sampling method randomly. Data were analyzed using Excel and Statistical Tool for Agricultural Research (STAR), version 2.0.1, January 2014. Least Significant Difference (LSD) has also been used for comparing the treatments.

## Results and Discussion

### Growth Parameters

Based on statistical analysis, sowing dates and varieties interaction significantly affected plant height, number of leaf plant<sup>-1</sup>, spike length, leaf area index and leaf nitrogen content at (P<0.01) (Table 1). The interaction of different sowing dates and varieties had no significant effect at (P<0.01) on the number of tillers per plant as well as the number of spikes per plant (Table 1). Treatment nine (V<sub>3</sub>S<sub>1</sub>) exhibited the tallest plants average (88.8 cm) and treatment four (V<sub>1</sub>S<sub>4</sub>) the shortest plant height average (66.6 cm). The largest number of leaves per plant was obtained (5.3) from treatment five (V<sub>2</sub>S<sub>1</sub>) and the lowest number (4.7) from treatment eleven. Treatment nine (V<sub>3</sub>S<sub>1</sub>) performed the largest number of tillers (6.8) and the lowest (5.2) in treatment four (V<sub>1</sub>S<sub>4</sub>). The largest number of spikes per plant was (6.8) in treatment five (V<sub>2</sub>S<sub>1</sub>) and (5.2) lowest in treatment four (V<sub>1</sub>S<sub>4</sub>). The longest leaf length (9.4 cm) observed in treatment two (V<sub>1</sub>S<sub>2</sub>). Treatment four (V<sub>1</sub>S<sub>4</sub>) performed the widest leaf area index and treatment ten (V<sub>3</sub>S<sub>2</sub>) the narrowest leaf area index (24). Highest amount of nitrogen (48.8 mg) was obtained from treatment four (V<sub>1</sub>S<sub>4</sub>) and the

lowest amount (41.5 mg) from treatment nine (V<sub>3</sub>S<sub>1</sub>) (Table 1). As a conclusion the different sowing dates in a combination with three promise varieties of wheat exhibited differences among treatments. We can say that the time of cultivation has an important role in wheat productivity. Time of cultivation of different agro-climatic zones is different for wheat cultivation in Afghanistan.

V<sub>1</sub>: Darolaman-07, V<sub>2</sub>: Mazar-99, V<sub>3</sub>: Chunta-1, S<sub>1</sub>: Sowing on 10-11-2020; S<sub>2</sub>: Sowing on 18-11-2020; S<sub>3</sub>: Sowing on 2, 6-11-2020; S<sub>4</sub>: Sowing on 05-12-2020.

Plant height is one of the important parameters in the determination of yield. Plant height was influenced by interaction of sowing dates and varieties at (P<0.01). Treatment nine (V<sub>3</sub> S<sub>1</sub>) exhibited the tallest plant (88.8 cm) and treatment four (V<sub>1</sub>S<sub>4</sub>) the shortest plant height (66.6 cm) (Table 1).

### Yield Parameters

Sowing dates performed significant effects (P<0.01) on grains spike<sup>-1</sup>, spikelet spike<sup>-1</sup>, grain weight spike<sup>-1</sup>, grain yield and different sowing dates had not significant influence (P<0.01) on 1000 grains weight, straw and biological produces (Table 2). The largest grains spike<sup>-1</sup> was obtained from treatment number four (V<sub>2</sub>S<sub>3</sub>), which is (46 grains per spike) and the lowest in treatment nine (38 grains per spike).

Treatment nine exhibited the largest number of spikelet spike<sup>-1</sup> (16.9) and the less in treatment four (14.2). Treatment two performed the heaviest grains per spike (12.6 g) and the lightest in treatment twelve (9.6 g). The heaviest 1000-grain weight was observed in treatment nine (29.5 g) and the lightest in treatment eight (24.6 g). The largest grain yield (3416.6 kg.ha<sup>-1</sup>) obtained from treatment five, and treatment four performed the lesser yield (1648.3 kg.ha<sup>-1</sup>). Treatment Four had the largest straw yield kg ha<sup>-1</sup> and treatment five the lesser (10083.3); it means a negative correlation between yield and straw is existed. The largest biological yield (20722.2.kg ha<sup>-1</sup>) was obtained from treatment four and the lowest in treatment twelve (6555.5). Based on the above discussion the sowing dates highly affected on yield parameters such as number of grains spike<sup>-1</sup>, number of spikelet spike<sup>-1</sup>, grains weight spike<sup>-1</sup>(g) thousand grain-weight (g) and grain yield (kg.ha<sup>-1</sup>) but no significant influence on straw yield (kg.ha<sup>-1</sup>) and biological yield (kg.ha<sup>-1</sup>) at (P<0.01) (Table 2).

Table 1. Cultivation dates and varieties influence on wheat growth parameters.

Treatments	Plant height (cm)	Number of leaves plant <sup>-1</sup>	Number of tillers plant <sup>-1</sup>	Number of spikes plant <sup>-1</sup>	Spike length (cm)	Leaf area index	Leaf nitrogen content (mg)
V1S1	83.5 <sup>a</sup>	5.2 <sup>ab</sup>	6.7 <sup>a</sup>	6.7 <sup>a</sup>	8.9 <sup>abc</sup>	27.3 <sup>ab</sup>	46.4 <sup>ab</sup>
V1S2	74.5 <sup>ab</sup>	5.0 <sup>ab</sup>	5.9 <sup>a</sup>	6.4 <sup>a</sup>	9.4 <sup>a</sup>	44.2 <sup>ab</sup>	46.2 <sup>ab</sup>
V1S3	74.7 <sup>ab</sup>	5.0 <sup>ab</sup>	5.6 <sup>a</sup>	6.0 <sup>a</sup>	9.3 <sup>ab</sup>	45.8 <sup>ab</sup>	46.0 <sup>ab</sup>
V1S4	61.7 <sup>b</sup>	5.0 <sup>ab</sup>	5.2 <sup>a</sup>	5.2 <sup>a</sup>	9.0 <sup>abc</sup>	49.9 <sup>a</sup>	48.8 <sup>a</sup>
V2S1	85.6 <sup>a</sup>	5.3 <sup>a</sup>	6.6 <sup>a</sup>	6.8 <sup>a</sup>	8.5 <sup>abc</sup>	28.1 <sup>ab</sup>	45.4 <sup>ab</sup>
V2S2	76.5 <sup>ab</sup>	5.1 <sup>ab</sup>	6.0 <sup>a</sup>	6.0 <sup>a</sup>	9.1 <sup>abc</sup>	30.4 <sup>ab</sup>	43.1 <sup>ab</sup>
V2S3	79.0 <sup>ab</sup>	4.9 <sup>ab</sup>	6.6 <sup>a</sup>	6.7 <sup>a</sup>	9.3 <sup>ab</sup>	26.4 <sup>ab</sup>	42.9 <sup>ab</sup>
V2S4	75.7 <sup>ab</sup>	5.2 <sup>ab</sup>	6.4 <sup>a</sup>	6.6 <sup>a</sup>	8.4 <sup>bc</sup>	25.9 <sup>ab</sup>	46.2 <sup>ab</sup>
V3S1	88.7 <sup>a</sup>	5.0 <sup>ab</sup>	6.8 <sup>a</sup>	6.7 <sup>a</sup>	8.1 <sup>c</sup>	30.9 <sup>ab</sup>	41.5 <sup>b</sup>
V3S2	76.7 <sup>ab</sup>	5.0 <sup>ab</sup>	6.4 <sup>a</sup>	6.2 <sup>a</sup>	8.4 <sup>bc</sup>	24.1 <sup>b</sup>	42.2 <sup>ab</sup>
V3S3	79.1 <sup>ab</sup>	4.7 <sup>b</sup>	6.1 <sup>a</sup>	7.0 <sup>a</sup>	8.7 <sup>abc</sup>	25.0 <sup>ab</sup>	44.5 <sup>ab</sup>
V3S4	74.5 <sup>ab</sup>	5.0 <sup>ab</sup>	6.4 <sup>a</sup>	6.4 <sup>a</sup>	8.4 <sup>bc</sup>	26.3 <sup>ab</sup>	43.9 <sup>ab</sup>
SD	7.8	0.2	1.1	1.4	0.6	13.9	4.0
CV (%)	10.7	3.9	18.4	21.6	6.7	41.2	8.9
ANOVA	**	**	ns	ns	**	**	**

Table 2. Interaction between cultivation and variety on yield and yield components of wheat.

Treatments	NGPS	NSPS	GWPS (g)	1000GW (g)	GY (kg.ha <sup>-1</sup> )	SY (kg.ha <sup>-1</sup> )	BY (kg.ha <sup>-1</sup> )
V1-S1	40.3 <sup>ab</sup>	16.5 <sup>ab</sup>	12.1 <sup>ab</sup>	27.1 <sup>ab</sup>	2995.5 <sup>ab</sup>	7948.8 <sup>a</sup>	10944.4 <sup>a</sup>
V1-S2	45.0 <sup>ab</sup>	16.4 <sup>ab</sup>	12.6 <sup>a</sup>	28.1 <sup>ab</sup>	1940.0 <sup>c</sup>	5393.3 <sup>a</sup>	7333.3 <sup>a</sup>
V1-S3	44.1 <sup>ab</sup>	16.3 <sup>abc</sup>	12.1 <sup>ab</sup>	27.6 <sup>ab</sup>	2170.0 <sup>bc</sup>	5441.1 <sup>a</sup>	7611.1 <sup>a</sup>
V1-S4	39.5 <sup>ab</sup>	14.2 <sup>c</sup>	11.1 <sup>ab</sup>	28.6 <sup>ab</sup>	1648.3 <sup>c</sup>	19073.8 <sup>a</sup>	20722.2 <sup>a</sup>
V2-S1	40.1 <sup>ab</sup>	16.7 <sup>abc</sup>	11.0 <sup>ab</sup>	27.8 <sup>ab</sup>	3416.6 <sup>a</sup>	10083.3 <sup>a</sup>	13500.0 <sup>a</sup>
V2-S2	41.8 <sup>ab</sup>	16.2 <sup>abc</sup>	10.1 <sup>ab</sup>	27.1 <sup>ab</sup>	2155.0 <sup>bc</sup>	5733.8 <sup>a</sup>	7888.8 <sup>a</sup>
V2-S3	46.0 <sup>a</sup>	16.9 <sup>a</sup>	11.8 <sup>ab</sup>	25.5 <sup>ab</sup>	2205.5 <sup>bc</sup>	6961.1 <sup>a</sup>	9166.6 <sup>a</sup>
V2-S4	38.3 <sup>ab</sup>	14.5 <sup>bc</sup>	9.8 <sup>b</sup>	24.6 <sup>b</sup>	1803.8 <sup>c</sup>	5307.2 <sup>a</sup>	7111.1 <sup>a</sup>
V3-S1	38.0 <sup>b</sup>	16.6 <sup>ab</sup>	10.8 <sup>ab</sup>	29.5 <sup>a</sup>	3393.8 <sup>a</sup>	7717.2 <sup>a</sup>	11111.1 <sup>a</sup>
V3-S2	41.3 <sup>ab</sup>	15.4 <sup>abc</sup>	11.1 <sup>ab</sup>	27.5 <sup>ab</sup>	2179.4 <sup>bc</sup>	5487.2 <sup>a</sup>	7666.6 <sup>a</sup>
V3-S3	44.1 <sup>ab</sup>	16.7 <sup>a</sup>	12.3 <sup>ab</sup>	27.5 <sup>ab</sup>	2141.1 <sup>c</sup>	5136.6 <sup>a</sup>	7277.7 <sup>a</sup>
V3-S4	38.8 <sup>ab</sup>	15.1 <sup>abc</sup>	9.6 <sup>b</sup>	27.0 <sup>ab</sup>	2435.5 <sup>abc</sup>	4120.0 <sup>a</sup>	6555.5 <sup>a</sup>
SD	4.9	1.3	1.4	3.0	587.6	4571.7	4759.5
CV (%)	12.0	8.2	13.3	11.0	24.6	41.4	35.2
ANOVA	**	**	**	ns	**	ns	ns

Number of grains per spike (NGPS), Number of spikelet per spike (NSPS), Grain weight per spike (GWPS), 1000 grain-weight (GW), Grain yield (GY), Straw yield (SY), Biological yield (BY) and Harvest index (HI), Standard error (SD), Coefficient of variation (CV), Variety (V) and Sowing (S), V<sub>1</sub>: Darolaman-07, V<sub>2</sub>: Mazar-99, V<sub>3</sub>: Chunta-1, S<sub>1</sub>: Sowing on 10-11-2020; S<sub>2</sub>: Sowing on 18-11-2020; S<sub>3</sub>: Sowing on 26-11-2020; S<sub>4</sub>: Sowing on 05-12-2020.

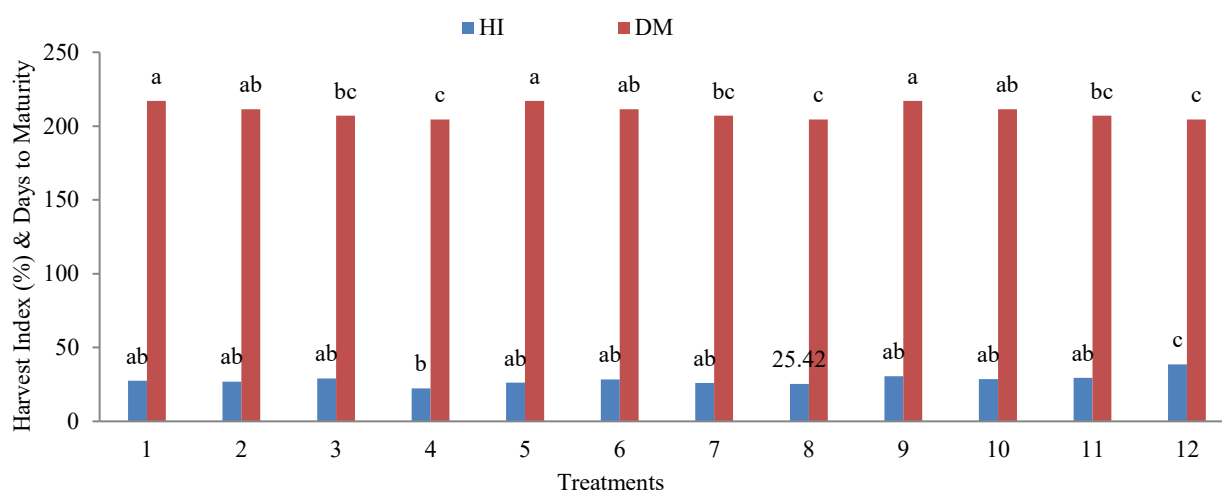


Figure 1. Combining effects of sowing dates and varieties on harvest index and days to maturity. Figure 1?

### Harvest Index and Days to Maturity

Based on statistical analysis of harvest index data, significant differences from interaction of cultivation times and varieties among treatments on harvest index were not observed at ( $P < 0.01$ ) but sowing dates and varieties combination exhibited variation among treatments at ( $P < 0.01$ ) (Figure 1).

### Correlation among the different quantitative traits of wheat

Correlations among traits were analyzed based on the mean value of each parameter. A significant positive correlation was among most of the traits (Figure 2). The highest significant correlation was obtained between biological yield and straw yield ( $r = 1$ ). The plant height at 90 days after sowing exhibited strong significant positive correlation with plant height at 60 days after sowing ( $r = 0.88$ ) and plant height at 120 days after sowing ( $r = 0.75$ ). A significant linear correlation was observed among the number of spikes per plant, the total number of tillers per plant ( $r = 0.72$ ), the plant height at 30 days after sowing and

the number of days to maturity ( $r = 0.72$ ), the number of grains per spike and spike length ( $r = 0.71$ ). Plant height at 60 days after sowing and the number of spikelet per spike ( $r = 0.69$ ) and the number of spikelet per spike and the number of grains per spike ( $r = 0.67$ ) performed a positive correlation.

A moderate correlation was observed in spike length among treatments and spikelet spike<sup>-1</sup> ( $r = 0.59$ ), grain yield, plant height at 15 days after sowing ( $r = 0.56$ ), grain yield, plant height at 30 days after sowing ( $r = 0.51$ ), grain yield and plant height at 60 days after sowing ( $r = 0.46$ ), grain yield and plant height at 90 days after sowing ( $r = 0.62$ ), grain yield and plant height at 120 days after sowing ( $r = 0.47$ ). Moderate positive correlation was also observed among the leaves plant<sup>-1</sup> at 15 days and plant height at 15 days ( $r = 0.51$ ), plant tallness at 60 days ( $r = 0.52$ ), plant height at 90 days ( $r = 0.51$ ). Negative significant correlations were observed between straw yields and harvest index ( $r = -0.50$ ), biological yield and harvest index ( $r = -0.46$ ), and leaf nitrogen content and plant height at 90 days after sowing ( $r = -0.44$ ) (Figure 2).





weight per spike, while sowing on November 10<sup>th</sup>, 2020 (Sowing 1) led to the shortest spike length. Sowing on December 4<sup>th</sup>, 2020 (Sowing 4) produced the lowest number of spikelets per spike, grains per spike, and grain weight per spike. Regarding days to maturity, sowing 1 required the longest duration (217 days), while sowing 4 had the shortest (204 days). Additionally, the highest yield was achieved with V<sub>2</sub>S<sub>1</sub> (3416.6 kg.ha<sup>-1</sup>), while the lowest yield (1962.59 kg.ha<sup>-1</sup>) was obtained from V<sub>1</sub>S<sub>4</sub>. Significant positive correlations were observed among most evaluated growth and yield parameters of wheat, highlighting key associations that could inform breeding and management strategies aimed at maximizing wheat productivity and quality in Afghanistan. Overall, these findings underscore the critical impact of sowing time on wheat production, emphasizing the need for careful consideration in agricultural planning to optimize crop productivity in Afghanistan. Given that wheat is a staple crop in Afghanistan and the country faces a wheat deficiency, with imports from neighboring countries annually, further research on factors that can increase wheat productivity in Afghanistan is recommended.

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## Potential of Biochar-Based Fertilizers for Increasing the Productivity of Okra in Gajuri, Dhading

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### ABSTRACT

Sustainable agricultural production depends on increasing crop productivity while preserving soil health and reducing environmental risk. The purpose of this study was to evaluate the potential of biochar (10 t ha<sup>-1</sup>) based organic and inorganic fertilizer for increasing okra productivity through a field experiment conducted in Gajuri, Dhading. A 130 m<sup>2</sup> area was divided into six treatment groups, each with four replications, using a Randomized Complete Block Design. The following were the treatments: i) inorganic fertilizer (RF); ii) biochar plus inorganic fertilizer (BF); iii) biochar plus vermicompost (BVC); iv) biochar plus poultry manure (BPM); v) biochar (BC); vi) control; neither biochar nor fertilizer (CK). The recommended rates of urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) were applied to the mineral NPK fertilizers in RF and BF. The rate whereby organic fertilizers were applied was 200 kg N ha<sup>-1</sup>. Plots treated with biochar and various fertilizer groups were compared in terms of growth and yield efficiency. The BVC treatment was found to exhibit poorer growth performance in terms of plant height, number of leaves, primary branches, and nodes compared to the combination of biochar and poultry manure. Fruit output rose by 170% over CK (7.13 mt ha<sup>-1</sup>) and by 53.26% over RF (12.58 mt ha<sup>-1</sup>) after BPM treatment (19.28 mt ha<sup>-1</sup>). While BF and RF did not significantly differ in terms of growth characteristics, BF outproduced RF by 29% and CK by 126.79% in terms of pod yield. BF and BPM offered greater financial rewards than alternative treatments.

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## Introduction

Okra (*Abelmoscus esculentus* L.) is one of the known and utilized species of the family Malvaceae. It is a chief summer vegetable widely grown from the tropics to subtropics and warmer parts of the temperate zone for its nutritious, tender pods. Okra can be grown in a wide variety of soil types, ranging from sandy loam to clay soil, and at temperatures ranging from 20°C to 30°C (Dada & Fayinminnu, 2010). It contains carbohydrates, proteins, and vitamin C in large quantities, as well as essential and non-essential amino acids that are comparable to those of soybeans (Adeboye and Oputa, 1996).

Okra is grown on 9584 ha of land nationwide, with production and productivity of 112260 metric tons and 11.95 metric tons ha<sup>-1</sup>, respectively. In the Dhading district, the total area under okra production is 168 ha, while production and productivity are 1399 metric tons and 8.39 metric tons per ha, respectively (MoALD 2022). In the last three years, the productivity of okra in Nepal has been on the rise while it has been decreasing in Dhading (MoALD

2022). Poor soil fertility (low pH, organic matter (OM), cation exchange capacity (CEC), base saturation, and available nutrients), inadequate use of chemical and organic fertilizers, and a lack of effective crop management practices by farmers are the main causes of decreased productivity (Acharya et al., 2022). Similarly, the degradation of land and poor soil health (acidic soil pH, low OM%, low N, and low K) is one of the major problems faced by farmers in Dhading with lowland and upland areas (Kharal et al., 2018). So, poor soil health and inadequate use of chemical and organic fertilizers have resulted in a decreased yield of okra in Dhading.

Research and application of various fertilizer and plant management techniques are being done in order to improve production and address the responsible causes. The positive effects of the simultaneous utilization of organic and inorganic fertilizer on okra yield and quality have been substantiated by several studies (Adekiya et al., 2020a; Akhter et al., 2019; Bharthy et al., 2017; Miah et al., 2020).

The highest quality and quantity of okra were recorded by Sachan et al. (2017) when a combination of NPK, Farm Yard Manure (FYM), poultry, and vermicompost was used concurrently. Other management practices include the use of mulching material to reduce weed density, conserve moisture, and increase yield (Ojiako et al., 2019; Puri et al., 2022; Shamim et al., 2018). One such nutrient management strategy could be the addition of biochar to the soil. Biochar is a carbon-rich, fine-grained product produced by the pyrolysis of organic matter such as wood, tree branches, agricultural waste, cow manure (Pandit et al., 2021). The addition of biochar significantly improves soil health and crop yield in poor to medium fertile soil and in acidic soil (El-Naggar et al., 2019; Van Zwieten et al., 2010).

Multiple studies have demonstrated the efficacy of biochar as a soil amendment, capable of enhancing degraded soil, improving soil fertility, and even boosting crop growth and yield (Murtaza et al., 2021). As the accumulation of soil organic C is vital for improving soil properties, biochar with a high carbon content (60–80%) can have a positive impact on soil properties (Blanco-Canqui, 2017). Blanco-Canqui (2017) reported that the application of biochar decreases soil bulk density by 3 to 31%, increases porosity by 14 to 64%, increases the availability of water by 4 to 130%, increases the stability of wet aggregates by 3 to 226%, and has variable effects on dry aggregate stability. Many studies have demonstrated the ability of biochar to increase the pH of the soil, particularly in acidic soil (Martinsen et al., 2015; Song et al., 2018; Van Zwieten et al., 2010). Liang et al (2014) suggested that the presence of a high amount of alkaline earth metals such as Mg<sup>+2</sup>, Ca<sup>2+</sup>, etc. in biochar is responsible for the increase in soil pH following biochar application. Therefore, biochar can act as a liming agent, neutralizing soil acidity and improving the availability of essential soil nutrients (El-Naggar et al., 2019). Many studies have provided strong evidence that the addition of biochar influences the NPK content of the soil. The incorporation of biochar increases N availability by reducing leaching, increasing retention (absorption of ammonium and nitrate ions), and reducing volatilization of ammonia (Major et al., 2012; Rondon et al., 2007). Joseph et al. (2021) found that the addition of biochar increased available P by a factor of 4.6, and an increase in plant yield was significantly high in low-nutrient P-sorbing acidic soil.

An increase in crop yield with the combined application of biochar and fertilizer has been demonstrated by numerous researchers. Acharya et al. (2022) investigated the effect of biochar-based fertilizers on soil fertility and productivity of okra and observed that biochar-blended goat manure resulted in the highest fruit yield, 88% more than the control. Rondon et al. (2007) reported a positive impact of biochar addition on Biological Nitrogen Fixation (BNF) in common beans and their pod yield. In a greenhouse experiment conducted by Uzoma et al. (2011) on the effect of cow manure biochar on maize productivity in sandy soil, they observed maximum yield and Water Use Efficiency (WUE) with cow manure biochar applied at a rate of 15 t/ha, with 150% more yield and 139% more WUE than the control.

These studies illustrate the positive impacts of adding biochar to soil health, productivity, and crop yield in addition to other organic or chemical fertilizers. The agronomic and financial consequences of co-applying biochar with organic or chemical fertilizers to okra plants in Nepal, however, have not been extensively studied. One such study, conducted in a controlled greenhouse, examines the effects of biochar on okra plants. The purpose of this study was to examine the effects of biochar-based inorganic and organic fertilizers on the vegetative and reproductive performance of okra, as well as to evaluate the impacts of biochar-based fertilizer on okra yield under open field conditions. This experiment's specific goal was to determine whether blended fertilizer containing biochar could increase okra production. Additionally, to evaluate the impact of adding biochar on okra productivity and identify the most efficient and economical method of raising okra yields.

**Materials and Methodology**

**Location, Site Weather and Soil Properties**

A field experiment was conducted in Gajuri-1, Dhading, Nepal, which falls under the PMAMP vegetable zone, Dhading from March to July 2023. The location of the experiment is situated at an altitude of 347 meters above sea level. Figure 1 and Figure 2 present information on the climatic conditions during the study's duration and the location of the experimental area, respectively. Table 1 illustrates the characteristic of soil from the research field.

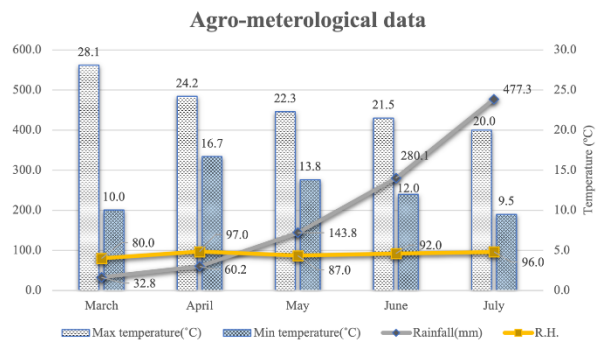


Figure 1. Agrometeorological information during the experimental period at Gajuri-1, Nepal from March 2023 to July 2023

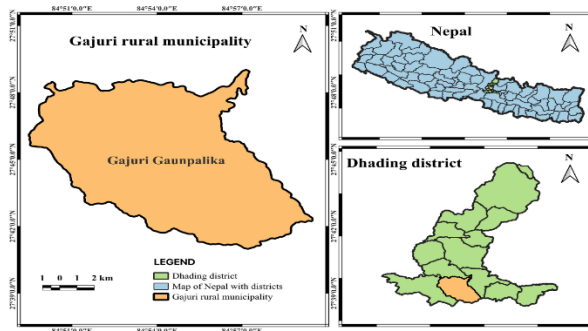


Figure 2. Location of the experiment field

Table 1. Characteristics of soil and biochar

Parameters	Soil	Biochar
pH	7.1	9.3
Texture	Sandy-loam	-
Organic matter (%)	0.23	-
Organic carbon (%)	-	13.6
Total Nitrogen (%)	0.23	0.81
Available Phosphorous (kg ha <sup>-1</sup> )	23.84	-
Available Potassium (kg ha <sup>-1</sup> )	244.8	-

Table 2. Description and Dosage of Different Treatment Used in the Experiment.

Treatment number	Description	Abbreviation	Dosage (per ha)
Treatment 1 (T <sub>1</sub> )	Control (no biochar and no NPK)	CK	n/a
Treatment 2 (T <sub>2</sub> )	Inorganic fertilizer	RF	MOP: 133 kg DAP: 391.2 kg Urea: 260.8 kg
Treatment 3 (T <sub>3</sub> )	Inorganic fertilizer + Biochar	BF	MOP: 133 kg DAP: 391.2 kg Urea: 260.8 kg Biochar: 10 ton
Treatment 4 (T <sub>4</sub> )	Vermicompost + biochar	BVC	8.89 ton + 10 ton
Treatment 5 (T <sub>5</sub> )	Poultry Manure + biochar	BPM	8.50 ton + 10 ton
Treatment 6 (T <sub>6</sub> )	Biochar	BC	10 ton

### Biochar Production

The biochar used in the experiment was produced by “Kon-tiki” method, a simple and effective method, especially for small farm holders (Dahal et al., 2021). The ideal temperature to produce biochar is 400-550°C (Baidoo et al., 2016; Naeem et al., 2014). Biomass materials such as wood, agricultural residues, and other organic waste were collected from nearby area around the research field and sun-dried for one day. A cone shaped pit with a diameter and depth of 1 m was made. A small fire was started at the bottom of the pit using dry kindling, and after the fire was established, the collected biomass material was added layer after layer, gradually and in small amounts, to maintain a controlled burn. This process was repeated until the pit was filled with biochar. Once the charcoal was fully charred, water was poured over the pit to stop the combustion process, and it was allowed to cool down for 24 hours. Then, the resulting biochar was ground into fine particles and used for the experiment.

### Experimental Setup and Cultivation Practices

The experiment investigated the effects of six treatments replicated three times in a Completely Randomized Block Design (RCBD). Three treatments consisted of a mixed biochar formulation: (i) biochar + mineral NPK fertilizer; (ii) biochar + poultry manure; (iii) biochar + vermicompost; the other two treatments consisted of the sole application of (iv) mineral NPK fertilizer; (v) biochar; and the remaining treatment was a non-fertilized control (neither biochar nor any fertilizer). Urea (46% nitrogen), di-ammonium phosphate (DAP - 46% P<sub>2</sub>O<sub>5</sub> and 18% nitrogen), and muriate of potash (60% K<sub>2</sub>O) were used as mineral fertilizers. Poultry manure and vermicompost were acquired from poultry farm and local agrovet respectively. Biochar was applied at a rate of 10 ton ha<sup>-1</sup> (Acharya et al., 2022; Dahal et al., 2021). The characteristics of biochar is represented in Table 1. NPK

content of poultry manure and vermicompost was determined by nutrient analysis. Poultry manure contained 2.35% nitrogen (N), 0.08% phosphorus (P), and 2.65% potassium (K), while vermicompost contained 2.25% nitrogen (N), 1.2% phosphorus (P), and 2.2% potassium (K). The fertilizers were mixed with biochar for 24 hours, two days prior to sowing, and then mixed with the soil one day prior to sowing. The other two treatments, involving sole applications of biochar and mineral fertilizer, were mixed with the soil one day before sowing took place. The application rate of various fertilizers was based on the recommended rate of fertilizer application for okra as 200:180:80 NPK ha<sup>-1</sup> (Bhattarai et al., 2020). Each treatment plot measured 1.5 \* 2.4 m<sup>2</sup> with a plant-to-plant spacing of 30 cm and row-to-row spacing of 50 cm.

The field was deeply tilled to break the soil, followed by cultivation and planking to achieve proper soil tilth. The treatment material was applied in line at a depth of 7-8 cm one day prior to sowing. The seeds of Rizwan Gorkha (*Abelmoschus esculentus*), a F1 hybrid plant, were used in this experiment. Seeds to be sown were soaked overnight and sown at a depth of 3-4 cm. The full dose of DAP and MOP and the half dose of urea were applied at the time of sowing. The remaining dose of urea was top dressed in two equal splits at 30 and 40 days after sowing (DAS). Intercultural operations, including weeding, irrigation, and thinning, were carried out at regular intervals. Practices of pest and disease management were carried out as and when needed uniformly across the treatment plots. The okra pods were harvested after reaching maturity and at regular intervals of 2-3 days.

### Analysis Method Used

Soil samples were collected from a depth of 10-15 cm at 20 different locations in a “W” shaped pattern to create one composite sample for soil parameter analysis. The collected soil sample was analyzed for pH, texture, organic

matter content (%), total nitrogen (%), available phosphorus ( $\text{kg ha}^{-1}$ ), and available potassium ( $\text{kg ha}^{-1}$ ). The biochar used in the experiment was analyzed for pH, organic carbon content (%), and total nitrogen (%). Similarly, poultry manure and vermicompost were analyzed for total nitrogen (%), available phosphorus ( $\text{kg ha}^{-1}$ ), and available potassium ( $\text{kg ha}^{-1}$ ).

The pH of the soil was measured with a digital pH meter using a soil-to-distilled water ratio of 1:2.5. Soil texture was assessed using the hydrometer method (Soil Management Directorate, 2017). Organic matter and organic carbon content were analyzed using the Walkley-Black method (Walkley & Black, 1934). The total nitrogen content was determined through the Kjeldahl method, involving digestion with concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and subsequent distillation with 40% sodium hydroxide (NaOH) followed by acid titration (Soil Management Directorate, 2017). Available phosphorus was measured using the modified Olsen method (Olsen et al., 1954), while available potassium was determined using a flame photometer after treating the soil samples with normal ammonium acetate (Soil Management Directorate, 2017).

#### Agronomic and Yield Parameters

Five plants in each plot were tagged for data collection. Vegetative parameters, namely plant height, number of leaves per plant, number of branches per plant, number of nodes in the main stem and stem diameter, were measured from 30 to 75 DAS at a 15-day interval. Reproductive parameters, namely pod length (cm), pod diameter (cm), fresh weight (gm), number of fruits per plot, days to 50% flowering, and days to 1<sup>st</sup> harvest, were also measured. Plant height and pod length were measured with a measuring scale; stem diameter and pod diameter were measured using a Vernier caliper; and fresh weight was measured using a weighing machine. Days to the first harvest were noted for each plant in the plot, and an arithmetic mean was calculated. Days to 50% flowering were documented as the days when more than 50% of the plants in the plot reaching flowering stage.

#### Economic Analysis

The costs incurred during the production of okra were both fixed (land lease) and variable (biochar, seed, fertilizers, field preparation, irrigation, intercultural activities, etc.). Based on data obtained from field trials, the

gross return (GR), net return (NR), and benefit-cost (BC) ratios were computed. The selling price of okra was set as per the market price during the harvesting period. Gross return (Eq. 1) was calculated as the total return generated before deducting cultivation-related costs, whereas net return (Eq. 2) was calculated as the total return generated after deducting all cultivation-related costs from the gross return. Net return was divided by total production costs to determine the benefit-cost ratio (Eq. 3).

Gross returns = Total marketable yield  $\times$  Selling price of okra (Eq. 1)

Net returns = Gross returns – Total cost of production (Eq. 2)

Benefit: Cost Ratio (B: C) = Net returns/Total cost of production (Eq. 3)

#### Data Analysis

Data collected from the sample plant was entered systemically in MS Excel (Office package 2019). Data was analyzed using R-studio version 4.2.2. Packages such as ggfortify, gvlma and agricolae were used for checking ANOVA assumptions and carrying out ANOVA and Least Significant Difference (LSD) analysis. One way ANOVA was performed to access the effect of various biochar-based fertilizer on the vegetative, reproductive, and phenological parameters of okra. Significant differences among the mean were analyzed by using Least Significant Difference (LSD) at 5% level of significance.

#### Results

##### Vegetative Parameters

The effect of fertilizer on the plant height of okra was found to be statistically significant at all stages of observation (Table 3). At 30 DAS, the highest plant height was observed in BVC treatment (10.74 cm), which was statistically similar to BPM treatment (10.54 cm). However, at other stages (45, 60, and 75 DAS), BPM treatment had the highest plant height, being statistically similar to BVC treatment at 45 DAS (26.175 cm) and 75 DAS (131.77 cm). Similarly, the lowest plant height was observed in the control treatment, followed closely by the BC treatment. Although BF and RF exhibited no statistical difference across all DAS, BF consistently showed higher plant height compared to RF.

Table 3. Plant Height of Okra Influenced by Different Fertilizers.

Treatment	Plant height (cm)			
	30DAS	45DAS	60DAS	75DAS
CK	8.75 <sup>d</sup>	22.60 <sup>c</sup>	66.40 <sup>b</sup>	82.60 <sup>d</sup>
RF	9.93 <sup>bc</sup>	26.18 <sup>b</sup>	75.86 <sup>a</sup>	121.77 <sup>bc</sup>
BF	9.86 <sup>bc</sup>	27.25 <sup>b</sup>	77.76 <sup>a</sup>	127.13 <sup>bc</sup>
BVC	10.74 <sup>a</sup>	26.18 <sup>b</sup>	77.50 <sup>a</sup>	131.77 <sup>ab</sup>
BPM	10.54 <sup>ab</sup>	29.92 <sup>a</sup>	81.38 <sup>a</sup>	137.26 <sup>a</sup>
BC	8.64 <sup>cd</sup>	25.53 <sup>b</sup>	68.30 <sup>b</sup>	89.54 <sup>d</sup>
LSD (0.05)	0.767	2.645	6.023	9.097
SEM ( $\pm$ )	0.106	0.362	0.825	1.245
F-probability	***	***	**	**
CV (%)	5.22	6.73	5.41	5.31

Note: Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; \*:significant at 5% probability level; \*\*: significant at 1% probability level; \*\*\*: significant at 0.1% probability level; CK : Control ; RF: Inorganic Fertilizer ; BF : Biochar + Fertilizer; BVC: Vermicompost + Biochar; BPM: Poultry Manure+ Biochar; BC: Biochar

Table 4. Number of Leaves per Plant of Okra Influenced by Different Fertilizers.

Treatment	Number of leaves per plant			
	30DAS	45DAS	60DAS	75DAS
CK	2.74	8.40 <sup>d</sup>	15.33 <sup>d</sup>	22.36 <sup>d</sup>
RF	3.05	11.75 <sup>bc</sup>	20.97 <sup>c</sup>	29.68 <sup>c</sup>
BF	3.20	11.70 <sup>bc</sup>	24.50 <sup>ab</sup>	32.01 <sup>bc</sup>
BVC	2.80	12.95 <sup>ab</sup>	22.69 <sup>bc</sup>	34.72 <sup>b</sup>
BPM	3.05	13.99 <sup>a</sup>	26.85 <sup>a</sup>	39.54 <sup>a</sup>
BC	2.90	10.55 <sup>c</sup>	17.80 <sup>d</sup>	24.13 <sup>d</sup>
LSD (0.05)	0.43	1.793	3.061	2.86
SEM (±)	0.045	0.246	0.419	0.392
F-probability	NS	**	**	**
CV (%)	7.32	10.4	9.61	6.30

Note: Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; NS: Non-significant; \*:significant at 5% probability level; \*\*: significant at 1% probability level; \*\*\*: significant at 0.1% probability level; CK : Control ; RF: Inorganic Fertilizer ; BF : Biochar + Fertilizer; BVC: Vermicompost + Biochar; BPM: Poultry Manure+ Biochar; BC: Biochar

Table 5. Stem Diameter of Okra Plant Influenced by Different Fertilizer.

Treatment	Stem diameter (cm)			
	30DAS	45DAS	60DAS	75DAS
CK	0.308	0.498 <sup>d</sup>	0.804 <sup>d</sup>	0.941 <sup>c</sup>
RF	0.331	0.552 <sup>bc</sup>	0.949 <sup>bc</sup>	1.135 <sup>b</sup>
BF	0.34	0.578 <sup>b</sup>	1.011 <sup>ab</sup>	1.233 <sup>a</sup>
BVC	0.355	0.656 <sup>a</sup>	1.07 <sup>a</sup>	1.246 <sup>a</sup>
BPM	0.349	0.546 <sup>bc</sup>	0.968 <sup>bc</sup>	1.122 <sup>b</sup>
BC	0.307	0.529 <sup>cd</sup>	0.91 <sup>c</sup>	1.073 <sup>b</sup>
LSD (0.05)	0.054	0.037	0.07	0.094
SEM (±)	0.037	0.032	0.06	0.081
F-probability	NS	***	***	***
CV (%)	8.61	4.40	4.91	5.56

Note: Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; NS: Non-significant; \*:significant at 5% probability level; \*\*: significant at 1% probability level; \*\*\*: significant at 0.1% probability level; CK : Control ; RF: Inorganic Fertilizer ; BF : Biochar + Fertilizer; BVC: Vermicompost + Biochar; BPM: Poultry Manure+ Biochar; BC: Biochar

Table 6. Number of Nodes per Plant of Okra Influenced by Fertilizer.

Treatment	Number of nodes on main stem per plant			
	30DAS	45DAS	60DAS	75DAS
CK	1.88	7.50	10.10 <sup>d</sup>	11.91 <sup>d</sup>
RF	2.19	8.60	12.37 <sup>bc</sup>	16.50 <sup>b</sup>
BF	2.13	8.10	12.70 <sup>bc</sup>	17.02 <sup>b</sup>
BVC	1.92	9.63	13.95 <sup>b</sup>	17.47 <sup>b</sup>
BPM	2.24	11.28	15.75 <sup>a</sup>	19.42 <sup>a</sup>
BC	2.02	7.45	11.60 <sup>cd</sup>	13.80 <sup>c</sup>
LSD (0.05)	1.08	3.91	1.701	1.28
SEM (±)	0.5330	0.459	0.233	0.176
F-probability	NS	NS	**	***
CV (%)	35.32	25.88	8.94	5.35

Note: Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; NS: Non-significant; \*:significant at 5% probability level; \*\*: significant at 1% probability level; \*\*\*: significant at 0.1% probability level; CK : Control ; RF: Inorganic Fertilizer ; BF : Biochar + Fertilizer; BVC: Vermicompost + Biochar; BPM: Poultry Manure+ Biochar; BC: Biochar

The number of okra leaves per plant exhibited significant variation ( $p < 0.01$ ) except at 30 DAS (Table 4). Across all days of observation, the maximum number of leaves per plant was found in response to BPM treatment, followed by BVC and BF treatments. In contrast, the lowest value was observed in response to control, closely followed by BC treatment. BF showed a higher number of leaves in comparison to RF in all cases, with RF being statistically similar to BF at 45 and 75 DAS.

Similarly, stem diameter varied significantly ( $p < 0.01$ ) except at 30 DAS (Table 5). Maximum stem diameter was found in response to BVC treatment, which was followed by BF and BPM treatments. The lowest values were observed in the case of control, followed by BC treatment, with control being at par with BC at 45 DAS. Moreover, the number of nodes on the main stem per plant in okra exhibited significant variation in response to fertilizers at 60 DAS and 75 DAS ( $p < 0.001$ ) but was non-significant at 30 DAS and 45 DAS (Table 6).



At 60 DAS and 75 DAS, BPM treatment exhibited superior performance, followed by BVC, RF, and BF treatments, with the latter three treatments showing no statistical difference between them. The lowest number of nodes was observed in control, followed closely by BF treatment. Additionally, the number of primary branches per plant significantly varied ( $p < 0.01$ ) except at 45 DAS (Table 7). The highest number was obtained under BPM, followed by BVC and BF, which were both statistically similar to each other. The lowest number was obtained under CK, which was statistically similar to RF and BC.

#### Reproductive and Phenological Parameters

Application of different fertilizers had a significant effect on reproductive and phenological parameters except for pod diameter (Table 8). The lowest number of days to the first harvest was observed in response to BF treatment (51.25), which was statistically similar to all other treatments except for the control (59). Similarly, the lowest number of days to 50% flowering was observed in the case of BVC treatment (48.50), which was statistically similar to other treatments except control (54.50). In addition, the highest productivity was observed in response to BPM treatment (19.28 mt/ha), which was statistically similar to BVC treatment (17.46 mt/ha). Conversely, the lowest productivity was reported in the control (7.13 mt/ha), which was on par with the BC treatment (9.23 mt/ha). The highest number of pods per plant was observed in the BPM treatment (18.70), which was statistically similar to BVC treatment (17.75). In contrast, the lowest number was observed in the case of the control (9.10), followed by the

BC treatment (10.85). The highest average fruit weight was observed in the case of BVC treatment (17.85 g), which was statistically similar to BPM treatment (17.32 g). Conversely, the lowest fruit weight was observed in response to the control (13.80 g) being statistically similar to BC treatment (14.44 g). The highest fruit length was observed in response to BPM treatment (13.97 cm), which was statistically at par with BVC treatment (14.41 cm). The lowest number was observed in control (12.23 cm), followed by BC treatment (13.02 cm).

#### Economic analysis

The rate per unit of all inputs was determined based on the perception of the farmers in the Gajuri area. The price of okra, set at NRs 30 per kg, was established according to the local selling price in the Gajuri vegetable market.

With the use of BC, RF, BF, BVC, and BPM, the gross return increased by 34.35%, 83.115%, 135.37%, 154%, and 180.64% compared to CK (NRs 206100). Similarly, compared with CK (NRs 126100), net return increased by 42.18%, 111.94%, 178.31%, and 244.278% with the use of BC, RF, BF, and BPM, respectively (Table 10). However, the net return decreased by 28.5% in BVC compared to CK due to the high per-unit cost of vermicompost. The economic analysis of okra specifically concentrated on evaluating its performance based on yield. The B:C ratio was found to be in the range of 1.209 to 4.01 with various organic and inorganic amendments (Table 10). For BPM and BF treatment, the gross return and net return per hectare were observed to be higher, consequently resulting in a higher B:C ratio, as indicated in Table 10.

Table 7. Number of Primary Branches per Plant Influenced by Different Fertilizers.

Treatment	Number of primary branches per plant		
	45DAS	60DAS	75DAS
CK	1.27	2.14 <sup>d</sup>	2.76 <sup>c</sup>
RF	1.84	2.56 <sup>bcd</sup>	3.17 <sup>bc</sup>
BF	2.18	2.78 <sup>bc</sup>	3.52 <sup>b</sup>
BVC	1.98	2.89 <sup>b</sup>	3.49 <sup>b</sup>
BPM	2.36	3.50 <sup>a</sup>	4.18 <sup>a</sup>
BC	1.55	2.28 <sup>d</sup>	2.96 <sup>bc</sup>
LSD (0.05)	1.12	0.594	0.635
SEM ( $\pm$ )	0.066	0.082	0.087
F-probability	NS	**	**
CV (%)	17.22	14.78	12.71

Note: Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; NS: Non-significant; \*: significant at 5% probability level; \*\*: significant at 1% probability level; \*\*\*: significant at 0.1% probability level; CK: Control; RF: Inorganic Fertilizer; BF: Biochar + Fertilizer; BVC: Vermicompost + Biochar; BPM: Poultry Manure + Biochar; BC: Biochar

Table 8. Different Yield Parameters and Productivity of Okra Influenced by Fertilizers.

Treatment	Pods per plant (number)	Average Fruit length (cm)	Fruit diameter (cm)	Fruit weight (gm)	Yield/ha (mt/ha)	Days to 50% flowering (d)	Days to 1 <sup>st</sup> harvest (d)
CK	9.10 <sup>c</sup>	12.23 <sup>d</sup>	1.93	13.80 <sup>d</sup>	7.13 <sup>d</sup>	54.50 <sup>a</sup>	59.00 <sup>a</sup>
RF	14.60 <sup>c</sup>	13.64 <sup>bc</sup>	2.08	16.64 <sup>bc</sup>	12.58 <sup>c</sup>	50.00 <sup>b</sup>	53.00 <sup>b</sup>
BF	16.85 <sup>b</sup>	13.38 <sup>bc</sup>	1.98	15.65 <sup>c</sup>	16.17 <sup>b</sup>	50.25 <sup>b</sup>	51.25 <sup>b</sup>
BVC	17.75 <sup>ab</sup>	14.41 <sup>a</sup>	2.01	17.85 <sup>a</sup>	17.46 <sup>ab</sup>	48.50 <sup>b</sup>	54.25 <sup>b</sup>
BPM	18.70 <sup>a</sup>	13.97 <sup>ab</sup>	2.17	17.32 <sup>ab</sup>	19.28 <sup>a</sup>	49.50 <sup>b</sup>	52.00 <sup>b</sup>
BC	10.85 <sup>d</sup>	13.02 <sup>c</sup>	1.93	14.44 <sup>d</sup>	9.23 <sup>d</sup>	49.50 <sup>b</sup>	54.00 <sup>b</sup>
LSD (0.05)	1.60	0.728		1.076	3.11	2.461	2.837
SEM ( $\pm$ )	0.096	0.065	0.05	0.146	0.421	0.334	0.385
F-probability	**	**	NS	**	**	*	*
CV (%)	7.25	2.13	11.91	4.47	14.87	3.242	3.46
Grand mean	14.64	13.44	2.02	15.94	13.84	50.375	54.46

Note: Means followed by the same letter(s) in a column are not significantly different by LSD at 5% level of significance; DAS: days after sowing; SEM: standard error of mean; LSD: Least significant difference; CV: coefficient of variation; NS: Non-significant; \*: significant at 5% probability level; \*\*: significant at 1% probability level; \*\*\*: significant at 0.1% probability level; CK: Control; RF: Inorganic Fertilizer; BF: Biochar + Fertilizer; BVC: Vermicompost + Biochar; BPM: Poultry Manure + Biochar; BC: Biochar

Table 9. Cost of Production (NRs ha<sup>-1</sup>) of Rizwan Variety of Okra Under Different Fertilizers.

S.N.	Particulars	Unit	Quantity	Rate (NRs)	Cost (NRs ha <sup>-1</sup> )
1)	Fixed cost			10000	10000
A)	Land lease	ha			
2)	variable cost				
A)	Seed	packet	77	400	30800
B)	Fertilizer				
	synthetic				36550
	• Urea	kg	270	40	10800
	• DAP	kg	387	50	19350
	• MOP	kg	128	50	6400
	Vermicompost	kg	11200	30	336000
	Poultry manure	kg	9333	5	46665
C)	Field preparation	man-hour	8	800	6400
D)	Tractor	hour	4	1200	4800
E)	Treatment application	man-hour	3	800	2400
F)	Intercultural operation	man-hour	8	800	6400
G)	Harvesting	man-hour	16	800	12800
H)	Biochar	ton	6	4000	24000

Table 10. Analysis of Benefit: Cost Ratio with Respect to Treatment.

S.N.	Treatment	Cost of cultivation per ha (NRs)	Market price of okra (NRs)	Gross return per ha (NRs)	Net return per ha (NRs)	B:C ratio
1	CK	80000	30	206100	126100	2.577
2	RF	110150	30	377400	267250	3.427
3	BF	134150	30	485100	350950	3.617
4	BC	97600	30	276900	179300	2.838
5	BVC	433600	30	523800	90200	1.209
6	BPM	144265	30	578400	434135	4.01

## Discussion

### Plant Morphological Traits

This study shows that the application of biochar-based fertilizer had a significant effect on the vegetative or morphological traits of okra. Application of BPM performed best in most of the growth parameters, such as plant height, number of leaves, number of primary branches, and number of nodes, which were closely followed by vermicompost treatment (Table 3 - 7).

The study results align with Wangmo et al. (2022) findings, showing that plant height was significantly higher in response to poultry manure and biochar in red chili, with the maximum plant height observed in BPM treatment (137.26 cm) and followed by BVC treatment (131.78 cm). Adhikari & Piya (2020) and Adekiya et al. (2020a) reported plant height being significantly greater in plants treated with poultry manure. Sharma et al. (2021) reported a significant increase in plant height of knolkhol with biochar applied in combination with vermicompost by 18.2%, and with cattle manure (FYM) by 15.8% compared to control, which corroborates our findings where biochar mixed with vermicompost, and poultry manure showed a superior effect on plant height. The increase in plant height could be attributed to improved soil pH, EC (electrical conductivity), and soil fertility, as well as plant-promoting effects and nutrient loadings stimulated by biochar (Acharya et al., 2022).

The number of leaves per plant was observed to be highest in BPM treatment (39.54) followed by BVC (Table 4). A higher number of leaves per plant in okra observed with the application of poultry manure followed by

vermicompost, reported by Bhandari et al. (2019), corroborates the above finding. Sarma et al. (2017) reported a significant increase in the number of okra leaves per plant treated with biochar in combination with vermicompost and synthetic fertilizer, which corroborates our finding that BVC and BF had a higher number of leaves in comparison to other treatments except BPM.

However, stem diameter was found to be significantly higher in BVC and BF than in BPM at 60 and 75 DAS (Table 5). This finding is consistent with Acharya et al. (2022) study, which demonstrated that combining vermicompost, biochar, and cow urine led to increased stem girth. The ability to retain nutrients due to the low bulk density and high-water holding capacity of biochar could explain the improvement in crop performance by increasing the stem girth of the okra plant, as observed in the findings.

Biochar enriched with poultry manure showed a significantly higher number of primary branches (4.18) in comparison to other treatments (Table 7). This is in line with the findings of the study conducted by Adhikari & Piya (2020), where a higher number of primary branches in okra was observed in poultry manure. Similarly, the number of nodes in the main stem per plant was found to be significantly higher in BPM at 60 and 75 DAS in comparison to other treatments (Table 6).

The study also suggests that plots treated with biochar showed better growth parameters than non-biochar-added plots, particularly in the cases of BF, RF, BC, and CK. Although BF, RF, BC, and CK showed statistically similar

results in all growth parameters, BF and BC showed better performance than RF and CK, respectively. Ahmed et al. (2017) reported significantly higher total branch/plant and higher node/meter in response to biochar combined with synthetic fertilizer in comparison to synthetic fertilizer alone. Similarly, Sarma et al. (2017) reported that biochar combined with vermicompost and synthetic fertilizer gave significantly higher plant height and leaf area for two consecutive years than their non-biochar counterparts did. Wu et al. (2019) reported that the application of potash fertilizer in combination with biochar in cotton significantly increased the number of effective branches, bolls, and buds compared to potash fertilizer alone. This finding illustrates the potential to improve the growth and development of crops through the co-application of fertilizer and biochar. This can be attributed to the ameliorative nature of biochar, which improves the physical, chemical, and biological properties of soil Murtaza et al. (2021), as well as its ability to store essential plant nutrients in its inner pore, making them available as and when needed by the plants (Dahal et al., 2021).

### **Reproductive and Phenological Parameters**

The findings of the study show a significant effect of fertilizer on the yield parameters and productivity of okra. Productivity was found to be significantly higher in poultry manure and vermicompost applied in combination with biochar than in the control and other fertilized groups (Table 8). In accordance with this, Khan et al. (2022) reported that the combined application of biochar at 20 t/ha and poultry manure at 150 kg/ha increased the grain yield in wheat by 62.9% in comparison to control and other treatments. Higher okra yield in response to poultry manure was observed in studies conducted by Adhikari & Piya (2020) and Adekiya et al. (2020a). This is because of the low lignin content, low C:N ratio, and low lignin: N ratio of poultry manure, which results in faster mineralization and early nutrient release, especially beneficial for a short-duration crop like okra. As a result, the superior performance in growth parameters directly translated into a greater yield compared to other treatments. Similarly, the susceptibility of N losses through volatilization, run-off, leaching, and denitrification in synthetic fertilizer, in contrast to the ability of poultry manure to conserve and supply N for a long time, may also have resulted in a higher yield (Adekiya et al., 2020a). However, a higher yield was observed in ginger in response to biochar combined with synthetic fertilizer than in poultry by (Adekiya et al., 2018).

Yield parameters such as fruit length, fresh pod weight, and number of pods per plant were found to be significantly higher in BPM, while fruit diameter was found to be non-significant among all treatments (Table 8). Neither the addition of biochar nor the change in fertilizer affected the days to 50% flowering or days to first harvesting, as all treatments, except the control, showed statistical similarity (Table 8).

Similarly, the yield of BF was found to be significantly higher than that of RF, and that of BC was higher than that of CK, although they were statistically similar to each other in the latter (Table 8). These findings suggest that biochar-added plots gave superior performance and yield to their non-biochar counterparts. This is in line with the study

conducted by Sarma et al. (2017), where, for two consecutive years, higher pod yields in okra were observed in plots fertilized with biochar only and synthetic fertilizer in combination with biochar than their respective non-biochar added counterparts. Remigius et al. (2022) found that plots fertilized with biochar either with organic or synthetic fertilizer gave better morphological parameters and yield than non-biochar fertilized plots for two consecutive years in rice under drip irrigation. Similar findings were reported by Timilsina et al. (2021), where biochar at 2 t ha<sup>-1</sup> in combination with mineral fertilizer outperformed the sole application of mineral fertilizer in the curd yield of cauliflower. Yield improvement with the addition of biochar can be attributed to enhanced nutrient cycling, maintenance of soil pH via the liming effect, increased CEC, nutrient and water retention, their use efficiency, and microbial activity (El-Naggar et al., 2019). Schmidt et al. (2015), in a multi-farm trial, found maximum pumpkin yield in response to biochar enriched with cow urine—300% more than urine alone—and speculated that high water holding capacity, increased mineralization and retention of compost minerals, and reduced leaching were the causes of the increased yield. Studies by Acharya et al. (2022), Dahal et al. (2021) and Frimpong et al. (2021) reported an increase in soil pH, N, P, K, and organic matter (OM) in plots treated with biochar. They also identified positive correlations between the improved soil properties and crop yield. The presence of inner pores in biochar enables the storage and timely supply of nutrients to plants when needed and reduces leaching loss as well (Dahal et al., 2021; El-Naggar et al., 2019). This, in consequence, leads to higher soil fertility and increased crop yield. So, these illustrate a complementary interaction with the co-application of biochar with organic or inorganic amendments.

### **Economic Analysis**

The economic analysis revealed BPM and BF treatment to be more profitable compared to other treatment as indicated by their higher B:C ratio and net return (Table 9-10). Although BVC ranked second in terms of gross return, higher cost of input associated with high price of vermicompost resulted in lower net return and lower B:C ratio. Dahal et al. (2021) also reported higher net return and B:C ratio with the application of biochar with compost and biochar with inorganic fertilizer which aligns with our result. In another similar study, Pandit et al. (2020) reported higher net return with the application of co-composted biochar at the rate of 60 ton ha<sup>-1</sup> in case of maize. This indicates the potential of BPM and BF treatment to increase the farm income at the household level.

### **Conclusion**

According to the study, okra's vegetative and reproductive development characteristics were greatly improved by using biochar in addition with vermicompost (BVC) and poultry manure (BPM). Application of biochar alone or in combination with synthetic fertilizer produced better results than non-biochar application, indicating the possible advantages of adding biochar for fertility, soil health, and crop productivity. Additionally, the economic

analysis shows that the treatments using biochar and poultry manure (BPM) and fertilizer (BF) are more profitable than the other treatments, which means that farmers who want to maximize crop output and return on investment can consider them. These results suggest the complementary interaction between biochar and fertilizers, which leads to balanced soil pH, higher nutrient retention, and the timely release of essential plant nutrients while minimizing potential nutrient loss, all of which result in higher crop yield. Using biochar with organic fertilizer can reduce the reliance on inorganic fertilizers, while combining biochar with inorganic fertilizer can maintain high yields and mitigate negative environmental impacts.

### Limitations of the study and suggestion for future

This research was carried out in specific soil type in a specific agricultural domain, which may limit the applicability of the findings to other soil types and agricultural settings. To, increase applicability, it would be beneficial for studies to investigate the agricultural and economic impacts of combining biochar with various organic and inorganic fertilizers across different types of soil and farming regions in Nepal. Conducting studies over years, in locations will offer more comprehensive insights and improve the overall applicability of the results.

Using biochar in grounded power form requires significant labor which could pose practical challenges. Therefore, it's important to look into different ways to apply biochar that would need less labor. Furthermore, further research is necessary to create biochar that is tailored to the needs of certain crops and soil fertility levels. This research should concentrate on preparation techniques and the best application rates.

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## Growth and Yield of Summer Squash (*Cucurbita pepo* var. *sunny house*) in Response to Organic and Inorganic Mulching Materials at Rampur, Chitwan

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### ABSTRACT

Mulching, a widely employed agricultural practice, has been recognized for its influence on soil moisture retention, temperature regulation, and weed suppression, thereby improving growth and yield of crops. An experiment was conducted to study the effect of mulching materials on growth and yield attributes of summer squash under water constraint condition during March to May 2023 in Chitwan, Nepal. The experiment was laid in single factor Randomized Complete Block Design (RCBD) with four different mulches (rice straw, wheat straw, rice husk and silver on black plastic mulch 30 microns) and control replicated 4 times. All the Plant growth parameters, including plant height, number of leaves per plant and plant spread, were highest in silver plastic mulch and the poorest in the un-mulched plots. Mulching also significantly influenced floral characters, with plastic mulch demonstrating superiority in traits such as days to flowering, number of flowers per plant, and sex ratio compared to organic mulches and the control. Fruit length, diameter, and weight exhibited the highest values under plastic mulch and the lowest values under the control, while all organic mulches showed similar results. The highest yield of 41.44 Mt ha<sup>-1</sup> was achieved with silver plastic mulch, highlighting its effectiveness in enhancing crop productivity, while the lowest yield of 11.77 Mt ha<sup>-1</sup> was recorded in the control plots. Despite its higher cost, silver plastic mulch exhibited highest net return and benefit-cost ratio. Rice husk mulch, with the second highest benefit-cost ratio and relatively low cost of production, emerged as promising alternative. Although mulching did not notably affect the soil pH, the varying soil organic matter percentages were observed, with the highest on rice straw mulch and the lowest on silver plastic mulch. The study suggests that while plastic mulch can significantly boost productivity during water scarcity, rice husk mulch offers a cost-effective alternative with substantial economic benefit.

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## Introduction

Summer squash, a member of the *Cucurbita pepo* L. species, is a versatile and economically important vegetable crop with a rich history and diverse horticultural groups (Paris 1996). Among these, the zucchini cultivar-group is particularly significant (Lust & Paris, 2016). The health benefits of summer squash, including its bioactive compounds and nutritional composition, are widely recognized (Tadros et al., 2023; Díaz et al., 2020). Additionally, the fruit of the summer squash is rich in fiber and vitamins and contains moderate amounts of mineral salts (Abdein, 2016).

Summer squash cultivation is quite popular in Nepal. However, the productivity of summer squash in Nepal is 14.3 Mt/ha, which is lower than the global average of 15.83

Mt/ha (MoALD, 2021/2022; FAO STAT 2021). In Nepal, summer squash is cultivated during the spring season, a particularly dry period (Regmi et al., 2021; Shrestha et al., 2021). Any degree of water stress can adversely affect the growth and yield of crops (El-Monayeri et al., 1984). Drought stress significantly decreases gas exchange parameters and physiological properties (Ors et al., 2016). Water stress in summer squash, as demonstrated by Ors et al. (2016) and Doklega et al. (2022), significantly reduces growth and yield parameters, including leaf chlorophyll reading values, leaf relative water content, stomatal conductance, photosynthetic rate, transpiration rate, fresh weight, and dry weight.



Research by Mahmood et al. (2014) found that mulching practices are highly effective in conserving soil moisture, leading to increased plant growth. The use of various mulching materials has significantly improved the growth and yield of summer squash (Kumar & Sharma, 2018). Mulching involves spreading organic or inorganic materials on the soil surface to create a more favorable environment for plant growth and development (Bobby et al., 2017). Locally sourced organic mulching materials, which are biodegradable, play a significant role in regulating temperature and water retention while also promoting higher levels of biological activity (Kwambe et al., 2015). Kumar & Sharma (2018) observed that the type of mulch used, the variety of summer squash, and their interaction had a significant impact on the total soluble solids (TSS) content in the fruit. Mahadeen (2014) found that mulching can lead to higher soil moisture content, positively affecting vegetative growth and yield by stimulating root growth through increased soil temperature and moisture. Sarmah et al. (2022) specifically noted the benefits of reduced weed problems and increased soil temperature due to mulching.

Youssef et al. (2021) found that organic mulches, including rice straw, positively impact soil properties, growth, and yield of squash plants. Barreda et al. (2023) observed that rice straw mulch effectively controls weeds and improves soil fertility, leading to increased grain yield. Parhizkar et al. (2021) further demonstrated the effectiveness of rice straw mulch in reducing surface runoff and soil loss, particularly at higher application rates. Paul et al. (2020) reported that straw mulching improves water use efficiency and soil water content, resulting in increased yields. Zhang et al. (2011) showed that mulching could reduce canopy temperature, increase relative humidity, and enhance the photosynthetic rate, all of which contribute to higher yields.

Noor et al. (2020) found that the increase in yield is attributed to the moderating influence of wheat straw mulch on soil temperature and its ability to enhance moisture retention. Peng et al. (2015) demonstrated that wheat straw mulch can increase soil water content and water use efficiency, leading to a substantial increase in wheat yield. Additionally, Aziz et al. (2022) found that wheat straw mulch can suppress weeds and conserve soil moisture, resulting in improved wheat growth and yield.

The application of rice husk mulch has been shown to improve germination percentage, yield, and the economics of pea production (Abonmai et al., 2023). Furthermore, the use of rice husk charcoal, a byproduct of rice husk, increased plant growth and soil carbon content in rice fields, although it did not significantly affect grain yield (Koyama & Hayashi, 2017). Rice husk mulch creates a favorable microclimate by reducing canopy temperature and increasing relative humidity, which enhances the photosynthetic rate and yield components (Zhang et al., 2011). It also conserves soil moisture, crucial for plant growth and development (Rahman & Khan, 2001). Additionally, rice husk mulch can improve soil physicochemical properties, such as reducing soil acidity and increasing water content, further supporting plant growth and yield (Mishra et al., 2017).

Plastic mulching material is widely used due to its convenient processability, exceptional chemical resistance,

durability, flexibility, and absence of harmful substances and odors (Lamont, 2017). Ahirwar et al. (2023) found that the reflective nature of silver mulch enhances photosynthesis and promotes uniform plant growth, leading to increased grain yield. This improvement is attributed to silver mulch's ability to maintain optimal root zone temperature, reduce heat stress, and suppress weed growth. Plastic mulches impede evaporation and moderate both soil temperature and moisture, promoting better root development and nutrient absorption by plants, resulting in enhanced growth (Kumar & Sharma, 2018). The elevated soil temperature from black plastic mulch creates a favorable microclimate for plant growth, enhancing photosynthesis and other metabolic activities, which in turn promotes earlier growth and development, leading to accelerated flowering and fruiting (Chaurasia et al., 2020). Consequently, plants grow better, absorb more nutrients, and produce a higher yield earlier than usual (Yaghi et al., 2013). Additionally, black polythene mulch acts as a physical barrier, preventing light from reaching the soil, which is essential for weed seed germination and growth, thereby minimizing weed density (Bobby et al., 2017).

It is evident that with the right microclimate surrounding the crop and soil through mulching, it is possible to achieve moisture conservation, improved soil structure, reduced incidence of pests and diseases, and better crop growth and yield. Therefore, with the objective of identifying the most beneficial and cost-effective mulching method and understanding the effects of different types of mulching materials on soil properties such as soil pH and organic matter, this experiment was conducted in the Horticulture field of Agriculture and Forestry University, Rampur, Bharatpur, Chitwan.

## Materials and Methodology

### Experimental Site

The experiment was conducted at Rampur, Chitwan district, at an altitude of 208 meters above sea level, during the spring-summer season from March to May 2020. The geographical coordinates of the research site are 27°39'00.9"N latitude and 84°21'09.8"E longitude (GPS). The research field was at located at Horticulture Research Field of Agriculture and Forestry University.

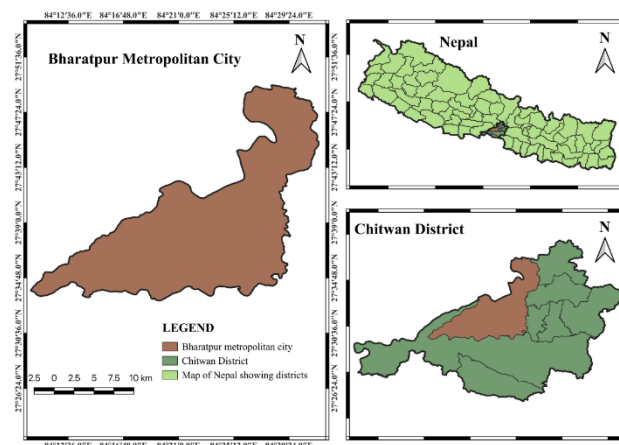


Figure 1. Map showing research area

### Climatic Condition and Soil Characteristics of The Experimental Site

The climatic data was taken from the meteorological station of National Maize Research Program, Rampur, Chitwan. Both maximum and minimum temperatures were in an increasing pattern from February to May (Figure 2). There was no precipitation in February and March. April and May received 24.3 mm and 43.9 mm of rainfall, respectively. The highest average temperature of 34°C was recorded in May. There was no precipitation in February and March. April and May received 24.3 mm and 43.9 mm of rainfall, respectively. Table 1 shows the soil characteristics of the study site before the experiment.

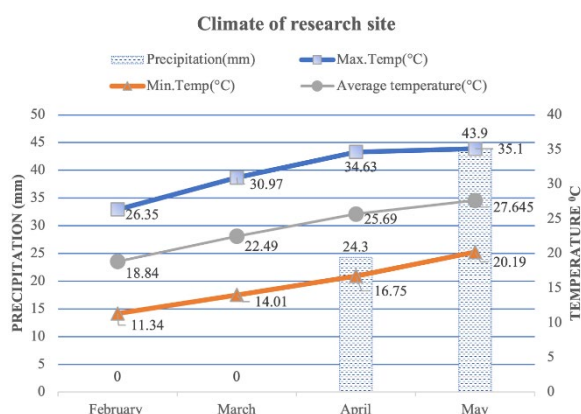


Figure 2. Cardinal Temperature and rainfall distribution in research area in 2023

Table 1. Description of Soil Status of the experimental field

Characteristics	Value
Soil depth (cm)	15-20 cm
Soil texture	Sandy-loam
Organic Matter (%)	2.1
pH	7.1
N (%)	0.32
P <sub>2</sub> O <sub>5</sub> (kg/ha)	37.20
K <sub>2</sub> O (kg/ha)	249.6

Source: Soil and Fertilizer testing laboratory, Hetauda, 2023

### Experimental Design

The experiment was laid out in Randomized Complete Block Design using five treatments, which were replicated four times. The treatments were T1: control (no mulching material), T2: rice straw, T3: wheat straw, T4: rice husk, and T5: silver on black plastic mulch (30 microns).

Sunny house variety of summer squash was used for this experiment. The total plot area of the experiment was 494.7 m<sup>2</sup>, with twenty plots each with an area of 16.2 m<sup>2</sup> (4.5\*3.6 m<sup>2</sup>). There was a 1 m distance between replications and 0.5 m distance between each plot within a replication. The spacing of the plants inside each plot was 90cm\*90cm (PP\*RR). There were four rows with five plants inside each plot, thereby accommodating 20 plants in each plot.

The seeds were sown in polybags under controlled protected conditions. The growing media used was prepared by using well rotten FYM and garden soil in a ratio of 2:1. The media was treated with the fungicide Saaf (Carbendazim 12% + Mancozeb 63% WP). A single seed was sown in each polybag. Regular watering was carried out as required. After complete germination of the seed,

seedlings bearing true leaves at 2–3 weeks of age were transplanted. The main field was ploughed twice, followed by planking to attain the good tilth required for planting. 5.4 kg FYM was applied per plot, and the recommended dose of N: P: K @ 12:9:3 kg/ropani was applied in the form of urea, DAP and MoP respectively (MoALD, 2021). The seedlings were transplanted at the required spacing of 90cm \* 90cm. The mulching material were added a day prior to transplanting and at the rate of 10 kg per plot (MoALD, 2021). Other intercultural operations such as weeding, pest, and disease management were uniformly carried out across the experimental plots. With respect to irrigation, it was carried out at two-week interval from the date of transplanting in the main field. Harvesting was done manually at the appropriate physiological maturity stage when the fruits were still green.

### Observation Taken

#### Plant growth parameters

- **Plant height:** It was measured from ground level to the tip of the main shoot with the help of scale at 20 days intervals (20,40 and 60 days after transplanting) and the average was calculated. The plant height was expressed in centimeters.
- **Number of leaves per plant:** The number of green, photosynthetically active leaves excluding senescent and emerging leaves per plant were counted at 20 days intervals (20,40 and 80 days after transplanting) and average were calculated.
- **Plant Spread:** Plant spread was measured at 20-day interval (20,40, 60 DAT) in both North-South and East-West directions. The maximum growth in each direction was recorded and the average plant spread was calculated.

#### Flowering Parameters

- **Days to opening of first male and first female flower:** The number of days from transplanting to the opening of the first male flower and first female flower was recorded.
- **Numbers of male and female flowers per plant:** Number of male and female flowers per plants was recorded in 3-4 days interval after initiation of flowering till the end of harvesting season from the same sample plants. Flowers that were either open or had bloomed since the last sampling day were the only ones counted. Petals were removed during each sampling period to ensure accurate counts in subsequent intervals. Plants with any abnormalities were excluded from the data analysis.
- **Sex ratio:** Sex ratio was calculated by dividing the total numbers of the male flowers by the total number of female flowers.

#### Yield parameters

- **Days to first harvest:** The number of days taken from transplanting to the first harvest was recorded and the average was calculated.
- **Number of fruits per plant:** The total number of fruits obtained from the sample plants from each harvest was divided by 5 to obtain the data.
- **Fruit length:** The length of each fruit harvested from the sample plant was measured individually from base of calyx to the tip and average was calculated.

- **Fruit diameter:** The diameter of each fruit harvested from the sample plant was measured individually at the widest point using vernier caliper and average was calculated.
- **Fruit weight:** The fruit weight was recorded by dividing the total weight of fruits harvested from the sample plants by the number of fruits in each harvest and average was calculated.
- **Productivity:** The net plot yield of the sample plants from each plot was calculated individually, averaged and converted to Mt/ha.

#### Soil Sampling and Analysis

After the final data recording the research field was left for 1.5 months. After that soil sampling was carried out using screw auger, tube auger and spade and the sample from the top 20 cm was taken from each of the plots. For the analysis of soil organic matter, the samples were subjected to wet digestion using 1 N Potassium Dichromate ( $K_2Cr_2O_7$ ) and 98% concentrated sulphuric acid and titrated against 0.5 N ferrous ammonium sulphate as per the protocols given by (Walkley & Black, 1934). Soil pH was determined at 1:2.5 soil water ratio using pH meter (Cottenie et. al., 1982).

#### Economic Analysis

The costs incurred during the production of summer squash were both fixed (land lease) and variable (seed, nursery preparation, fertilizers, field preparation, intercultural activities, etc.). Based on data obtained from field trials, the gross return (GR), net return (NR), and benefit-cost (BC) ratios were computed. The selling price of summer squash was set as per the market price during the harvesting period. Gross return (Eq. 1) was calculated as the total return generated before deducting cultivation-related costs, whereas net return (Eq. 2) was calculated as the total return generated after deducting all cultivation-related

costs from the gross return. Net return was divided by total production costs to determine the benefit-cost ratio (Eq. 3).

Gross returns = Total marketable yield  $\times$  Selling price of summer squash (Eq. 1)

Net returns = Gross returns – Total cost of production (Eq. 2)

Benefit: Cost Ratio (B: C) = Gross return/Total cost of production (Eq. 3)

#### Data Analysis

Recorded data was systematically arranged based on various observed parameters. Data entry was done in MS Excel following standard format then data was subjected to one way Analysis of variance (ANOVA) using R Studio. Duncun's Multiple Range Test (DMRT) test was used for mean separation at 5% level of significance.

#### Results

##### Plant Growth Parameters

Mulching materials significantly influenced plant height at all stages of observation (Table 2). Silver plastic mulch exhibited the highest plant height at all stages of observation, while the lowest plant height was observed under control. After silver plastic mulching, wheat straw mulch, rice straw mulch, and rice husk mulch resulted in higher plant heights at 20, 40, and 60 DAT, respectively.

The number of leaves per plant was significantly influenced by different mulching materials (Table 3). The maximum number of leaves per plant was observed under silver plastic mulch, which was statistically similar to rice straw mulch and wheat straw mulch at 20 and 60 DAT, respectively. In contrast, the lowest number of leaves per plant was reported under the control condition. After silver plastic mulching, a higher number of leaves were observed under wheat straw mulch at all stages of observation.

Table 2. Effect of different mulching materials on plant height of summer squash.

Treatments	Plant height (cm)		
	At 20 DAT	At 40 DAT	At 60 DAT (cm)
Control	15.19 <sup>c</sup>	19.93 <sup>c</sup>	36.17 <sup>d</sup>
Rice Straw mulch	18.00 <sup>b</sup>	27.35 <sup>b</sup>	43.15 <sup>bc</sup>
Wheat Straw Mulch	18.32 <sup>b</sup>	22.86 <sup>c</sup>	38.20 <sup>cd</sup>
Rice Husk mulch	18.15 <sup>b</sup>	23.23 <sup>c</sup>	44.65 <sup>b</sup>
Silver on black plastic mulch	20.52 <sup>a</sup>	31.025 <sup>a</sup>	51.55 <sup>a</sup>
LSD (0.05)	1.80	3.30	5.63
SEM ( $\pm$ )	0.26	0.48	0.81
F-probability	<0.001	<0.001	<0.001
CV %	6.50	8.62	8.56

NOTE: Means followed by the same letter in a column are not significantly different by DMRT at a 5% level of significance. LSD: Least Significant difference, SEM: Standard Error of Mean, CV: Coefficient of Variation, DAT: Days after transplanting.

Table 3. Effect of different mulching materials on number of leaves per plant of summer squash.

Treatment	Number of leaves per plant		
	20 DAT	40 DAT	60 DAT
Control	7.90 <sup>c</sup>	19.30 <sup>c</sup>	28.30 <sup>b</sup>
Rice Straw mulch	8.92 <sup>ab</sup>	21.15 <sup>bc</sup>	30.55 <sup>b</sup>
Wheat Straw Mulch	10.15 <sup>bc</sup>	24.65 <sup>b</sup>	34.05 <sup>ab</sup>
Rice Husk mulch	8.62 <sup>bc</sup>	19.9 <sup>bc</sup>	28.70 <sup>b</sup>
Silver on black plastic mulch	11.05 <sup>a</sup>	28.72 <sup>a</sup>	38.00 <sup>a</sup>
LSD (0.05)	1.95	3.60	6.04
SEM ( $\pm$ )	0.28	0.52	0.87
F-probability	<0.001	<0.001	<0.05
CV %	13.60	10.28	12.29

NOTE: Means followed by the same letter in a column are not significantly different by DMRT at a 5% level of significance. LSD: Least Significant difference, SEM: Standard Error of Mean, CV: Coefficient of Variation, DAT: Days after transplanting

Table 4. Effect of different mulching materials on plant spread of summer squash squash.

Treatment	Plant Spread (cm)		
	20 DAT	40 DAT	60 DAT
Control	56.55 <sup>c</sup>	100.37 <sup>c</sup>	106.45 <sup>c</sup>
Rice Straw mulch	65.77 <sup>b</sup>	122.88 <sup>b</sup>	127.73 <sup>b</sup>
Wheat Straw Mulch	63.32 <sup>b</sup>	115.50 <sup>b</sup>	121.57 <sup>b</sup>
Rice Husk mulch	63.97 <sup>b</sup>	122.25 <sup>b</sup>	128.20 <sup>b</sup>
Silver on black plastic mulch	74.30 <sup>a</sup>	135.30 <sup>a</sup>	145.05 <sup>a</sup>
LSD (0.05)	5.72	11.44	14.35
SEm (±)	0.83	1.66	2.08
F-probability	<0.001	<0.001	<0.001
CV %	5.73	6.23	7.40

NOTE: Means followed by the same letter in a column are not significantly different by DMRT at a 5% level of significance. LSD: Least Significant difference, SEM: Standard Error of Mean, CV: Coefficient of Variation, DAT: Days after transplanting

Table 5. Effect of different mulching materials on floral characteristics of summer squash.

Treatment	Days to 1 <sup>st</sup> male flower (DAT)	Days to 1 <sup>st</sup> female flower (DAT)	Total no. of male flowers per plant	Total no. of female flowers per plant	Sex ratio
Control	25.30 <sup>ab</sup>	30.95 <sup>a</sup>	7.12 <sup>a</sup>	2.625 <sup>d</sup>	3.13 <sup>a</sup>
Rice Straw mulch	23.35 <sup>c</sup>	26.20 <sup>bc</sup>	5.67 <sup>b</sup>	2.975 <sup>cd</sup>	2.03 <sup>b</sup>
Wheat Straw Mulch	26.05 <sup>a</sup>	28.05 <sup>ab</sup>	6.5 <sup>a</sup>	4.10 <sup>ab</sup>	1.74 <sup>b</sup>
Rice Husk mulch	24.20 <sup>bc</sup>	26.10 <sup>bc</sup>	5.7 <sup>b</sup>	3.50 <sup>bc</sup>	1.77 <sup>b</sup>
Silver on black plastic mulch	22.90 <sup>c</sup>	23.35 <sup>c</sup>	5.67 <sup>b</sup>	4.75 <sup>a</sup>	1.32 <sup>c</sup>
LSD (0.05)	1.51	3.63	0.63	0.72	0.41
SEm (±)	0.22	0.527	0.09	0.10	0.06
F-probability	<0.01	<0.01	<0.001	<0.001	<0.001
CV %	4.02	8.76	6.72	13.16	13.49

NOTE: Means followed by the same letter in a column are not significantly different by DMRT at a 5% level of significance. LSD: Least Significant difference, SEM: Standard Error of Mean, CV: Coefficient of Variation, DAT: Days after transplanting

Table 6. Effect of different mulching materials on yield and yield parameters of summer squash.

Treatment	Days to 1 <sup>st</sup> picking (DAT)	Average fruit diameter (mm)	Average fruit length (cm)	Average fruit weight (kg)	Productivity (mt/ha)
Control	41.85 <sup>a</sup>	59.24 <sup>c</sup>	26.36 <sup>c</sup>	0.49 <sup>c</sup>	11.77 <sup>c</sup>
Rice Straw mulch	34.45 <sup>bc</sup>	63.89 <sup>b</sup>	31.67 <sup>b</sup>	0.76 <sup>ab</sup>	25.03 <sup>b</sup>
Wheat Straw Mulch	36.90 <sup>b</sup>	62.88 <sup>b</sup>	31.02 <sup>b</sup>	0.66 <sup>b</sup>	24.21 <sup>b</sup>
Rice Husk mulch	33.95 <sup>bc</sup>	63.45 <sup>b</sup>	32.09 <sup>b</sup>	0.70 <sup>b</sup>	23.12 <sup>b</sup>
Silver on black plastic mulch	31.10 <sup>c</sup>	66.84 <sup>a</sup>	34.43 <sup>a</sup>	0.81 <sup>a</sup>	41.44 <sup>a</sup>
LSD (0.05)	4.17	2.42	2.02	0.09	8.70
SEm (±)	0.605	0.35	0.29	0.01	1.263
F-probability	<0.01	<0.001	<0.001	<0.001	<0.001
CV %	7.59	2.48	4.22	9.35	22.49

NOTE: Means followed by the same letter in a column are not significantly different by DMRT at a 5% level of significance. LSD: Least Significant difference, SEM: Standard Error of Mean, CV: Coefficient of Variation, DAT: Days after transplanting

Table 4 shows the effect of different mulching materials on the plant spread. The maximum plant spread was observed under silver plastic mulching at stages of observation, while the minimum plant spread was reported under the control plot. After silver plastic mulching, rice straw mulching resulted in a higher plant spread at 20 and 40 DAT, while rice husk straw resulted in a higher plant spread at 60 DAT.

#### Flowering Parameters

Mulching material had a significant effect on the floral characteristics of summer squash (Table 5). The fastest day to the first male flower was obtained under silver plastic mulch, which was statistically similar to rice straw mulch. Wheat straw mulch was the latest on days to the first female flower, which was statistically similar to no

mulching. Similarly, plastic mulching was the fastest for the first pistillate flower, followed by rice husk mulch, which was statistically similar to rice straw mulch. Control was late among all treatments. Control produced the highest number of male flowers per plant, which was statistically similar to wheat straw mulch. Other treatments had a statistically similar number of male flowers per plant. In contrast, plastic mulch condition resulted in maximum number of female flowers per plant. The highest sex ratio was observed in no mulching, and plastic mulch showed the lowest sex ratio.

#### Yield and Yield Parameters

Mulching material had a significant effect on the yield and yield parameters of summer squash (Table 6). The earliest days to first picking was observed in silver plastic

mulch (31.10) followed by rice husk mulch (33.95), rice straw mulch (34.45) and wheat straw mulch (36.90). In contrast, the control resulted in the maximum number of days to reach first harvesting (41.85). Fruit diameter, fruit length, and fruit weight were observed to be highest in silver plastic mulch and lowest in no control. Other treatments had statistically similar average fruit diameter, length, and weight, with rice straw mulching exhibiting higher fruit diameter and fruit weight and rice husk mulch, resulting in longer fruit length. Productivity values varied with silver plastic mulch (41.44 mt/ha) showing the highest, followed by rice straw mulch (25.03 mt/ha), wheat straw mulch (24.21 mt/ha), rice husk mulch (23.12 mt/ha), and control (11.76 mt/ha). Other treatments showed statistically similar productivity (Table 6).

#### Soil Organic Matter and Soil pH

There was significant variation in the soil organic matter percentage in response to various mulching materials (Table 7). The highest soil organic matter percentage was observed in rice straw mulch (3.31%), followed by rice husk mulch (2.74%), wheat straw mulch (2.71%), and the control condition (2.18%). Silver plastic mulch recorded the lowest organic matter percentage (1.24%). However, there was no significant difference in soil pH in response to different mulching materials.

#### Economic Analysis

The economic analysis of summer squash production was carried out in two sections. In the first section, the general cost of cultivation for summer squash was calculated (Table 8) that excluded the cost of mulching materials, and later on, the total cost of cultivation was calculated by adding the general cost of cultivation and the cost of mulching materials according to treatments. In the second section, the B:C ratio concerning the treatments was carried out (Table 9). The rate per unit of all the input is based upon the perception of the farmers and the local rate that exists in the Bharatpur area, and the price of summer squash, fixed at NRs 20 per kg, is based on the wholesale selling price in the Khestrapur vegetable market in Chitwan.

Gross return with the use of rice straw mulch, wheat straw mulch, rice husk mulch, and silver plastic was increased by 112.65%, 105.7%, 96.43% , and 252.08%, respectively, compared with control (Table 9).

Similarly, the net return with the use of rice straw mulch, wheat straw mulch, rice husk mulch, and silver plastic increased by 1467.08%, 1564.31%, 1515.7%, and 3438.27%, respectively, compared with control (Table 9).

Highest benefit to cost ratio was found in silver plastic mulch followed by rice husk mulch, wheat straw mulch and rice straw mulch control being the lowest (Table 9).

Table 7. Effect of mulching materials on Soil pH and Organic matter.

Treatment	Soil organic matter (%)	Soil pH
Control	2.18 <sup>d</sup>	6.86
Rice Straw mulch	3.31 <sup>a</sup>	6.89
Wheat Straw Mulch	2.71 <sup>c</sup>	6.91
Rice Husk mulch	2.74 <sup>b</sup>	6.88
Silver on black plastic mulch	1.24 <sup>e</sup>	6.82
LSD (0.05)	0.02	NS
SEm (±)	0.003	0.026
F-probability	<0.001	0.83
CV %	0.66	1.69

NOTE: Means followed by the same letter in a column are not significantly different by DMRT at a 5% level of significance. LSD: Least Significant difference, SEm: Standard Error of Mean, CV: Coefficient of Variation

Table 8. Cost of cultivation for summer squash (NRs ha<sup>-1</sup>).

S.N.	Particulars	Unit	Rate per unit	Quantity used	Cost (NRsha <sup>-1</sup> )
1	Land preparation by tractor	hour	1500	10	15000
2	Land Rent				32000
	Seeds	kg	23000	1.961	45080
4	Nursery raising	Lumpsum			15450
5	Fertilizer (Synthetic)	kg			
*	Urea		25	235.29	5882.25
*	DAP		50	176.47	8823.50
*	MOP		50	58.82	2941
6	Fertilizer (Organic)	kg	3	29411.76	88235.28
7	Fruit fly traps		50	40	2000
8	Harvesting labor	man hour	150	40	6000
				General Cost of Cultivation	221412.03
	Mulching materials				
1	Silver on black plastic mulch	meter	13.5	8333	112495.5
2	Wheat straw	kg	5	6000	30000
3	Rice straw	kg	10	6000	60000
4	Rice husk	kg	5	3000	15000

Table 9. Benefit Cost Ratio Analysis

Treatments	Cost of cultivation (NRs ha <sup>-1</sup> )	Productivity (kg/ha)	Average price per kg	Gross return (NRs ha <sup>-1</sup> )	Net return (NRs ha <sup>-1</sup> )	B:C ratio
Control	221413	11770	20	235400	13987	1.07
Rice straw	281413	25030	20	500600	219187	1.78
Wheat Straw	251413	24210	20	484200	232787	1.93
Rice husk	236413	23120	20	462400	225987	1.96
Silver on black plastic mulch	333903	41440	20	828800	494897	2.49

## Discussion

The increased performance of summer squash plants under mulching conditions can be attributed to soil moisture conservation, lower evaporation, a lower weed population, and increased soil temperature. The mulching materials act as a barrier to evaporating moisture vapor, cool the soil surface, and reduce the impact of sunlight energy (Jabran, 2019). McMillen (2013) reported that mulching material up to 5 cm can reduce surface evaporation by 40% compared to bare soil. Similarly, mulching materials cover the soil surface, creating a physical barrier that shades the emerging weed and hinders their access to sunlight, which is necessary for their germination and growth (Hussain & Luqman, 2022). This reduces the competition for essential resources such as sunlight, nutrients, and water, which leads to better plant growth. Plastic mulches absorb the incoming solar radiation and transfer a considerable part of it to the soil, increasing the soil temperature. This increased temperature can lead to better nutrient and water absorption by plants by influencing soil physiochemical properties and microbial activities. Elevated temperatures can increase the availability of essential nutrients and organic carbon (Islam et al., 2020) as well as stimulate microbial activity, accelerate the decomposition process, and facilitate nutrient cycling (Auwal et al., 2021; Dai et al., 2020).

The highest plant height was observed under silver plastic mulching at all stages of observation, and the lowest under control (Table 1). This is in line with the study conducted by Ekinici & Dursun (2009) who reported the highest plant height under plastic mulch in melon (*Cucumis melo L.*). Similar results were observed by (Hallidri, 2000) on cucumber and (Chaurasia et al., 2020) on summer squash. The use of plastic mulch inhibits evaporation, moderates soil temperature, and maintains favorable moisture conditions, thereby promoting improved root development and nutrient absorption, ultimately enhancing overall plant growth (D. Kumar & Sharma, 2018).

Similarly, silver plastic mulch resulted in the maximum number of leaves per plant and was statistically similar to rice straw mulch and wheat straw mulch at 20 and 60 DAT, respectively (Table 2). This finding is consistent with findings (Rajablariani et al., 2012) who reported the maximum number of leaves per plant in mulched soil compared to bare soil in tomatoes. Similar results were obtained by (Bhatt et al., 2011) on summer squash and by Sil et al. (2022) on cauliflower. The increase in leaves per plant is directly linked to the absorption of solar radiation by mulches, especially plastic mulch, and the transfer of a significant portion of it to the soil, thereby elevating temperatures to levels conducive to increased leaf formation (Radhika Regmi et al., 2021).

In addition, the maximum plant spread was observed under silver plastic mulch and the lowest under the control condition (Table 3). Similar findings were reported by (Bhatt et al., 2011) and (Kumar et al., 2012) in summer squash and strawberry, respectively. Mulching helps in maintaining soil moisture, temperature, and nutrient level, which are essential for optimal plant spread (Sharma & Menon, 2020).

Furthermore, the lowest number of days to first staminate and pistillate flowers was observed under the plastic mulch condition, while the highest number was reported under the control condition (Table 4). Bhatt et al. (2011) observed earliness in flowering in plastic mulches, followed by organic mulches. He concluded that higher soil temperature under plastic mulch improves the plant microclimate, leading to early growth and development, which advanced flowering. Similarly, control resulted in the maximum number of male flowers per plant, while plastic mulch resulted in the highest number of female flowers per plant. The highest sex ratio was observed in no mulching, and plastic mulch showed the lowest sex ratio (Table 4). Karki et al. (2020), in a similar study, reported the highest number of female flowers per plant under plastic mulch conditions and the lowest under control.

Enhanced fruit dimensions, including length and diameter, as well as increased average fruit weight, were observed under silver plastic mulch. This observation aligns with the findings of Reddy et al. (2022) and Hossen et al. (2023), who reported superior fruit length and diameter under plastic mulch conditions. Additionally, Bhatt et al. (2011) documented a greater average fruit weight under plastic mulch. Plastic mulch resulted in the highest yield (41.44 mt ha<sup>-1</sup>) while control resulted in the lowest yield (11.77 mt ha<sup>-1</sup>) (Table 5). Similar results were reported by Chaudhary et al. (2023), Rajablariani et al. (2012), and Chaurasia et al. (2020) in okra, tomato, and summer squash, where the highest yield was obtained under plastic mulch conditions. The better dimension and weight of fruit and yield under plastic mulch can be attributed to the improved moisture retention, soil temperature regulation, increased nutrient availability, and effective weed suppression (Han et al., 2021; Samphire et al., 2023).

Organic mulching increased soil organic matter%, with the highest under rice straw mulch while the lowest was reported under plastic mulching (Table 6). This is in line with the findings of Bajorienė et al. (2013) where a higher content of SOC (soil organic carbon) was established in all mulched experimental plots compared with the unmulched plots. Zhai et al. (2008) reported that the concentration of soil organic matter, at a soil depth of 0–20 cm, was considerably higher under the mulching treatment as



compared to the control. Similarly, Kumar (2014) observed the highest soil organic carbon in farmyard manure, followed by brankad (*Adhotada vassica*), maize straw, bajra straw, and palah leaves, and the lowest without mulch. Samphire et al. (2023) reported an increased organic matter decay rate and a higher soil CO<sub>2</sub> efflux rate under plastic film mulching and suggested that plastic film mulching can accelerate the decomposition rate of organic matter content. This might explain the lower soil OM content in plastic mulch compared to the control.

Despite the higher production costs associated with silver plastic mulch, it demonstrated the highest net return (NRs/ha) and a benefit-cost ratio of 2.49. This result is consistent with the findings of Thapliyal et al. (2014) and Bhatt et al. (2011), who also reported higher net returns and benefit-cost ratios under plastic mulch conditions. Regmi et al. (2021) similarly observed higher net returns and benefit-cost ratios with silver plastic mulch, further supporting our findings. Conversely, rice husk mulch, as an organic alternative, offers a more economical cost of production and the highest benefit-cost ratio (1.96) among the remaining treatments.

## Conclusion

Overall, this study presents mulching as a suitable and efficient agricultural practice that can enhance the growth and yield performance of summer squash. Silver plastic mulch was superior to other mulching materials in promoting plant height, number of leaves, plant spread, and floral characteristics. Notably, silver plastic mulch resulted in the highest yield and productivity, followed by rice straw mulch, wheat straw mulch, and rice husk mulch. The increased performance under mulching conditions can be attributed to soil moisture conservation, reduced evaporation, lower weed population, and soil temperature regulation. Organic mulches, such as rice straw, significantly increased soil organic matter percentage, indicating their potential to enhance soil health. However, plastic mulching recorded the lowest organic matter percentage, likely due to accelerated decomposition rates. Despite its higher expense, silver plastic mulch yielded the greatest gross and net returns and a favorable benefit-cost ratio, highlighting its economic viability. Rice husk mulch emerges as a notable alternative as it offers a lower production cost and the second highest benefit-cost ratio among the tested treatments. Additionally, as an organic option, rice husk mulch can also enhance soil organic matter and contributes positively to soil health. Thus, this study emphasizes the importance of selecting the appropriate mulching material to maximize plant growth, yield, and economic returns.

## Limitations of the study and suggestions for future study

This study was conducted on a single soil type, focusing only on soil organic matter and pH. Hence, it is recommended that future investigations should analyze impacts of diverse kinds of mulching materials on various soil types as well as climatic conditions for long times. The results would be more generalized if they involved experiments done at different sites and during multiple

seasons. Moreover, the economic analysis carried out in this research was short-term. To examine how it affects the economy over a longer period would require an extensive approach that involves both short-term and long-term cost-benefits analysis. This will help to provide some clarity on whether or not the use of mulching materials are economically viable in long run. The scope of tested mulching materials was narrow. Further inquiries are needed to encompass a wider array of organic and inorganic mulches that better inform their efficiency across crops and conditions. Similarly, a wider range of such properties like microbial activity, soil structure etc., should be evaluated alongside environmental hazards related to biodegradable plastics. Investigating how different mulching materials interact with various irrigation practices is another crucial area for future research. This would provide valuable insights into the water efficiency of mulching materials, particularly in regions where water is scarce.

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## Why do Farmers not Use Fertilizer? A Case of Multistorey Cropping System with Abaca under Coconut in Zamboanga Peninsula, Philippines

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### ABSTRACT

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The Zamboanga Peninsula region significantly contributes to the Philippine coconut production, wherein abaca is also cultivated as an additional crop within coconut plantations. Still, coconut farmers belong to the country's poverty sector. Abaca plays a significant role in providing an alternative source of income as a perennial intercrop in agroforestry systems like coconut. However, the productivity and sustainability of this farming system are affected by practices such as nutrient management, which is not explicitly known. Using a mixed-methods approach, this study applies a combination of quantitative and qualitative data-gathering techniques to assess the nutrient management practices adopted by the 33 qualified respondents, including challenges encountered and perceptions to abaca under the coconut farming system and fertilizer use. Quantitative data were analyzed using IBM SPSS Statistics version 29, and thematic analysis was utilized for qualitative data. Results indicate that most (88%) of the farmers in the area did not apply fertilizers for their abaca. Yet, they still consider abaca as one of the income-generating crops besides coconut. The lack of financial resources and irregular harvesting operations are significant challenges that hinder fertilizer application. Findings revealed the need to develop low-cost and practical nutrient management technology for abaca under coconut, which shall be complemented with an effective human resource management system of skilled harvesters for the continuous harvesting operation of abaca to translate good crop growth into good yield and income. This will serve as a basis for government institutions and other stakeholders in developing and implementing programs and policies to improve the abaca and coconut industries.

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## Introduction

There is no denying that farming has become more challenging over time. Decreased agricultural land area, low productivity, low income, extreme weather conditions, and climate change threaten food security and agricultural production. These issues, which have impacted the world's major agricultural systems, are unresolved and only worsen with time.

In the Philippines, coconut farming is one of the country's critical agricultural systems and accounts for most agricultural exports. It is cultivated by 2.5 million coconut farmers in 69 out of 82 provinces with a total land area of 3.6 million hectares, an annual nut production of 14.7 metric tons, and average export earnings of 91.4 billion pesos for 2014–2018 (PCA, n.d.). However, most coconut growers and their families face the most

challenging obstacles to achieving a steady living above the country's poverty line (Andriessse, 2018). According to Mendoza et al. (2018), the low income from coconut is attributed mainly to the low yield. Diversifying coconut farms can help improve productivity, especially in places where there are old coconuts. Farm diversification is a strategy to address the challenges of stagnant yields, soil degradation, environmental concerns, diseases, pests, and weeds (Hufnagel et al., 2020).

Intercropping is a common form of diversification applied in coconut-growing regions across the Philippines. The suitability of intercrops shall be based on light requirements. Light transmitted under coconut stands varies with planting distance and age of coconut trees; at 40 years of age, 37% of light is transmitted and is suitable

for shade-tolerant crops as well as crops with a wide range of light requirement such as bananas and sweet potatoes, (Dauzat et al., 1997). Similar to bananas, abaca thrives in the presence of coconut and serves as a lucrative intercrop (Eroy, 2012). As a shade-loving plant, abaca is a potential component of farm diversification. Abaca, also known by its scientific name *Musa textilis* Nee, is a native perennial fiber crop of the Philippines that belongs to the Musaceae family, like banana. It grows well in the shade of coconut, ipil-ipil, anii, dapdap, and similar trees (PhilFIDA, n.d.-a). Abaca has many economic uses and is one of the country's agricultural exports. More than 80% of the world's supply of abaca fiber comes from the Philippines. Exports of abaca fiber and products generated an annual average of 119 million US dollars (2010–2019) from pulp, cordage, fabrics/yarns, fiber crafts, and raw fiber (PhilFIDA, n.d.-b). Harvesting abaca typically spans 16-24 months from planting, followed by consecutive harvests every three to four months, resembling the pattern observed in well-established coconut farms.

For perennial plants like abaca and coconut, a continuous nutrient supply is essential for long-term productivity. As the plant grows, it requires an increasing quantity of crucial elements. Soil health and nutrient management are necessary for plant growth and development. It is associated with several plant responses, including enhancements to crop yield and quality (Miner et al., 2020) and resistance to plant pests and diseases (Thakur et al., 2021). According to Crisostomo et al. (2023), most coconut plantations in the Philippines do not usually use fertilizer, which leads to significant differences in yield of 31 to 87% between farmers' areas and well-managed farms.

Although abaca is endemic in the country, there is limited available knowledge of nutrient management in multistorey cropping under coconut. Abaca planted under coconut in the region is observed to have poor growth. High fertilizer cost had hindered the farmers to apply fertilizer for maintenance. Therefore, it is vital to identify the practical methods farmers use and examine their perspectives on nutrient management of abaca under coconut. This information will provide the foundation for national government agencies such as the Philippine Fiber Industry Development Authority (PhilFIDA), the Philippine Coconut Authority (PCA), and the Department of Agriculture (DA), as well as local government units, and other stakeholders, to develop and implement policies, programs, and projects aimed at enhancing crop productivity and income of abaca and coconut farmers while also addressing food security, promoting a climate-resilient farming system, and encouraging sustainable agriculture.

## Materials and Methods

### Study Area

The study was conducted in the southernmost part of the Philippines at the Zamboanga Peninsula Region, island of Mindanao (Figure 1). The study areas included two provinces comprising one city and twelve municipalities, specifically the municipalities of Aurora, Bayog, Josefina, Kumalarang, Lakewood, Tigbao, and Sominot in the province of Zamboanga del Sur, and the city of Dapitan and municipalities of Baliguian, Jose Dalman, Mutia,

Pinan, and Sindangan in the province of Zamboanga del Norte. These provinces are among the country's top-producing provinces of coconut (PCA, n.d.), where abaca is also grown. In 2018, the area planted to coconut was 127,354 hectares in Zamboanga del Sur province and 240,298 hectares in Zamboanga del Norte, (PCA, n.d.). According to the land suitability map for abaca developed by the Bureau of Soils and Water Management (BSWM), the provinces of Zamboanga del Sur (BSWM, 2019b) and Zamboanga del Norte (BSWM, 2019a) have the potential of planting abaca under coconut areas by 88,923 and 133,033 hectares respectively. However, the total abaca areas planted in the entire Zamboanga Peninsula region is only 2,501 hectares with an annual average (2010-2019) fiber production of 614 metric tons that is 0.9% from the national production of 67,545 metric tons (PhilFIDA, n.d.-b).

### Participants of the Study

The participants of this study were the farmers who cultivated abaca under coconut for at least two years. Participants did not receive prior assistance in terms of cash incentives and fertilizer subsidies for abaca. This purposive identification of participants provided a better exploration of data from the farmers who utilized their own capabilities and resources to establish and maintain their farms. This also facilitated the identification of original farmers' practices in abaca under coconut farming. The identification of beneficiaries was coordinated with the Philippine Fiber Industry Development Authority Regional Office IX. This study utilized total enumeration for the qualified participants identified in the list. Out of the 37 farmers listed, only 33 had participated.

### Method of Data Collection

This study employed a mixed-methods approach, incorporating both quantitative and qualitative data. Quantitative data collection used a farmer's survey, reinforced by qualitative data from in-depth interviews and focus group discussions (FGD). The interview questionnaires and guide questions were tested outside the locale of the study with similar farming system and local dialects at Sibantang, Talisayan, Misamis Oriental, Philippines and were finalized. The survey was done in an interview format composed of sections on personal and farm profiles, nutrient management practices, and their perceptions of abaca farming and fertilizer use. The in-depth interview was conducted using open-ended questions that focused on describing the nutrient management practices of abaca and its challenges. To further expound and validate the data, the focus group discussion was conducted after the in-depth interview in areas that involved several numbers of farmers in a barangay such as in Bolisong, Kumalarang, Zamboanga del Sur and Nanganangan, Tigbao, Zamboanga del Sur. Before conducting the survey and in-depth interview, a courtesy call was made to local officials in the vicinity of the respondents. At the start of the interview, the participants were informed about the purpose of the research. Their consent was obtained, including permission to use video, camera, and recorders and utilize the data obtained for publication. Audio-video recording and a reflective notebook were done to support data collection. Data gathering started from October 2023 to December 2023.



Figure 1. Map of the study area in Zamboanga Peninsula Region of Mindanao, Philippines modified from Google Earth Pro

### Data Analysis

After data collection, quantitative data were analyzed using IBM SPSS Statistics version 29 for frequency and weighted arithmetic mean statistical measurements. Using the same software, a Kruskal-Wallis Test was done to evaluate the differences in fiber yield across the different nutrient management practices and a Fisher's exact test was done for the nonparametric evaluation of socio-demographic profiles across nutrient management. In order to provide additional support for the findings derived from the quantitative data, a qualitative analysis was conducted. Qualitative analysis offers a distinct and valuable understanding of individuals' experiences beyond quantitative analysis (Ayre & McCaffery, 2022). It is instrumental in exploring the challenges encountered by the respondents in the nutrient management of abaca under coconut. The qualitative analysis technique employed in this study was thematic analysis, following the guidelines established by Kiger and Varpio (2020). These guidelines involved familiarizing oneself with the data, coding the data, identifying themes, reviewing the themes, defining the themes, and finally, producing the report.

## Results and Discussion

### Socio-demographic and farm profile

The descriptive analysis (Table 1) shows that farmers who cultivated abaca under coconut in the Zamboanga Peninsula Region were predominantly male (64%). Most (84%) were above 40 years old and were generally married. Most of the respondents (36%) were at the elementary level, and only 12% had graduated from college. Regarding ethnicity, Bisaya (76%) was the dominant group, compared to Subanen (24%). Regarding farm profiles, almost all the respondents (91%) own the farmland, and only 9% were tenants. Moreover, more than half of the respondents were cultivating abaca under coconut in relatively small areas, less than 5 hectares, with the majority (94%) planting the Kutaykutay cultivar of abaca than the Lunhan cultivar (6%). Meanwhile, the maximum number of years recorded in abaca cultivation under coconut in the region was 23 years. However, most (79%) respondents had planted abaca under coconut within 2-10 years. It also shows that the coconut areas planted with abaca were already old, with

coconut established already ahead of abaca more than ten years ago (24% were planted 10-19 years ago, and 30% were planted more than 50 years ago).

### Influence of socio-demographic profile on nutrient management

The Fisher's exact test (Table 2) revealed no significant difference in the response distribution across the variables of age, sex, marital status, education, and ethnicity against the variable of nutrient management, as indicated in all P-values greater than 0.05. The result showed that the distribution of responses among males and females, married or not, with high educational attainment or not, young or old, and whatever ethnicity they belong to does not influence the nutrient management of abaca under coconut. This result is similar to the observation of Valleser et al. (2020) in cacao plantations where similar socio-demographic profiles did not affect their performance. Socio-demographic factors do not influence the decision to use fertilizer for abaca under coconut. Future interventions in this regard may not be discriminatory to the said sociodemographic profiles.

### Nutrient management practices and yield

Three nutrient management practices for abaca under coconut were identified in this study: 1) applying no fertilizer at all; 2) using synthetic fertilizer exclusively in the first year of farm establishment; and 3) using both synthetic and organic fertilizers in the first year of farm establishment only. Result shows (Figure 2A) that majority of the farmers (88%) in the region did not apply fertilizer throughout the growth duration of abaca under coconut, only 6% of the respondents applied purely synthetic fertilizers like urea (46-0-0) and complete fertilizer (14-14-14) and the remaining 6% used both organic and synthetic fertilizer (carbonized rice hull + 14-14-14 and chicken manure + 16-20-0 + 14-14-14). Fertilizers were applied only during the first year of farm establishment and no succeeding application followed. Since majority of the respondents did not use fertilizer, result indicates that most farmers rely solely on the inherent capacity of the soil to supply nutrients to the crops. Bande et al. (2013) stated that supplementing NPK fertilizer during the early stage of abaca growth positively impacted crop growth and net assimilation rates. According to Armejin (2008), the predominant decline in soil fertility of abaca areas due to the depletion of nutrients as a result of no fertilizer application causes a significant reduction in fiber yield and income.

Figure 2B comparatively illustrates the corresponding yield of the three nutrient management practices. Respondents that did not apply fertilizer at all have reported an average annual fiber yield of 681.67 kg, while respondents who applied synthetic fertilizer obtained 700 kg per year and respondents who applied both synthetic and organic fertilizer obtained an annual average production of 900 kg. However, the Kruskal-Wallis Test revealed no significant difference in fiber yield across three nutrient management practices. This indicates that all practices are still deemed insufficient, falling short of the attainable yield of 1700 kg per hectare set by the Bureau of Agriculture and Fishery Standards (2019) for the Kutaykutay cultivar under good agricultural practices.

Table 1. Socio-demographic profile of farmers and farm characteristics of abaca under coconut in Zamboanga Peninsula Region

Variables		Frequency <sup>N=33</sup>	Percentage%
Sex	Male	21	64
	Female	12	36
Age	Below 18	0	0
	18-29	2	7
	30-39	3	9
	40-49	10	30
	50-59	11	33
	60 and above	7	21
Marital Status	Single	2	6
	Married	29	88
	Widow/er	2	6
Educational Attainment	Elementary Level	12	36
	High School Level	8	24
	College Level	9	27
	Graduate Level	4	12
Ethnicity	Subanen	8	24
	Bisaya	25	76
Land Ownership Status	Landowner	30	90.91
	Tenant	3	9.09
Area Planted with Abaca under Coconut (hectares)	Less than 1	5	15.15
	1-2	13	39.40
	3-4	8	24.24
	5-6	4	12.12
	7-10	2	6.06
	More than 10	1	3.03
Age of Abaca from Planting (Years)	2	5	15.15
	3-5	13	39.40
	6-10	8	24.24
	10-15	2	6.06
	15-20	2	6.06
	More than 20	3	9.09
Abaca cultivar used	Kutaykutay	31	93.94
	Lunhan	2	6.06
Age of Coconut from Planting (Years)	Less than 10	0	0
	11-19	8	24.24
	20-29	6	18.18
	30-39	6	18.18
	40-49	3	9.10
	50 and more	10	30.30

Table 2. Fisher’s exact test on the correlation of age, sex, ethnicity, educational attainment, and marital status to nutrient management of abaca under coconut

Variables	P-value
Age	0.586
Sex	0.271
Marital Status	1.000
Education	0.587
Ethnicity	1.000

This result is comparable to the observation of Armeccin et al. (2011) in Leyte, where abaca fiber yield in the same cropping system is low. Similarly, Calica et al. (2024) also observed low production for abaca in North Cotabato, Mindanao, Philippines at 600 Kg per hectare per year with less farm inputs like fertilizer and chemicals. Gliessman (2016) explained that in an agroecosystem like this, a substantial amount of nutrients is lost due to crop removal, so sustainability requires nutrient recycling mechanisms and soil amendments.

With poor fiber production, the income is also low. Based on the findings of Calica et al. (2024), the 600 kilogram per hectare per year fiber output can generate a net income of Php7,600. This is calculated by taking into account the purchasing price of fiber at Php55 per kilo with a gross sale of Php33,000, deducted by the expenses on harvesting or the share of harvesters (60% of the gross sales or Php19,800), weeding expense (Php5,000), and transportation costs (Php1 per kilo or Php600).



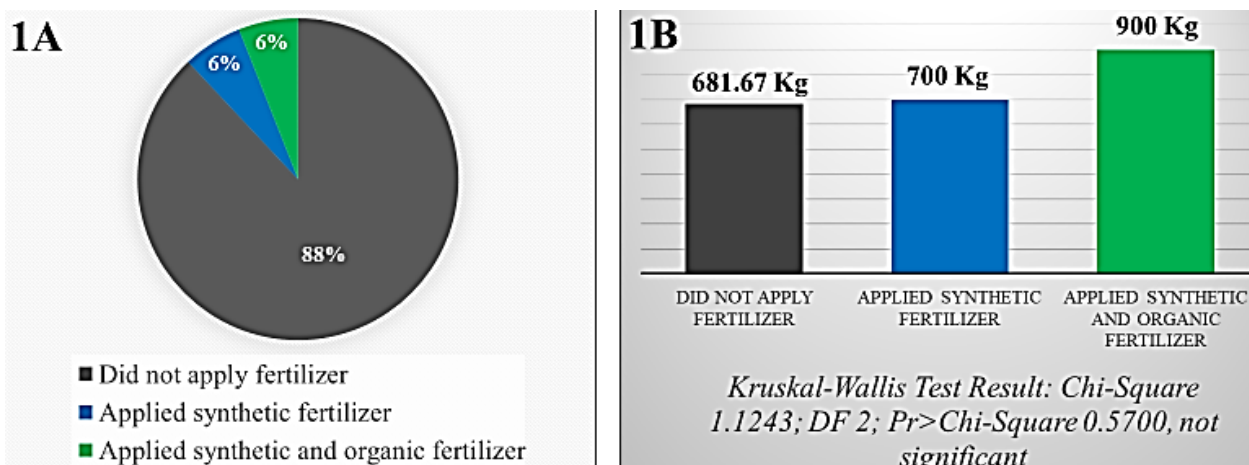


Figure 2. Percentage of respondents who adopted fertilizer application in abaca under coconut (1A) and the corresponding annual average yield per hectare (1B)

Using similar price and costs, a farmer who did not apply fertilizer in this study can earn an income of Php9,315 while the farmers who applied pure synthetic fertilizer earned Php9,700 and the farmers who applied both synthetic and organic fertilizer earned Php13,900. The earnings of farmers who utilized fertilizers do not include fertilizer costs since they only used fertilizer during the first year of abaca farm establishment, and the respondents' abaca farms were more than two years old, hence maintenance costs omit fertilizer. Moreover, considering the potential yield set by BAFS at 1700 kilograms, a gross sale of Php93,500 per hectare per year can be attained using good agricultural practices which includes fertilizer application.

#### **Factors Affecting Fertilizer Use**

Two themes emerged from the qualitative analysis regarding the challenges in nutrient management of abaca under coconut: insufficient financial resources and inconsistent harvesting. We described insufficient financial resources as the most prevalent challenge of nutrient management among the respondents who did not apply fertilizer. Farmers bear the financial strain of high fertilizer capital requirements, whereas abaca can continue to generate income for them even without fertilizer application. This belief is well elaborated in the statement of Bienvenido,

“Nowadays, prices of household needs are extremely high, including fertilizers. Our income is good only for our basic household needs and the school needs of our children. That is why we cannot afford to buy fertilizer for our abaca. Also, abaca can still grow without fertilizer, it only needs to be free from weeds”. This result conforms to the findings of Ayoo (2022), where the lack of financing is the major constraint for economic prosperity in many developing countries. The results of this study illustrated the actual circumstances experienced by the respondents as they struggled with the financial requirements of sustaining an ideal farm, explicitly concerning the substantial expense of fertilizers given their limited economic means and other competing needs. Moreover, this result also conformed to the findings of Calica et al. (2024) in North Cotabato and Cortez et al. (2015) in Catanduanes, wherein farmers provided only low farm inputs in their abaca farms. Camilo also remarked,

“If the government provides fertilizer support for abaca, it would boost our yield. However, if it is only us, we should prioritize our household needs before buying fertilizer”.

For sustainability and productivity to take place, there should be a wise consideration between what is ideal and what is real. In this case, the insufficient funds for fertilizer must be considered. In addition, aside from fertilizers, other factors such as planting density, climatic and environmental factors, and other cultural and farm management practices must also be optimized for sustainable productivity, as Bande et al. (2016) explained in their study with the abaca-based agroecosystem.

Meanwhile, farmers who applied fertilizer during the farm establishment observed good growth of their plants. However, the absence of harvesting or inconsistent harvesting schedules discouraged them, neglecting the farm. Pablo, a farmer who applied organic and commercial fertilizers during the first year of his abaca farm establishment, explained that the delays and irregular harvesting of his abaca due to the lack of skilled harvester had discouraged him to continue providing appropriate care and management for his abaca including fertilizer application.

“Establishing a farm is not only expensive, but it also requires time and attention; seeing your plants grow well is very inspiring, but knowing that it could not be harvested due to the lack of a skilled harvester is disheartening,” he said.

Abaca takes two years from planting before its first harvest and requires skilled harvesters in the extraction of fiber; the delay in harvesting can be frustrating, especially for small-scale farmers like him who expect an immediate return to operationalize the farm continually. Harvesting or fiber extraction of abaca is a labor-demanding activity that requires skill in its processes, including machine operation. Small-scale farmers in the area do not have the machines and harvesters and rely only on the group of harvesters to harvest the farm. These groups are pretty few and also engaged in several farm activities. Nevertheless, he observed that applying both organic fertilizers (chicken manure) and commercial fertilizers (16-20-0 and 14-14-14) improved the growth of abaca and the nut yield of coconut.



Table 3. Perception of farmers to abaca under coconut farming system and fertilizer use

Indicators	Mean	Description
Abaca under coconut farming system		
1. Planting abaca under coco provides another source of income.	4.61	SA
2. Planting abaca under coco minimizes weeds.	4.39	SA
3. Planting abaca under coco improves soil fertility.	4.12	A
4. Planting abaca under coco increases the yield of coconut.	3.97	A
5. Planting cover crops in abaca under coco is possible even when abaca grows taller.	2.67	U
Fertilizer use		
1. Organic fertilizer is more economical than synthetic fertilizer	4.03	A
2. Organic fertilizer can improve the growth and yield of abaca and coconut.	4.00	A
3. Soil analysis is necessary for the nutrient management of abaca under coconut.	3.88	A
5. Abaca and coconut can still be productive even without fertilizer.	3.79	A
4. Continuous fertilizer application is essential for abaca and coconut's long-term productivity.	3.73	A
6. Fertilizer application is necessary for abaca under coco.	3.70	A
8. A combination of organic fertilizer and synthetic fertilizer is more economical and more effective.	3.58	A
7. Synthetic fertilizer is more effective than organic fertilizer.	3.23	U

Legend: Mean Value

Score	Mean Range	Description
5	4.21-5.00	Strongly Agree (SA)
4	3.41-4.20	Agree (A)
3	2.61-3.40	Uncertain (U)
2	1.81-2.60	Disagree (DA)
1	1.00-1.80	Strongly Disagree (SD)

#### ***Farmers' Perception of Abaca Under Coconut Farming and Fertilizer Use***

The respondents in this study expressed their high agreement that abaca provides another source of income and minimizes weeds based on the weighted arithmetic mean of 4.61 and 4.39, respectively, as shown in Table 3. Additionally, the respondents agreed with the succeeding statements as indicated in the corresponding means that planting abaca under coconut improves soil fertility (4.12) and increases the yield of coconut (3.97). On the contrary, the respondents are uncertain about planting under-story crops (2.67) in this farming system.

Despite the low yield obtained by the respondents, they still highly agreed that abaca provides another source of income. Since abaca is planted under coconut, it contributes as a supplementary source of income in the uplands where most of the coconut and abaca are grown. This finding aligns with the research of Lacuna-Richman (2002), which states that abaca serves as an additional source of income for the coconut households in Eastern Visayas, and it only requires minimum input in terms of fertilizers, pesticides, and irrigation as well as in processing. While the respondents see abaca farming as a means of income generation with low input or minimal cost, it can be a potential component of agroecological sustainability. This type of agroforestry encompasses economic, social, and ecological benefits. According to Fahad et al. (2022), agroforestry enhances soil structure, microclimate, and hydrologic functions by promoting soil-related microbial activity and mitigating the effects of rainfall. Additionally, it diminishes the process of soil erosion and the degradation of nutrients. Agroforestry has the potential to enhance and revive soil-based ecosystem services, promote sustainability in agriculture, and mitigate disease risks and vulnerability to climate change.

Although the majority of the respondents did not use fertilizer in their actual farms, as they agreed that abaca and

coconut can still be productive even without fertilizer (3.79), they also believed that continuous application of fertilizer is needed for long term productivity of abaca and coconut (3.73) and that fertilizer application is required for this type of farming system (3.70). With regards to nutrient management, the respondents agreed that organic fertilizer is more economical than synthetic fertilizer (4.03), it can improve the growth and yield of abaca and coconut (4.00), and they also agreed that soil analysis is necessary for nutrient management of abaca under coconut (4.0) as well as on the statement that the combination of organic fertilizer and synthetic fertilizer is more economical and more effective (3.58). However, they are uncertain about the effectiveness of synthetic fertilizer over organic fertilizer (3.23). This result is further elucidated by Diosdado, a farmer who has been cultivating abaca for about nine years, and Pablo on their observations that, in contrast to costly and non-ecofriendly synthetic fertilizers, organic fertilizers like rice hull and chicken manure, though not quick acting but have long-term effects in plant nutrition which is valuable for perennial crops like abaca and coconut. According to them,

“Synthetic fertilizers are effective, but only for a shorter period; you need to apply again for continuous nutrition compared to the organic fertilizers, which are slow but long-lasting”.

The respondents believed organic fertilizer is more advantageous than synthetic fertilizers in the perennial cropping system of abaca and coconut. This statement is supported by Maitra et al. (2021), who stated that using organic fertilizer in multiple cropping is also suitable because this system promotes less incidence of pests and disease and maintains soil fertility. Shah and Wu (2019) also pointed out that organic fertilizers are the best practical alternative to synthetic fertilizers, improving crop productivity and ecological conservation. In addition, Rosati et al. (2021) proved that organic farming exhibits

greater resilience in the face of challenging conditions, such as drought, due to its substantial organic matter content and superior water retention ability. Moreover, organic fertilizer is more resistant to nutrient leaching. This is particularly enhanced in agroforestry systems like abaca and coconut, where nutrients are contained and leaching is prevented. Agroforestry enhances soil fertility through the deposition of leaf litter while also mitigating soil acidity and preventing erosion. Even though there have been extensive discussions on this matter, it remains uncertain which specific type of organic fertilizer or fertilizer combination is best for abaca in multistorey cropping under coconut, that is both practical for farmers' needs and can result in a yield comparable to the standards set by BAFS.

## Conclusion

Abaca, under coconut farming, can still provide income to respondents even without using fertilizer. However, farmers believed using fertilizer would enhance productivity that is crucial for sustainability. Due to other competing needs and the high cost of fertilizer, insufficient funds prevent them from using fertilizers on abaca, and the absence of harvesting operations due to a lack of competent harvesters resulted in the farm being neglected, with care and management activities, including fertilizer application, discontinued. Developing and providing low-cost and practical nutrient management technology and an efficient management system for skilled harvesters are vital to ensure continuous abaca harvesting that will translate good crop growth into sustainable income. This will be the basis for government institutions and relevant stakeholders to develop appropriate interventions such as but not limited to fertilizer subsidy program and support for technology and production of organic fertilizer to increase yield. To maintain a continuous harvesting system, feasible options include developing and providing labor and cost-efficient fiber extraction machinery, training out-of-school youth in abaca harvesting skills, and incentivizing harvesting operations.

## Declarations

### *Ethical Approval Certificate*

The experimental procedures of this study were approved by the Institutional Ethics Review Committee of Central Mindanao University, Musuan, Bukidnon, Philippines, under permit number 0656.s.2023.

### *Author Contribution Statement*

Aladin Repaso: Conceptualization, data collection, analysis, and writing the original and final draft

Raquel Salingay: Conceptualization, supervision, review and editing

Zabdiel Zacarias: Conceptualization, data collection, review and editing

Myrna Pabiona: Conceptualization, review, and editing

Maria Estela Detalla: Conceptualization, review, and editing

Ma. Stella Paulican: Conceptualization, review, and editing

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## **Conflict of Interest**

“The authors declare no conflict of interest.”

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## Morphological and Phenological Attributes of Chickpea (*Cicer arietinum* L.) Affected by Different Growing Conditions, Zeolite and Nitrogen Applications

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### ABSTRACT

The present study investigated the effects of two different zeolite applications and different nitrogen-based fertilizers on chickpea's yield and yield components in dry and irrigated conditions. The field experiment was conducted during 2019 and 2020 in the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Türkiye. The experimental design was a split-split plot with four replicates. The main plots were grown under dry-irrigated conditions. At the same time, subplots received zeolite applications (zeolite<sup>+</sup>- zeolite<sup>-</sup>), and sub-sub plots received nitrogen applications [control, traditional, chemical, farmyard manure, and Isabion, (an animal collagen-derived biostimulant)]. The experiment found that irrigation caused a delay in phenological characters but had a favorable impact on morphological characters and yield. The effect of zeolite applications was different in the first and second years of the experiments for the investigated characters. In the first year, the application of zeolite had a significant impact on grain yield, but there was no discernible effect in the second year. The experiment demonstrated that both chemical fertilizer and farmyard manure positively impacted phenological and morphological characteristics. In both years, the farmyard manure plots produced the highest grain yield. Farmers in Türkiye are advised to apply nitrogen to their crops as the profitability of chickpeas has risen in recent years. If the high cost of farmyard manure renders its use impracticable, farmers can opt for chemical fertilizer as an alternative.

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### Introduction

Pulses have been extensively grown crops from prehistoric times across the globe. They provide a substantial amount of protein, dietary fiber, and minerals, and have numerous health advantages (Kaur & Prasad, 2021). Studies on legumes have demonstrated their significance in preventing diseases such as type 2 diabetes, cancer, cardiovascular disorders, and obesity. Chickpeas (*Cicer arietinum* L.), often known as garbanzo beans, are a type of leguminous food that has been valued for its protein content and has been a significant source of nutrition for humans for a long time (Gupta et al., 2017). Within agricultural systems, it functions as a substitute for fallow in cereal rotations, hence promoting production sustainability and diminishing the requirement for nitrogen fertilization by fixing atmospheric nitrogen.

The extensive utilization of synthetic fertilizers in agricultural activities leads to numerous environmental issues. Ammonia volatilization is the primary factor responsible for nitrogen depletion in agricultural systems on a global scale (Bouwman et al., 2002). Zeolites are a class of hydrated aluminosilicate minerals that possess an unending three-dimensional crystal lattice. They consist of cations derived from alkaline elements and alkaline soil

elements, together with other less frequently occurring cations (Jarosz et al., 2022). Zeolites have a high porosity in their crystalline structure, allowing them to retain water molecules that make up to 60% of their weight (Mumpton, 1985). Zeolites can alter the amount of water in the soil by changing its bulk density and aeration porosity (Ramesh & Reddy, 2011). Zeolites have been utilized in agriculture since the 1960s because of their efficacy as soil additives for promoting plant growth, their ability to exchange cations, and their capability to release fertilizers gradually (Vassilina et al., 2023). In their study, Sangeetha and Baskar (2016) found that zeolites have a high level of selectivity for the ammonium cation (NH<sub>4</sub><sup>+</sup>). As a result, the use of zeolites can help reduce ammonium loss.

Farmyard manure not only supplies soil with essential nutrients for plants but also enhances its physical characteristics. It increases the quantity of organic matter and promotes microbial activity in the soil. Farmyard manure contains a high concentration of organic matter and essential plant nutrients. Organic fertilizers are also the source of nitrogen for plants (Karayel et al., 2020) and nitrogen can be bound by organic compounds.

Biostimulants enhance plants' resilience to abiotic stressors by promoting plant growth and development. They have been employed in agriculture as a viable substitute for synthetic fertilizers to enhance the productivity and nutritional quality of food crops. Biostimulants enhance nitrogen assimilation by stimulating the initiation and transcription of nitrogen metabolism (Ertani et al., 2009). Biostimulant application significantly improved grain yield, nutrient content in plant and seed (Mukherjee et al., 2022). Gomez et al. (2024) reported that biostimulants positively affected germination in chickpea.

The aim of this work is to evaluate the influence of two different zeolite treatments and different nitrogen-based fertilizers on the morphological and phenological traits of chickpeas in both dry and irrigated conditions.

## Material and Methods

The field experiment was conducted during 2019 and 2020 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39°48' N; 30°31' E, 798 m above sea level). Figure 1 shows climatic data for the research area. The research area experienced a long-term average rainfall of 165.6 mm and an average temperature of 16.23°C from March to August. Total precipitation for the 2019 and 2020 growing seasons was 163.9 mm and 169.5 mm, respectively. Total precipitation was close to the long-term in both years, but there were differences in precipitation distribution in months. The total precipitation in June was more than 2.5 times the long-term total precipitation in the second year of the experiment. Chickpeas are sensitive to water deficiency at the start of flowering and pod setting (Adak, 2021). As a result, the plants benefited greatly from the second year of June precipitation. The mean temperatures were 16.13°C in the first year and 16.15°C in the second year (Table 1). In the first year, the Transitional Zone Agricultural Research Institute analysed soil samples from the research area and in the second year, the New Water Soil Analysis Laboratory did so. Soil samples were taken separately from both dry and irrigated plots in both years of the experiment. In the first year, the soil sample was slightly alkaline, very low in organic matter, moderately calcareous, unsalted, low in nitrogen, high in potassium, and low in phosphorus. Dry areas have clay loam soil, while irrigated areas have loamy soil (Anonymous, 2019). In the second year, the soil sample is slightly alkaline, very low in organic matter, moderately calcareous, unsalted, low in nitrogen, potassium high, and

phosphorus sufficient. It is loamy in dry areas and clay-loam in irrigated areas (Anonymous, 2020).

The experimental design was a split-split plot with four replicates. The main plots were grown under dry-irrigated conditions, subplots received zeolite applications (zeolite<sup>+</sup>, zeolite<sup>-</sup>), and sub-sub-plots received nitrogen applications [control, traditional, chemical, farmyard manure, and Isabion (an animal collagen-derived biostimulant)]. The Azkan chickpea variety was used as genetic material. Zeolite in the form of clinoptilolite was obtained from Manisa Gördes (Enli Mining Company). Diammonium Phosphate (DAP) (18N-46P%) was used as traditional fertilizer, and Ammonium Sulfate (AS) (21N%) + Triple Super Phosphate (TSP) (44% P<sub>2</sub>O<sub>5</sub>) was used as chemical fertilizer. The amount of nitrogen and phosphorus in the experiment was applied at equal doses in traditional and chemical fertilization. Farmyard manure was obtained from Mahmudiye district of Eskişehir province, and Isabion was obtained from Syngenta Company. Table 2 presents some of the physical and chemical properties of zeolite, Isabion and farmyard manure.

Sowing was done at a 30 cm row spacing at a seeding rate of 60 seeds per m<sup>2</sup> on April 26 and 15, 2019 and 2020, respectively. The seeds were sprayed to prevent root rot and anthracnose diseases before sowing. Table 3 provides the application times and ratios of the materials used in the experiment. The experiment irrigated the plants at emergence time, before flowering, during the flowering period, during the pod formation period, and the grain filling period. Herbicide application was done for weed control. A fungicide containing 25% Boscalid and 12% Pyraclostrobin was applied against *Ascochyta rabiei* before flowering and before grain filling period. The harvest was done by hand in the first year, August 26, 2019, and August 23, 2020, in the second year.

For all the sub-sub plots, the number of days to emergence, days to flowering and days to maturity were determined. The plant height (cm), first pod height (cm), number of branches, and branch diameter (mm) were evaluated on 10 randomly selected plants in each sub-sub plot. Each sub-sub plot was harvested and threshing, and grain yield (kg ha<sup>-1</sup>) was estimated (Tosun & Eser, 1975).

The experiments were analyzed with the MSTATC statistical programs. Means were compared by the Least Significant Differences (LSD) test (Steel & Torrie, 1980).

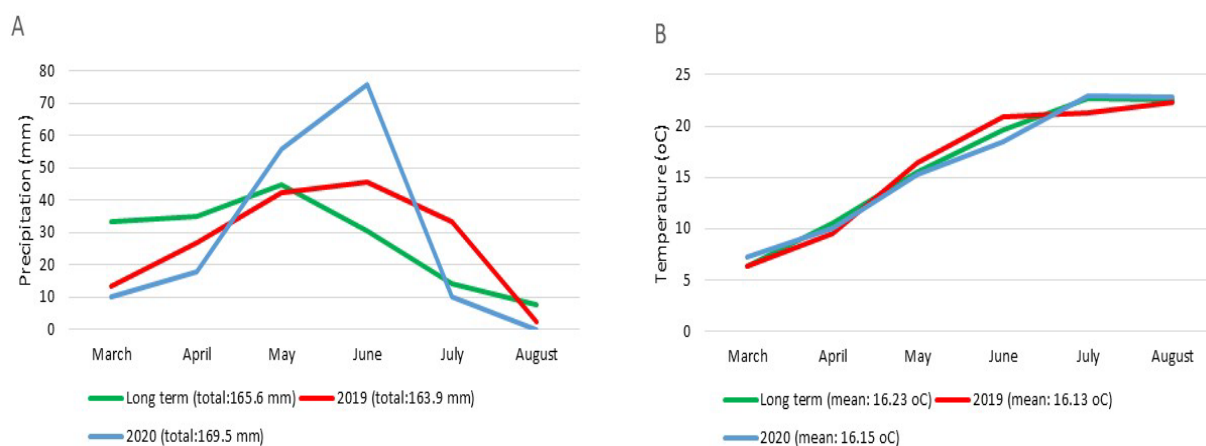


Figure 1. Climatic data of research area

Table 1. Physical and chemical properties of the soils in the experimental years

Year		Depth (cm)	pH	Lime (%)	Salt (%)	Organic matter (%)	N (%)	P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> )	K <sub>2</sub> O (kg ha <sup>-1</sup> )	Texture
2019	Dry	0-30	7.78	5.60	0.02	0.93	0.04	23.4	2720	Clay-loam
	Irrigation	0-30	7.80	5.46	0.01	1.23	0.06	22.0	3910	Loamy
2020	Dry	0-30	8.22	6.73	0.01	1.19	0.05	62.7	3500	Loamy
	Irrigation	0-30	8.20	4.75	0.02	1.26	0.06	93.8	4430	Clay-loam

Table 2. Physical and chemical properties of zeolite, isabion and farmyard manure

Properties	Zeolite	Isabion	Farmyard Manure
Organic matter (%)	20	62	48.2
Maximum humidity	20		
pH	6-8		7.2
Organic nitrogen (%)		10	
Organic carbon (%)		30	
Amino acids (%)		11	
N(%)			1.6
P (%)			0.35
K (%)			1.8

Table 3. Application times and ratio of the materials used in the experiment.

Material	Application time	Application ratio
Zeolite	Before sowing	1000 kg ha <sup>-1</sup>
Diammonium Phosphate (DAP)	Before sowing	140 kg ha <sup>-1</sup>
Ammonium Sulfate (AS)	Before sowing	25 kg ha <sup>-1</sup>
Triple Super Phosphate (TSP)	Before sowing	60 kg ha <sup>-1</sup>
Farmyard manure	Before sowing	20 tons ha <sup>-1</sup>
Isabion	Before flowering	3000 ml ha <sup>-1</sup>

## Results and Discussion

### *Phenological Observations (Seed germination time, flowering time, maturity time)*

In the first year, the growth conditions considerably impacted both the flowering time and maturity time. However, in the second year, only the growth conditions affected the maturity time. The seed germination period was determined to be minimal in both years due to the growing conditions. In the first year of the experiment, irrigation caused a delay in both the flowering and maturity time. However, in the second year, irrigation only affected the maturity time (Table 4, 5). Oğuz & Erman (2021) found that chickpeas experienced a delay in their flowering time when exposed to high relative humidity levels. Yolcu (2008) reported that the maturity time of chickpeas was delayed by irrigation. In the second year, there was a significant amount of rainfall, especially in June. The abundant rainfall hindered the ability to observe irrigation's impact on the flowering period's duration in the second year. The use of zeolite considerably impacted flowering time and maturity time in the first year, but these effects were not significant in the second year. The effect of zeolite applications on seed germination time was statistically negligible in both years, as seen in Table 4 and 5. Applying zeolite in the first year resulted in a postponement of both flowering and maturity time. Zeolite, a type of mineral, can absorb moisture present in the soil. This property of zeolite helps decrease the negative effects of drought on plants, known as drought stress (Mahmoud et al., 2023). Nitrogen applications considerably impacted seed germination time and maturity time in the first year. However, in the second year, only maturity time was

significantly affected by nitrogen applications (Table 4,5). The first year of the experiment resulted in the earliest occurrence of seed germination time in the farmyard manure plots. Akal (2016) states that using farmyard manure enhances soils' ability to absorb solar radiation, resulting in a faster warming process. Oğuz (2008) suggested raising the temperature of cold soils is advisable. Farmyard manure raises the soil's warmth. Consequently, the seed germination occurred early. The seed emergence period in the second year was not influenced by farmyard manure due to climate and soil conditions. The decreased temperature in April during the first year resulted in a more pronounced manifestation of the impact of farmyard manure. The farmyard manure plots in the first year exhibited the longest time to reach maturity, whereas the chemical plots in the second year offered the same results. In the first year, the farmyard manure assimilated moisture from the soil, delaying the maturity period. Abundant rainfall may have hindered the ability to observe the impact of farmyard manure in the second year.

Flowering time was postponed in all nitrogen applications, except in the control application, because of the water-absorbing characteristics of zeolite in the dry + zeolite<sup>+</sup> plots. Figure 2 demonstrates that using farmyard manure resulted in the most recent flowering time. Demir (2021) found that using organic fertilizers in chickpeas leads to delayed flowering compared to using chemical fertilizers. The hydrophilic nature of zeolite in the dry + zeolite<sup>+</sup> plots (Figure 2) caused a delay in the maturation period for all nitrogen treatments, except the conventional method.

Table 4. Effects of different growing conditions, zeolite applications and nitrogen applications on some traits of chickpea in 2019.

	SGT (day)	FT (day)	MT (day)	PH (cm)	FPH (cm)	NB	BD (mm)	GY (kg ha <sup>-1</sup> )
Dry	12.3	50.1 b	112.8 b	46.2 b	30.9	1.88 b	3.82 b	1180 b
Irrigated	12.3	52.5 a	119.0 a	48.7 a	31.7	2.05 a	4.76 a	2140 a
Mean	12.3	51.3	115.9	47.4	31.3	1.96	4.29	1660
Zeolite <sup>+</sup>	12.3	51.8 a	116.3 a	47.7 a	30.7 b	1.95	4.28	1730 a
Zeolite <sup>-</sup>	12.2	50.8 b	115.5 b	47.2 b	31.8 a	1.98	4.30	1590 b
Mean	12.3	51.3	115.9	47.4	31.3	1.96	4.29	1660
Control	12.5 a	50.6	116.1 b	46.5 d	30.9 c	1.91 b	4.24 b	1380 e
Traditional	12.3 a	50.6	115.1 c	47.4 bc	30.5 c	1.98 ab	3.97 c	1630 c
Chemical	12.7 a	50.4	114.9 c	48.6 a	32.6 a	2.03 a	4.35 b	1720 b
Farmyard man.	11.2 b	52.6	117.1 a	47.8 b	30.8 c	1.94 b	4.61 a	2070 a
Isabion	12.6 a	50.9	116.3 b	47.0 cd	31.5 b	1.96 ab	4.28 b	1510 d
Mean	12.3	51.3	115.9	47.4	31.3	1.96	4.29	1660
General Mean	12.3	51.3	115.9	47.4	31.3	1.96	4.29	1660
Growing cond. (A)	ns	**	**	**	ns	*	**	**
Zeolite app.(B)	ns	*	**	*	**	ns	ns	**
A × B	ns	ns	ns	ns	ns	*	*	ns
Nitrogen app. (C)	**	ns	**	**	**	*	**	**
A × C	ns	*	**	**	**	**	**	**
B × C	ns	*	**	**	**	ns	**	*
A × B × C	ns	**	**	**	**	ns	ns	ns

ns: non-significant, \*: p≤0.05, \*\*: p≤0.01. SGT: Seed germination time FT: Flowering time MT: Maturity time; PH: Plant height FPH: First pod height NB: Number of branches BD: Branches diameter GY: Grain yield

Table 5. Effects of different growing conditions, zeolite applications and nitrogen applications on some traits of chickpea in 2020.

	SGT (day)	FT (day)	MT (day)	PH (cm)	FPH (cm)	NB	BD (mm)	GY (kg ha <sup>-1</sup> )
Dry	12.4	67.0	130.8 b	69.4 a	34.2	2.38	6.47 b	2504 b
Irrigated	12.5	67.0	136.1 a	67.4 b	33.5	2.13	6.63 a	3253 a
Mean	12.5	67.0	133.5	68.4	33.9	2.25	6.55	2879
Zeolite <sup>+</sup>	12.4	67.0	133.7	69.5 a	34.1 a	2.35 a	6.75 a	2876
Zeolite <sup>-</sup>	12.5	67.0	133.3	67.3 b	33.6 b	2.16 b	6.35 b	2880
Mean	12.5	67.0	133.5	68.4	33.9	2.25	6.55	2879
Control	12.5	67.1	133.0 b	70.2 a	34.9 a	2.14 b	6.30 b	2467 c
Traditional	12.4	67.1	133.4 b	68.0 bc	33.6 b	2.45 a	6.44 b	2880 b
Chemical	12.2	66.8	134.3 a	67.2 c	32.8 c	2.27 ab	6.73 a	2867 a
Farmyard man.	12.4	67.0	133.1 b	68.1 bc	34.3 ab	2.22 b	6.91 a	3360 a
Isabion	12.5	66.9	133.6 ab	68.4 b	33.8 b	2.19 b	6.37 b	2817 b
Mean	12.5	67.0	133.5	68.4	33.9	2.25	6.55	2879
General Mean	12.5	67.0	133.5	68.4	33.9	2.25	6.55	2879
Growing cond. (A)	ns	ns	**	*	ns	ns	*	**
Zeolite app. (B)	ns	ns	ns	**	**	*	**	ns
A × B	ns	ns	**	ns	ns	ns	**	ns
Nitrogen app. (C)	ns	ns	*	**	**	**	**	**
A × C	ns	ns	ns	*	**	**	**	**
B × C	ns	ns	ns	**	**	**	**	**
A × B × C	ns	ns	ns	**	**	**	**	**

ns: non-significant, \*: p≤0.05, \*\*: p≤0.01. SGT: Seed germination time FT: Flowering time MT: Maturity time; PH: Plant height FPH: First pod height NB: Number of branches BD: Branches diameter GY: Grain yield

Among the plots tested, those with zeolite and without irrigation had the lowest values for maturity time, while the plots with zeolite and irrigation had the greatest values in the second year. Hence, the correlation between the growing conditions and the zeolite application might have played a significant role (Figure 3).

#### **Plant Height, First Pod Height**

The growing conditions considerably impacted the plants' height in both the first and second years. The first pod height was determined to have no substantial impact

on the growing conditions in both years, as seen in Table 4 and 5. During the first year of the experiment, the plant height exhibited a positive correlation with the application of irrigation. Irrigation is anticipated to promote greater development of the vegetative components of chickpeas. Chickpea has drought tolerance, nevertheless, irrigation exerts a beneficial impact on the plants. Togay et al. (2005) found that irrigation or enough soil moisture led to an increase in the height of chickpea plants. The excessive amount of rainfall in the second year of the experiment hindered the ability to observe the impact of irrigation,



leading to a decrease in plant height in the irrigated plots. Zeolite applications were significant for plant height and first pod height in first and second year, as shown in Table 4.5. The application of zeolite may have inhibited the leaching of plant nutrients into the soil. Consequently, plants could more readily obtain nutrients, resulting in increased plant height in the zeolite<sup>+</sup> plots. Bybordi (2016) and Amiri et al. (2021) reported that applying zeolite resulted in an augmentation of plant height. In their study, Erdin and Kulaz (2014) found a direct correlation between plants' height and the first pod in chickpeas. Nevertheless, during the first year of the experiment, the zeolite plots had a greater height for the first pod. In the second year of the experiment, the zeolite<sup>+</sup> plots had a greater height for the first pod. This outcome is anticipated. Nitrogen applications had a considerable impact on plant height and first pod height throughout the first and second years, as shown in Table 4.5. Applying chemical manure in the first year of the experiment resulted in the greatest plant height and the highest height of the first pod. According to Gul et al. (2015), plant height was greater in the chemical plots than in the control plots. In their study, Kaya et al. (2007) found that the control plot had the shortest first pod height, measuring 16.3 cm. However, the first pod height increased as the chemical fertilizer doses were raised, ranging from 18.2 cm to 19.2 cm in chickpeas. During the

second year of the trial, the control plots exhibited the greatest plant height and first pod height. The climate and environmental factors may have influenced the unforeseen outcome.

In the first year of the experiment, the plots treated with dry + zeolite<sup>-</sup> + farmyard manure had the lowest plant height, while the plots treated with dry + zeolite<sup>+</sup> + farmyard manure showed higher plant height (Figure 3). In the second year of the experiment, the plots irrigated and treated with zeolite had a higher plant height than those only irrigated and treated traditionally. However, the plots irrigated and treated with zeolite had the lowest plant height overall. The diagram is shown in Figure 4. For this reason, the interaction between nitrogen applications, zeolite applications, and growing conditions may have been of considerable importance. During the first year of the experiment, the plots treated with dry, zeolite<sup>-</sup>, and chemicals had the shortest first pod height. In contrast, the plots treated with irrigation, zeolite, and chemicals had the tallest first pod height (Figure 4). The irrigated + zeolite<sup>-</sup> + chemical plots had the lowest first pod height, whereas the irrigated + zeolite<sup>+</sup> + chemical plots had a greater first pod height in the second year of the experiment (Figure 5). For this reason, the interaction between nitrogen applications, zeolite applications, and growing conditions may have been significant.

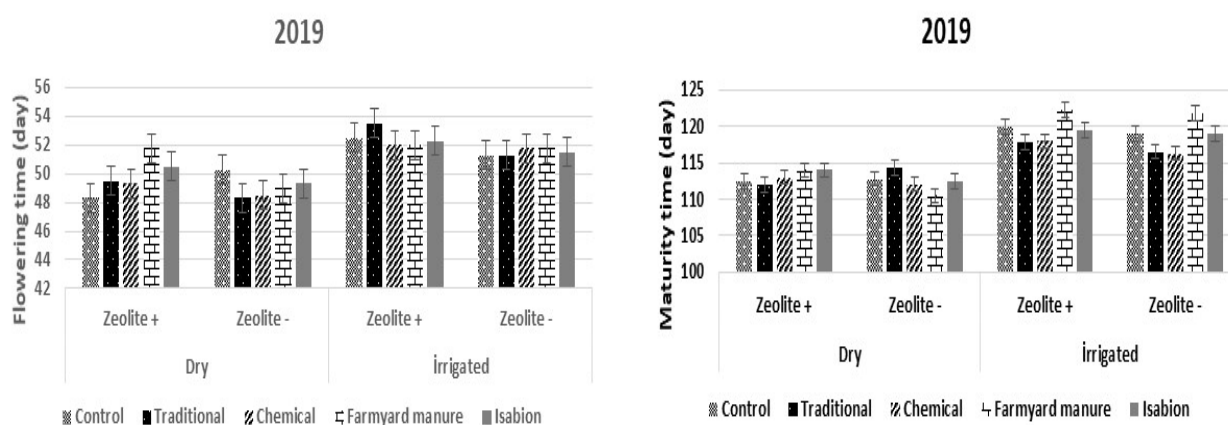


Figure 2. The interaction between growing conditions, zeolite applications and nitrogen applications for flowering and maturity time of chickpea.

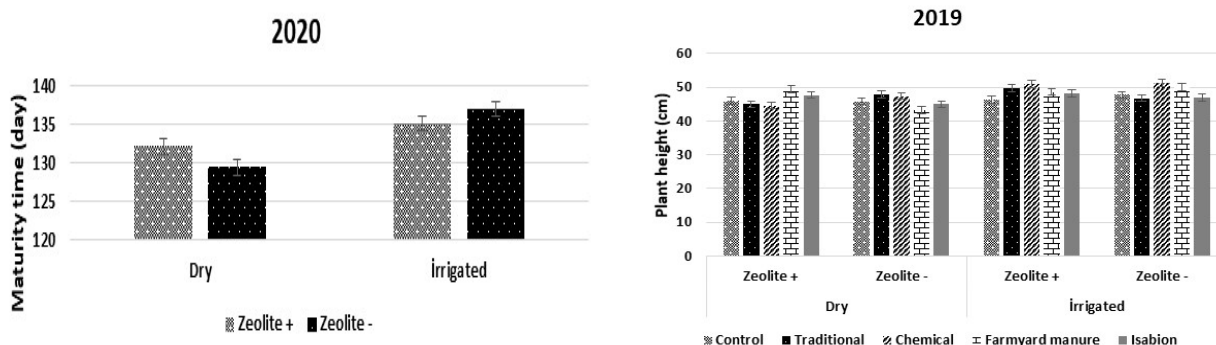


Figure 3. The interaction between growing conditions and zeolite applications for maturity time (2020); the interaction between growing conditions, zeolite applications and nitrogen applications (2019) for plant height of chickpea.

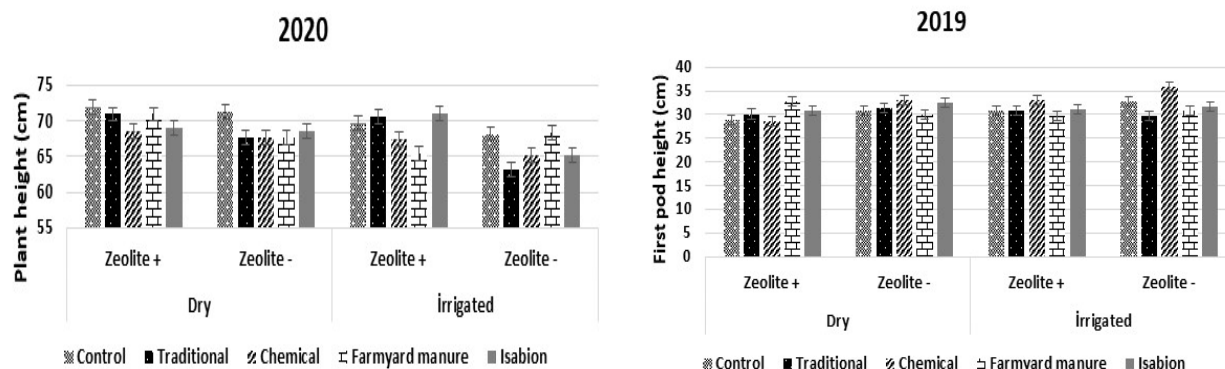


Figure 4. The interaction between growing conditions, zeolite applications and nitrogen applications for plant height (2020); the interaction between growing conditions, zeolite applications and nitrogen applications (2019) for first pod height of chickpea.

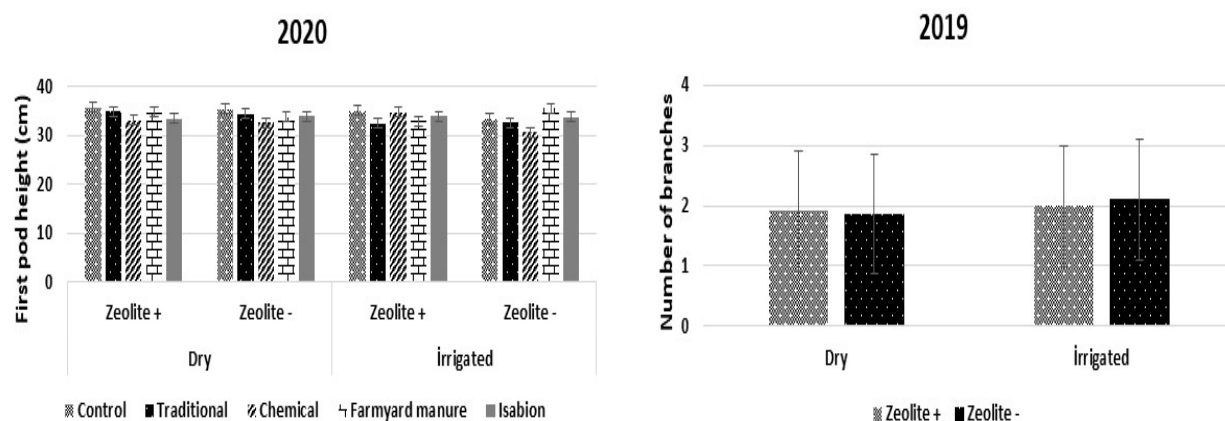


Figure 5. The interaction between growing conditions, zeolite applications and nitrogen applications for first pod height (2020); the interaction between growing conditions and zeolite applications (2019) for number of branches of chickpea.

### Number of Branches, Branch Diameter

In the first year, growing conditions were significant for the number of branches and branch diameter, but only branch diameter was significant for the second year (Table 4,5). The number of branches is higher in irrigated plots than in dry plots in the first year. Togay et al. (2005) found that the number of branches increased with irrigation in chickpeas. The impact of growing conditions was negligible due to considerable precipitation during the second year of the experiment. Yolcu (2008) found no disparity in the number of branches between control and irrigated plots in chickpeas. In the first and second years, the branch diameter is greater in irrigated plots compared to dry plots. The experiment demonstrated that irrigation of had a beneficial impact on the diameter of the branches. Although zeolite applications had little impact on the number of branches and branch diameter in the first year, these characteristics became substantial in the second year (Table 4,5). In the second year, the zeolite application plots exhibit more branches and larger branch heights. Zahedi et al. (2009) found that applying zeolite positively impacted the number of branches in their study. Chemical fertilizer plots yielded the highest number of branches in the first year, while traditional fertilizer plots yielded the highest value in the second year (Table 4,5). The number of branches in the control plots was the lowest during the first and second years of the experiment. The nitrogen applications increased the number of branches. The

application of nitrogen in chickpeas increased the number of branches (Doğan, 2019; Demir, 2021). The farmyard manure plots produced the largest branch diameter in both years, perhaps because they included more organic matter.

In the first year, irrigated + zeolite<sup>-</sup> plots showed the highest value, while dry + zeolite<sup>-</sup> plots yielded the lowest number of branches. The lowest and maximum number of branches were obtained from zeolite<sup>-</sup> plots, indicating the importance of the relationship of the growing conditions x zeolite applications. (Figure 5). The dry + Isabion plots produced the fewest branches in the first year, whereas the irrigated + Isabion plots displayed the highest value. The Isabion plots yielded the lowest and largest number of branches, indicating the importance of the interaction of growth conditions x nitrogen applications (Figure 6). The irrigated + zeolite<sup>+</sup> + chemical plots produced the fewest branches, but other chemical applications yielded higher numbers in the second year. Thus, it's possible that the relationship between the growth conditions, zeolite applications, and nitrogen applications was significant (Figure 6). The irrigated + zeolite<sup>+</sup> plots showed the highest value in the first year, while the dry + zeolite<sup>+</sup> plots produced the lowest branch diameter. While zeolite application negatively affected the branch diameter in dry plots, zeolite application increased the branch diameter in irrigated plots. As a result, it was discovered that the relationship between growing conditions and zeolite

applications was significant (Figure 7). Irrigated plots yielded larger branch diameters for every nitrogen application in the first year than dry plots, which yielded lower branch diameters. Thus, it's possible that the relationship between the nitrogen applications and the growing applications was significant (Figure 7). The zeolite<sup>+</sup> + farmyard manure plots showed the highest value in the first year, while the zeolite<sup>+</sup> + traditional plots produced the lowest branch diameters. Thus, it's possible that the relationship between nitrogen applications and zeolite applications was significant (Figure 8). The lowest branch diameter was produced by irrigation + zeolite<sup>+</sup> + Isabion plots; in contrast, other Isabion applications showed a larger value in the second

year. Thus, it's possible that the relationship between the growth conditions, zeolite applications, and nitrogen applications was significant (Figure 8).

### Grain Yield

Growing conditions significantly impacted grain yield in the first and second years. (Table 4,5). The grain yield was greater in irrigated conditions than in dry conditions in both years. The grain yield of chickpeas is greatly affected by environmental factors. Chickpea exhibits a high tolerance to drought conditions, yet, it also demonstrates a favorable response to irrigation (Ceyhan et al. 2012; Kahraman et al., 2016; Arif et al., 2021).

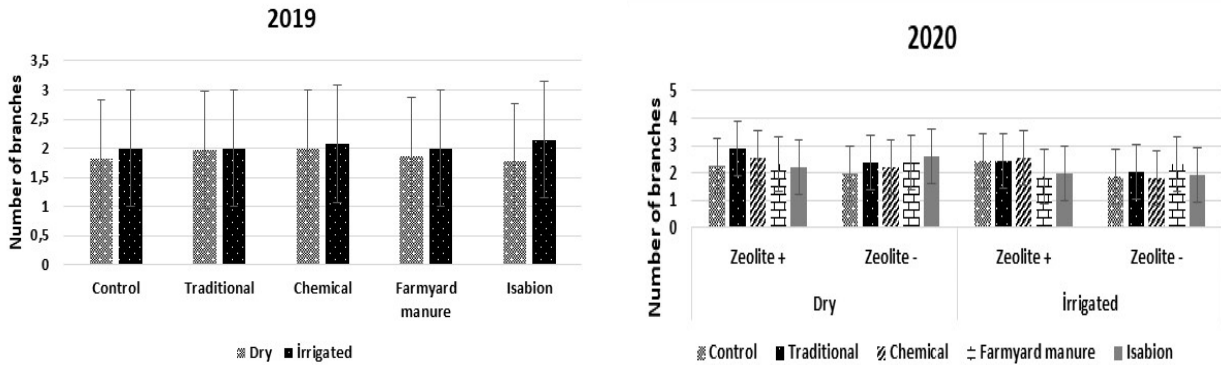


Figure 6. The interaction between growing conditions and nitrogen applications for number of branches (2019); the interaction between growing conditions, zeolite applications and nitrogen applications (2020) for number of branches of chickpea.

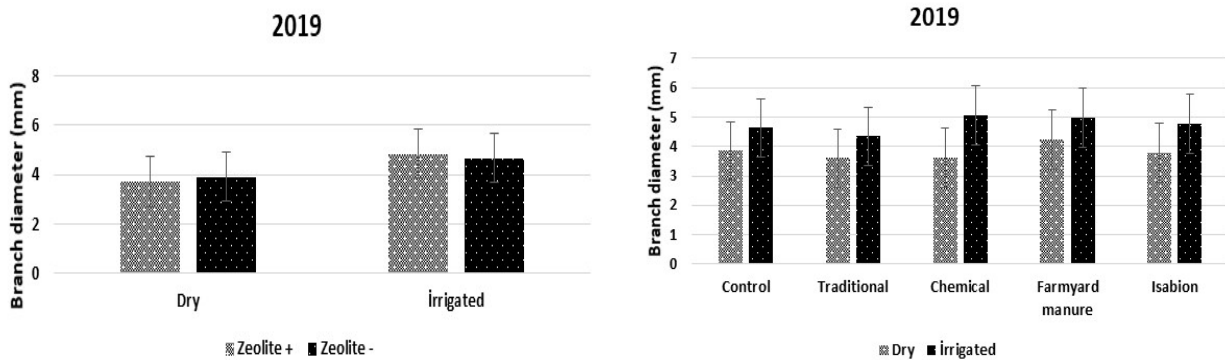


Figure 7. The interaction between growing conditions and zeolite applications for branches diameter (2019); the interaction between growing conditions and nitrogen applications (2019) for branches diameter of chickpea.

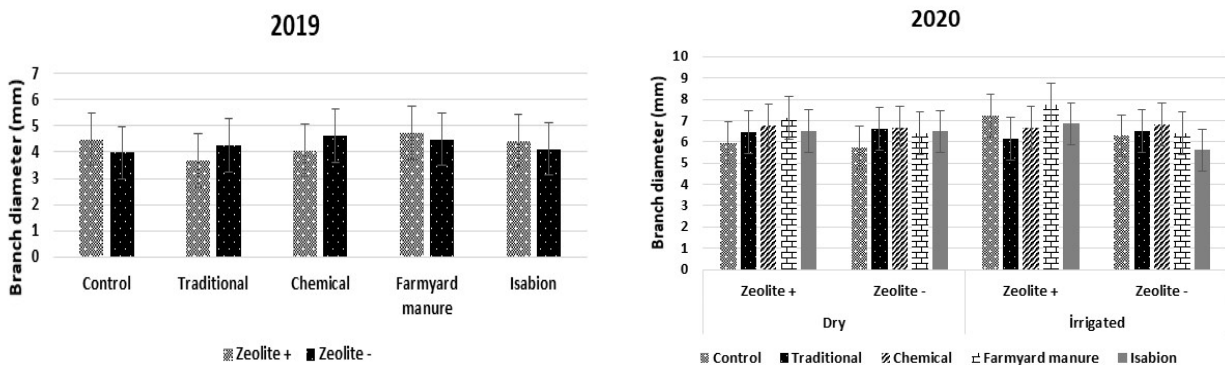


Figure 8. The interaction between zeolite applications and nitrogen applications for branches diameter (2019); the interaction between growing conditions, zeolite applications and nitrogen applications (2020) for branches diameter of chickpea.

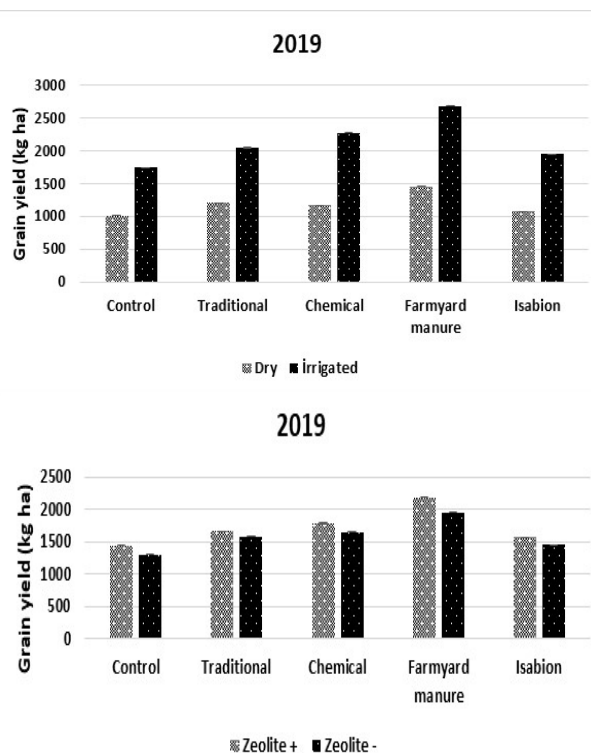


Figure 9. The interaction between growing conditions and nitrogen applications for grain yield (2019); the interaction between zeolite applications and nitrogen applications (2019) for grain yield of chickpea.

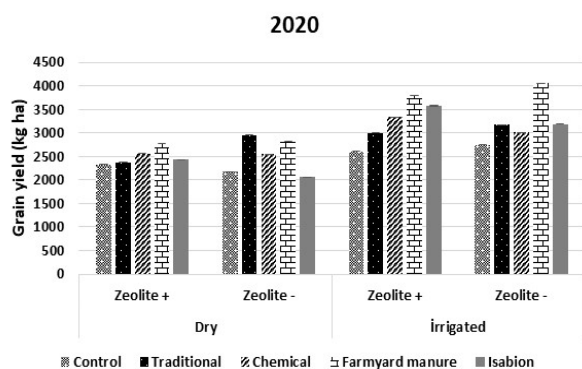


Figure 10. The interaction between growing conditions, zeolite applications and nitrogen applications for grain yield of chickpea.

In the first year, the irrigated plots produced 81% more grain compared to the dry plots, whereas in the second year, the irrigated plots yielded 29% more grain. In a study conducted by Muruiki et al. (2021), it was discovered that the application of irrigation resulted in a significant 60.3% increase in chickpea grain yield. In the first year, zeolite applications substantially impacted grain yield; however, they were insignificant in the second year (Table 4,5). The grain yield in the zeolite<sup>+</sup> treatment was measured at 1730 kg ha<sup>-1</sup>, while in the zeolite<sup>-</sup> treatment it was 1590 kg ha<sup>-1</sup> in the first year. Zeolite enhances plant growth by effectively retaining water and nutrients, limiting their loss through leaching. According to Mondal et al. (2021), zeolite enhances the ability of plants to withstand drought conditions in semi-arid locations. According to Amiri et al. (2021), using zeolite in soybeans resulted in a 64% increase in grain yield compared to the control plots. Applying

zeolite resulted in increased grain yield during the first year, but no effect was observed in the second year. During the second year of the experiment, the presence of adequate rainfall and favorable climatic conditions made it impossible to assess the impact of the zeolite (Figure 1). In their study, Hoseini et al. (2020) found that the application of zeolite did not have any significant impact on the grain yield of chickpeas. The application of nitrogen had a notable effect on grain yield during the first and the second seasons, as indicated in Tables 4 and 5. The application of farmyard manure yielded the most favorable outcomes in both years, whereas the control plots exhibited the least satisfactory results regarding nitrogen applications. Farmyard Grain yield benefits from the presence of farmyard manure, which includes an abundance of organic matter and plant nutrients. According to Janmohammadi's (2018) research, the use of organic fertilizer for chickpeas resulted in a significant increase in crop output. According to Demir's (2021) findings, the plots treated with chicken dung produced the most significant amount of grain, while the control plots produced the lowest amount.

In the first year, nitrogen applications in dry regions resulted in low values, but irrigated regions exhibited large grain yields. Therefore, the interaction of growing conditions x nitrogen applications may have been significant. farmyard manure + irrigated plots gave the best results (Figure 9). Zeolite<sup>+</sup> plots achieved high grain yields, whereas zeolite<sup>-</sup> plots produced poor values for all nitrogen applications in the first year. Therefore, the interaction between zeolite applications x nitrogen applications may have been significant. Farmyard manure + zeolite<sup>+</sup> plots gave the best results (Figure 9). While the highest grain yield was obtained in irrigated + zeolite<sup>+</sup> + farmyard manure plots, dry + zeolite<sup>-</sup> + Isabion manure plots showed lowest grain yields in the second year of the experiment (Figure 10). The grain yield was more significant in the traditional and farmyard manure plots than in the plots where zeolite was not used, specifically during the second year of the experiment when the growth conditions were dry. The zeolite's water adsorption capacity may have been compromised in the second year of the experiment due to significant precipitation (Figure 1). Under arid growth conditions, Isabion had a more favorable response to zeolite. The grain yield was more significant in plots treated with zeolite<sup>+</sup> than plots treated with zeolite<sup>-</sup> under dry conditions at the Isabion plots.

## Conclusions

The experiment found that irrigation caused a delay in phenological characteristics, but had a favorable impact on morphological characteristics and yield. A surplus of rainfall was recorded in the second year, particularly in June. The abundant rainfall hindered our results to observe the impact of irrigation on the studied traits. The effect of zeolite applications were different in the first and second years of the experiments for the investigated characters. Zeolite application had a positive impact on grain yield in the first year but had no impact in the second year. During the second year of the experiment, the presence of adequate rainfall and favorable climatic circumstances made it impossible to determine the impact of the zeolite. The

experiment demonstrated that chemical fertilizer and farmyard manure had a good impact on the phenological and morphological traits. The farmyard manure plots gave the highest yield values of grains in both years. Farmers in Türkiye should apply nitrogen to their crops due to the recent rise in their earnings from chickpeas. Farmers have the option of substituting chemical fertilizers when the exorbitant cost of farm manure prevents its use.

## Declarations

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## Determination of the Effects of Modified Atmosphere Packaging and 1-Methylcyclopropene Applications on the Storability of 'Farfia' Apricot Fruits

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 30.07.2024 Accepted : 27.09.2024</p> <p><b>Keywords:</b> <i>Prunus armeniaca</i> MAP 1-MCP Storage Quality</p>	<p>The study aimed to determine the effects of modified atmosphere packaging (MAP) and 1-methylcyclopropene (1-MCP) on the postharvest resistance of 'Farfia' apricot variety fruits. The study was carried out in five different applications; a) Control, b) MAP, c) 1-MCP, d) MAP + 1-MCP, e) RipeLock™ MAP packaging + 1-MCP. 1-MCP was applied at 625 ppb for 24 hours. 'Farfia' apricot fruits were stored at 0°C and 90% relative humidity for 8 weeks. Weight loss, color, fruit flesh firmness, total soluble solids, acidity, pH, total phenol content, antioxidant activity, respiration rate, ethylene production, sensory evaluation and decay development were determined in samples taken at 2-week intervals before and during storage after the packages were opened and kept on the shelf life (20°C) for 2 days. It was observed that the treatments containing MAP significantly reduced the weight loss of apricot fruits during storage and shelf life. Co-treatments of MAP and 1-MCP were effective in preserving fruit flesh firmness. The respiration rate of the fruits was found to be lower in the treatments containing MAP. Single and combined applications of MAP and 1-MCP slowed the ethylene release of apricot fruits. As a result of the study, it was determined that 'Farfia' apricot fruits in the treatments in which MAP and 1-MCP combination could be successfully stored for 42 days.</p>

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## 'Farfia' Kayısı Meyvelerinin Depolanabilirliğine Modifiye Atmosfer Paketleme ve 1-Metilsiklopropen Uygulamalarının Etkilerinin Belirlenmesi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 30.07.2024 Kabul : 27.09.2024</p> <p><b>Anahtar Kelimeler:</b> <i>Prunus armeniaca</i> MAP 1-MCP Depolama Kalite</p>	<p>Bu çalışmada, modifiye atmosfer paketleme (MAP) ve 1-metilsiklopropen (1-MCP) 'Farfia' kayısı çeşidi meyvelerinin hasat sonrası dayanımlarına etkilerinin belirlenmesi amaçlanmıştır. Çalışma; a) Kontrol, b) MAP, c) 1-MCP, d) MAP + 1-MCP, e) RipeLock™ MAP ambalaj + 1-MCP olacak şekilde beş farklı uygulama gerçekleştirilmiştir. 1-MCP, 24 saat 625 ppb olarak uygulanmıştır. 'Farfia' kayısı çeşidine ait meyveler 8 hafta süreyle 0°C'de %90 nemde depolanmıştır. Depolama öncesi ve süresince 2 haftalık periyotlarla alınan örneklerde ambalajların ağzı açılarak 2 gün raf ömründe (20°C) bekletildikten sonra ağırlık kaybı, renk, meyve eti sertliği, suda çözünür kuru madde miktarı, asitlik, pH, toplam fenol miktarı, antioksidan aktivitesi, solunum hızı, etilen salınımı, duyuşal değerlendirme ve çürüklük gelişimi belirlenmiştir. MAP'ın yer aldığı uygulamalar, kayısı meyvelerinin ağırlık kaybını depolama ve raf ömrü süresince önemli derecede azalttığı görülmüştür. Meyve eti sertliğinin korunmasında, MAP ile 1-MCP'nin birlikte uygulamaları etkili olmuştur. MAP'ın yer aldığı uygulamalarda meyvelerin solunum hızları daha düşük bulunmuştur. MAP ve 1-MCP'nin teksele ve birlikte uygulanmaları kayısı meyvelerinin etilen salınımını yavaşlatmıştır. Çalışma sonucunda MAP ile 1-MCP'nin birlikte yapıldığı uygulamalardaki 'Farfia' kayısı meyvelerinin 42 gün başarıyla saklanabileceği saptanmıştır.</p>

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## Giriş

Kayısı, ülkemizin hemen hemen tüm bölgelerinde yetiştiriciliği yapılan, sofralık ve kurutulmuş olarak sevilerek tüketilen bir meyve türüdür. İçeriğinde yüksek oranda bulunan A vitaminin yanı sıra, diyet lifi, potasyum, kalsiyum, demir ve fosfor bakımından da zengin olan kayısının insan sağlığına etkisi oldukça fazladır (Muzzaffar ve ark., 2018).

Türkiye hem yaş ve hem de kuru kayısı üretiminde dünya birinci sırada yer alırken, sahip olduğu gen kaynakları ve üretim alanları bakımından büyük bir potansiyele sahiptir. Son yıllarda tüketicinin pazarda uzun süre yaş kayısı bulma isteğinin yanında tat, aroma, renk ve irilik açısından da farklı özelliklere sahip yeni çeşitlere talep fazlalaşmıştır (Asma ve ark., 2017). Ülkemizde farklı zamanlarda olgunlaşan sofralık kayısı çeşitleri ile tesis edilen bahçe sayısı gün geçtikçe artmaktadır. Mayıs ayı ortalarından itibaren hasadına başlanan kayısı, geç olgunlaşan çeşitler ile Ağustos ayının sonlarına kadar tezgâhlarda bulunmaktadır. Özellikle geç olgunlaşan sofralık kayısı çeşitleri ile tesis edilen bahçe sayısının artması, depolama ve pazarlama sürecinde de meyve kalitesinin korunmasını zorunlu kılmaktadır. Geç olgunlaşan kayısı çeşitlerinin depolanması ile birlikte pazarlama süreci uzayacağından uzun süre piyasada ürün bulunabilecektir.

Kayısı, genel olarak çabuk olgunlaşan, çok çabuk bozulabilen ve hasat sonrası ömrü sınırlı olan klimakterik bir meyve olup çeşide bağlı olarak hasattan sonra 0°C'de 2-4 hafta muhafaza edilebilir (Stanley, 1991; Crisosto & Kader, 1999; Karaçalı, 2016). Kayısı çeşitlerinin çoğunda, depolama sırasında meyvelerde üşüme hasarı semptomları, çürüklük gelişimi, meyve etinde yumuşama ve asitlikte azalış, suda çözünür kuru madde (SÇKM) miktarında artış gözlenir (Stanley, 1991, Holb ve ark., 2006, 2011; Ezzat ve ark., 2012). Kayısı meyvelerinde depolama ve pazarlama sürecini uzatmak için söz konusu bu değişimlerin geciktirilmesi ve yavaşlatılması büyük önem arz etmektedir. Bunlardan özellikle meyvenin olgunlaşmasıyla birlikte ortaya çıkan yumuşama, etilen yoğunluğunun artmasından kaynaklanmaktadır (Khan & Singh, 2007). Bu durumun engellenebilmesi için etilen ve etilenin etkilediği değişimlerin sınırlandırılması gerekmektedir.

Söz konusu etkilerin sınırlandırılması farklı uygulamalar ve yöntemlerin kullanılması ile mümkün olabilmektedir. Günümüzde en yaygın olarak kullanılan uygulama, bitki dokusunun etilen salgılamasını engellemektir. Etilen salgılanmasını önlemek için; sıcaklığın mümkün olan en düşük dereceye düşürülmesi, CO<sub>2</sub> konsantrasyonunun yükseltilmesi ve etilen inhibitörü olarak 1-MCP'nin kullanılması en başarılı yöntemler olarak bildirilmektedir (Watkins, 2006).

Modifiye atmosferin prensibi, ambalaj içindeki ürünün solunum esnasında O<sub>2</sub> alıp CO<sub>2</sub> vererek O<sub>2</sub> konsantrasyonunun bir miktar düşmesine CO<sub>2</sub> konsantrasyonunun ise bir miktar yükselmesini sağlayarak yaşlanmayı yavaşlatmaktadır. Ayrıca MAP ambalajları ürünün etrafındaki nem miktarını yükselterek, nem kaybını azaltarak ürünlerde buruşma, kırışma, büzüşme gibi kayıpları sınırlandırmaktadır. MAP ambalajlarının bu olumlu sonuçları nedeniyle kayısı meyvelerinin hasat sonrası ömrüne olumlu katkılarının olduğu rapor edilmiştir

(Muftuoğlu ve ark., 2012; Özdoğru ve ark., 2014; Ezzat, 2018). Ancak MAP ambalajlarındaki nem geçirgenliğinin ürünler için uygun olmaması durumunda meyveden ortama verilen nem ambalaj içinde doymuş hale gelerek çürümeyi teşvik edebilmekte (Nunes, 2008), uygun olmayan gaz bileşimleri ise fizyolojik bozulmalar oluşturabilmektedir (Crisosto ve ark., 2009).

1-MCP, yaş meyve ve sebzelerde kalitenin korunması ve hasat sonrası ömrünün uzatılmasında, etilen salgısını sınırlandırılması ve yaşlanmanın geciktirilmesinde etkilidir (Watkins, 2006). 1-MCP uygulandığı meyvelerdeki etilen alıcılarına bağlanıp, etilenin bu kısma bağlanmasını engelleyerek etilenin tetiklediği biyokimyasal reaksiyonların hızını yavaşlatmaktadır (Sisler & Serek, 1997). Kayısı meyvelerinde 1-MCP uygulamasının kayısının olgunlaşmasını geciktirme potansiyeline sahip olduğunu, ancak çeşidin, meyve olgunluğunun ve uygulama zamanının dikkatli seçilmesi gerektiğini göstermiştir (Dong ve ark., 2002). 1-MCP uygulaması bazı kayısı çeşitlerinde depolama ve raf ömrü süresini uzattığı ve kaliteyi arttırdığı rapor edilmiştir (Dong ve ark., 2002; Cao ve ark., 2009; Shi ve ark., 2013; Rebeaud ve ark., 2015; Wu ve ark., 2015; Muzzaffar ve ark., 2018).

Kayısı meyvelerinin hasat sonrası dayanımları çeşitlere ve yetiştikleri bölgelere göre önemli farklılıklar gösterebildiğinden bu bağlamda yapılacak çalışmalar büyük önem arz etmektedir (Karaçalı, 2016). Ayrıca 1-MCP ile MAP'ın birlikte uygulanmasının bu kayısı çeşidinde soğuk depolamada ve raf ömrü sürecinde olumlu katkıları olacağı düşünülmektedir. Bu çalışmada, MAP ambalajları ve 1-MCP uygulamalarının 'Farfia' geççi kayısı çeşidinin depolanabilirliğine etkilerinin saptanması amaçlanmıştır.

## Materyal ve Yöntem

Bu çalışma, Denizli ili Tavas ilçesinde 'Myrobolan 29-C' anacı üzerine aşılı 'Farfia' geççi kayısı (*Prunus armeniaca*) çeşitleri ile 4,5 m × 2,5 m dikim sıklığında kurulmuş olan Ülkü Meyvecilik San. Tic. A.Ş. firmasına ait 10 yaşındaki meyve bahçesinde yürütülmüştür.

Çalışmada iki farklı modifiye atmosfer paketleme (MAP) ambalajı (torba-poşet) kullanılmıştır. Bunlardan biri polietilen (PE) bazlı 20 µ kalınlığındaki MAP ambalajıdır (LifePack, Aypek, Bursa). Diğeri ise 20 µ kalınlığındaki RipeLock™ ambalajı, 1-MCP uygulamaları için özel geliştirilmiş MAP özelliğine sahip bir ambalajdır (SmartFresh™, Agrofresh, ABD).

## Hasat

Kayısı meyvelerinin hasadı, kabuk rengi yanında meyve eti sertliği ve suda çözünür kuru madde (SÇKM) miktarları dikkate alınarak sert olum döneminde yapılmıştır. Hasat edilen kayısı meyvelerinden şekli düzgün, zarar görmemiş, birbirine benzer irilikte olanlar seçilip sert plastik kasalara [525 × 368 × 201 (h) mm] koyularak hemen Ege Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü'ne getirilmiştir.

### Ön soğutma ve uygulamalar

Kayısı meyveleri her mukavva kutuda 3 kg olacak şekilde, MAP ambalajına konulmuş veya ambalajsız olacak şekilde çekirdek sıcaklığı 4°C'ye ininceye kadar ön soğutmaya alınmıştır. Ön soğutması yapılan meyveler; 1) Kontrol (hiç uygulamaya yapılmayan), 2) 1-MCP uygulaması (24 saat süreyle 4°C 625 ppb uygulanmıştır), 3) MAP (PE bazlı 20 µ kalınlığında, LifePack, Aypek, Bursa, Türkiye) uygulaması, 4) MAP + 1-MCP uygulaması (MAP ambalajlarının ağzı açık olarak 24 saat süreyle 5°C 625 ppb uygulandıktan sonra ambalajların ağzı kapatılmıştır), 5) RipeLock™ MAP (RL) + 1-MCP uygulaması (RL MAP ambalajlarının için 1-MCP jeneratörü konarak ağzı kapatılmıştır).

1-MCP uygulaması, 1 m<sup>3</sup> hacminde gaz geçirmez fermuarlı PVC çadır içinde 4°C sıcaklıkta 24 saat süreyle 625 ppb (0.084 g/m<sup>3</sup>) konsantrasyonuna ulaşacak şekilde 1 adet mavi tablet, 2 adet pembe tablet (aktivatör) ve 20 mL çözgen sıvı (SmartFresh™, Agrofresh, ABD) kullanılarak yapılmıştır.

### Depolama

Kontrol ve uygulama yapılan kayısı meyveleri 0°C sıcaklık ve %90 nemde 8 hafta muhafaza edilmiştir (Crisosto ve ark., 2009). Depolamanın başlangıcında ve depolama süresince her kayısı çeşidinden de 2 hafta aralıklarla alınan örnekler (MAP ve RL ambalajı olanların ağzı açık olarak) 2 gün raf ömrü koşullarında (20°C ve %60-70 oransal nem) bekletildikten sonra bazı kalite değişimleri araştırılmıştır. Çalışma tesadüf parselleri deneme desenine göre dört tekrarlı olarak planlanmış, her bir mukavva kutu bir tekrür olarak kabul edilmiştir.

### Kalite analizleri

Ağırlık kaybı, depolama başlangıcında ağırlıkları dijital hassas terazide belirlenen nektarin meyveleri, depolama dönemlerine ilaveten raf ömrü sonrası ağırlıkları tekrar belirlenmiş, bu verilerden ağırlık kayıpları hesaplanarak sonuçlar yüzde (%) olarak sunulmuştur.

Kayısı meyvelerinin kabuk rengi, meyvenin ekvator kısmındaki farklı noktalarından Chroma Meter ölçüm cihazı (CR-400, Konica Minolta, Japonya) kullanılarak CIE L\*, a\*, b\* cinsinden belirlenmiştir (McGuire, 1992).

Kayısıların meyve eti sertliği, meyvelerin ekvator bölgesinden kabuğun uzaklaştırıldığı bölgeden meyve tekstür ölçer cihazının (GS-15, GÜSS Manufacturing Ltd., Güney Afrika) 7,9 mm çaplı silindirik ucu, 10 cm/dak hızla 10 mm derinliğe kadar batırılarak belirlenmiş, sonuçlar Newton (N) olarak ifade edilmiştir.

SÇKM miktarı, kayısı meyve sularında dijital refraktometre (PR-1, Atago, Japonya) kullanılarak ölçülmüştür (Karaçalı, 2016). Titre edilebilir asit (TA) miktarı, 10 mL meyve suyunun pH değeri 8,1 gelinceye kadar 0,1 N NaOH ilave edilerek titrasyon yapılmış, harcanan NaOH miktarından g malik asit/100 mL cinsinden hesaplanmıştır. Meyve suyunun pH değeri, bir pH metre yardımıyla belirlenmiştir (Karaçalı, 2016).

Kayısıların meyve etinden alınan 5 g örneğin metanol ile ekstrasyonu yapılmıştır (Thaiponga ve ark., 2006). Toplam fenolik madde (TF) miktarı; Folin-Ciocalteu kolorimetrik yöntemi modifiye edilerek (Zheng & Wang, 2001), antioksidan aktivitesi (AA); Ferric Reducing Antioxidant Power (FRAP) yöntemi (Benzie & Strain, 1996) kullanılarak spektrofotometre ile belirlenmiştir. TF miktarı mg gallik asit eşdeğeri (GAE)/100 g, AA µmol trolox eşdeğeri (TE)/g olarak verilmiştir.

### Solunum hızı ve etilen salınım miktarı

Kayısı meyvesi gaz geçirmez plastik kavanozlarda (IKEA®, İtalya) 3 saat süreyle 20°C sıcaklıkta bekletildikten sonra alınan gaz örneği gaz kromatografisine (6890 N, Agilent Technologies, ABD) enjekte edilmiştir. GS-GASPRO kolonu, ısı iletkenlik detektörü ve alev iyonlaşma detektörü kullanılarak solunum hızı mL CO<sub>2</sub>/kg.sa. ve etilen salgı miktarı ise µL C<sub>2</sub>H<sub>4</sub>/kg.sa. olarak hesaplanmıştır.

### Duyusal analiz

Kayısı meyvelerinin görünüş, tekstür ve tat durumları, beğeni 1-9 skalasına göre eğitilmiş 5 panelist tarafından değerlendirilmiştir (Altuğ Onoğur ve Elmacı, 2011).

### Çürüklük gelişimi

Her tekerrürde çürüklük gelişimi gösteren meyve sayısı tespit edilmiş, bu sayı toplam meyve sayısına orantılanarak çürük meyve oranı yüzde (%) olarak bulunmuştur.

### İstatiksel analiz

Bu çalışmadan edilen verilerin varyans analizi IBM® SPSS® Statistics 22 (IBM, NewYork, ABD) istatistik paket programı kullanılarak yapılmış, her depolama dönemindeki farklılıklar Duncan testi (P≤0.05) kullanılarak saptanmıştır.

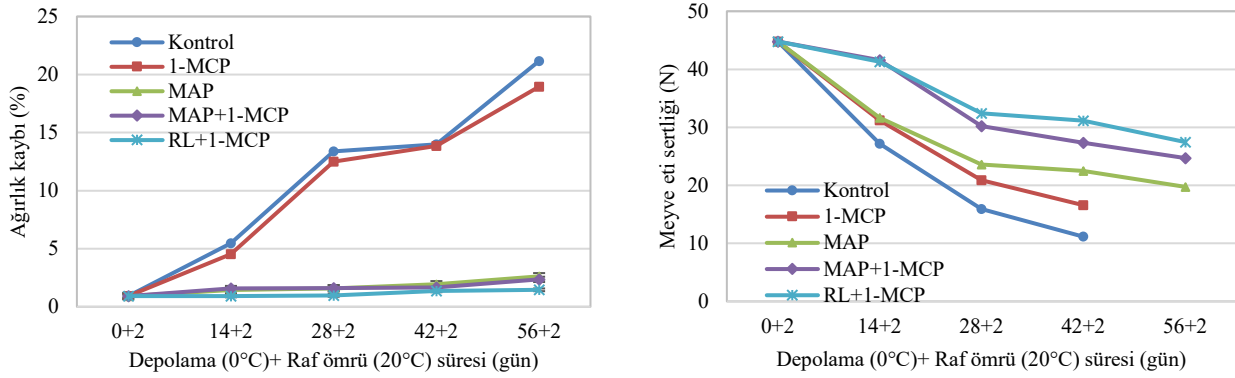
### Bulgular

#### Kalite analizleri

'Farfia' kayısı meyvelerinde MAP ve 1-MCP uygulamalarına göre depolama periyotlarına ek olarak raf ömrü sonrası saptanan ağırlık kayıpları Şekil 1'de verilmiştir. Depolama periyotlarına ek olarak raf ömrü sonrası kayısuların ağırlık kaybına farklı uygulamaların etkisi istatistiksel olarak önemli (P≤0,01) bulunmuştur. Söz konusu bu dönemlerde MAP ambalajı kullanılan kayısı meyvelerinin ağırlık kayıplarının kontrol ve 1-MCP uygulananlara göre önemli düzeyde daha düşük olduğu saptanmıştır. 56 günlük depolama süresine ilaveten 2 günlük raf ömrü sonunda MAP ambalajlarının yer aldığı uygulamalarda kayısı meyvelerinin ağırlık kaybı %1,46 ile %2,62 arasında değişirken, kontrol ve 1-MCP uygulamalarında ise bu değerler sırasıyla %21,17 ve %18,96 olarak belirlenmiştir.

Farklı uygulamaların kayısuların meyve eti sertliğine etkisi önemli bulunmuş, 14, 28, 42 gün depolamaya ilaveten raf ömrü sonrası MAP+1-MCP ve RL+1-MCP uygulanan kayısuların meyve eti sertlik değeri en yüksek, kontrolde ise bu değer en düşük bulunmuştur. MAP ve 1-MCP uygulanan kayısularda meyve eti sertliğinin 28+2 ve 42+2 günde kontroldekilerden daha yüksek olduğu saptanmıştır. Depolama sonunda (56+2 gün) MAP+1-MCP ve RL+1-MCP uygulanan kayısuların meyve eti sertliği MAP uygulananlardan daha yüksek olmuştur.

'Farfia' kayısı meyvelerinin L\*, a\* ve b\* değerlerine uygulamaların etkisi tüm depolama periyotlarına ek olarak raf ömrü sonrası birbirine benzerlik gösterdiği saptanmıştır. Depolama süresince kayısı meyvelerinin renk değerlerindeki değişimler sınırlı olmuş L\*, a\* ve b\* değerleri sırasıyla 50,47-57,56, 16,21-21,74 ve 39,40-45,17 arasında bir değişim göstermiştir (Tablo 1).



Şekil 1. MAP ve 1-MCP uygulamalarının depolama periyotlarına ek olarak raf ömrü sonrası kayısı meyvelerinin ağırlık kaybı ve meyve eti sertliğine etkileri

Figure 1. Effects of post-harvest MAP and 1-MCP treatments on weight loss and fruit flesh firmness of apricot fruits after shelf life in addition to storage periods

Çizelge 1. Hasat sonrası MAP ve 1-MCP uygulamalarının depolama periyotlarına ek olarak raf ömrü sonrası kayısı meyvelerinin  $L^*$ ,  $a^*$  ve  $b^*$  değerlerine etkileri

Table 1. Effects of post-harvest MAP and 1-MCP treatments on  $L^*$ ,  $a^*$ , and  $b^*$  values of apricot fruits after shelf life in addition to storage periods

Parametreler	Uygulamalar	Depolama (0°C) + Raf ömrü (20°C) süresi (gün)				
		0+2	14+2	28+2	42+2	56+2
$L^*$ değeri	Kontrol	57,26 <sup>ö.d.</sup>	54,99 <sup>ö.d.</sup>	50,47 <sup>ö.d.</sup>	56,95 <sup>ö.d.</sup>	–
	1-MCP	57,26	55,57	51,37	56,85	–
	MAP	57,26	56,83	56,91	55,64	55,29 <sup>ö.d.</sup>
	MAP+1-MCP	57,26	57,23	55,87	55,80	55,84
	RL+1-MCP	57,26	55,50	55,89	54,46	57,56
$a^*$ değeri	Kontrol	17,19 <sup>ö.d.</sup>	21,36 <sup>ö.d.</sup>	21,74 <sup>ö.d.</sup>	18,87 <sup>ö.d.</sup>	–
	1-MCP	17,19	19,77	20,89	18,23	–
	MAP	17,19	19,56	18,31	19,19	18,70 <sup>ö.d.</sup>
	MAP+1-MCP	17,19	17,72	19,55	19,44	16,21
	RL+1-MCP	17,19	19,52	20,16	20,64	16,82
$b^*$ değeri	Kontrol	43,33 <sup>ö.d.</sup>	39,40 <sup>ö.d.</sup>	41,11 <sup>ö.d.</sup>	45,17 <sup>ö.d.</sup>	–
	1-MCP	43,33	40,44	41,91	44,53	–
	MAP	43,33	41,82	43,60	40,45	40,36 <sup>ö.d.</sup>
	MAP+1-MCP	43,33	42,52	41,94	41,51	40,66
	RL+1-MCP	43,33	41,27	42,47	40,04	43,59

<sup>ö.d.</sup> önemli değil.

Kayıslı meyvelerinde depolama süresince saptanan SÇKM, TA miktarı ve pH değerindeki değişimler Çizelge 2’de verilmiştir. Farklı uygulamaların kayısların SÇKM miktarına etkisi 28 ve 42 günlük depolamaya ilave raf ömrü sonrası istatistiksel olarak önemli ( $P \leq 0,05$ ) olurken, incelenen diğer depolama dönemlerinde önemsiz olmuştur. Kontroldeki meyvelerin SÇKM miktarı, 28+2 günde MAP’ın yer aldığı uygulamalara, 42+2 günde ise birlikte yapılan uygulamalara (MAP+1-MCP, RL+1-MCP) göre daha yüksek bulunmuştur. Kayıslı meyvelerinde TA miktarına farklı uygulamaların etkisi tüm depolama dönemlerinde birbirine benzerlik göstermiş, 42+2 günde meyvelerin TA miktarı 0,67-0,74 g/100 ml arasında değişmiştir. 14, 28 ve 42 günlük depolamaya ilaveten 2 günlük raf ömrü sonrasında uygulamaların pH değerine etkisi istatistiksel olarak önemli ( $P \leq 0,05$ ) farklılıklar göstermiş, bu dönemlerde MAP+1-MCP uygulanan meyvelerin pH değeri RL+1-MCP uygulananlara göre daha yüksek bulunmuştur.

Uygulamalara göre depolama süresince kayıslı meyvelerinin TF miktarı ve AA’daki değişimler Çizelge 3’te sunulmuştur. Kayıslı meyvelerinin TF miktarına uygulamaların etkisi 14 günlük muhafazaya ilaveten raf

ömrü sonrası istatistiksel olarak önemli ( $P \leq 0,05$ ) farklılık gösterirken ilerleyen depolama dönemlerinde bu farklılıklar kaybolmuştur. Bu dönemde kontrol ve 1-MCP uygulanan meyvelerin TF miktarı, MAP uygulananlara (76,50 mg GAE/100 g) göre sırasıyla %23 ve %20 oranında daha yüksek bulunmuştur. Farklı hasat sonrası uygulamalarının kayıslı meyvelerinin AA değerlerine etkisi 28 günlük muhafazaya ilaveten 2 günlük raf ömrü sonrasında etkisi istatistiksel olarak önemli ( $P \leq 0,05$ ) olmuş, kontroldeki meyvelerin AA 8,01  $\mu\text{mol TE/g}$  ile en yüksek iken 1-MCP (5,33  $\mu\text{mol TE/g}$ ) ve MAP (4,98  $\mu\text{mol TE/g}$ ) uygulananlarda ise en düşük olduğu saptanmıştır.

#### Solunum hızı ve etilen salınım miktarı

Kayıslı meyvelerinde depolama süresine ilaveten raf ömrü sonrasında uygulamalara göre solunum hızının ve etilen salınım miktarının değişimleri Şekil 2’de verilmiştir. Farklı uygulamaların kayıslı meyvelerinin solunum hızına 14, 28 ve 42 günlük muhafazaya ilaveten günlük raf ömrü sonrası etkisi önemli bulunmuş, kontroldeki kayıslı meyvelerinin solunum hızı MAP’ın yer aldığı uygulamalara göre ortalama %36 daha yüksek bulunmuştur.

Çizelge 2. MAP ve 1-MCP uygulamalarının depolama periyotlarına ek olarak raf ömrü sonrası kayısı meyvelerinin SÇKM, TA miktarına ve pH değerine etkileri

Table 2. Effects of post-harvest MAP and 1-MCP treatments on TSS, TA content and pH value values of apricot fruits after shelf life in addition to storage periods

Parametreler	Uygulamalar	Depolama (0°C) + Raf ömrü (20°C) süresi (gün)				
		0+2	14+2	28+2	42+2	56+2
SÇKM miktarı (%)	Kontrol	12,40	14,13 <sup>ö.d.</sup>	15,57 a <sup>z*</sup>	13,73 a <sup>*</sup>	–
	1-MCP	12,40	13,37	14,47 ab	13,30 a	–
	MAP	12,40	14,61	12,30 c	13,63 a	13,83 <sup>ö.d.</sup>
	MAP+1-MCP	12,40	14,60	13,57 b	12,73 b	13,33
	RL+1-MCP	12,40	14,00	13,37 bc	12,80 b	12,73
TA miktar (g/100 ml)	Kontrol	0,87	0,89 <sup>ö.d.</sup>	0,75 <sup>ö.d.</sup>	0,74 <sup>ö.d.</sup>	–
	1-MCP	0,87	0,85	0,92	0,72	–
	MAP	0,87	0,73	0,90	0,68	0,63 <sup>ö.d.</sup>
	MAP+1-MCP	0,87	0,75	0,81	0,70	0,63
	RL+1-MCP	0,87	0,84	0,74	0,67	0,72
pH değeri	Kontrol	4,34	4,49 ab <sup>*</sup>	4,71 a <sup>*</sup>	4,79 a <sup>*</sup>	–
	1-MCP	4,34	4,46 ab	4,70 a	4,81 a	–
	MAP	4,34	4,52 a	4,69 ab	4,77 ab	4,76 <sup>ö.d.</sup>
	MAP+1-MCP	4,34	4,53 a	4,73 a	4,79 a	4,84
	RL+1-MCP	4,34	4,42 b	4,63 b	4,72 b	4,78

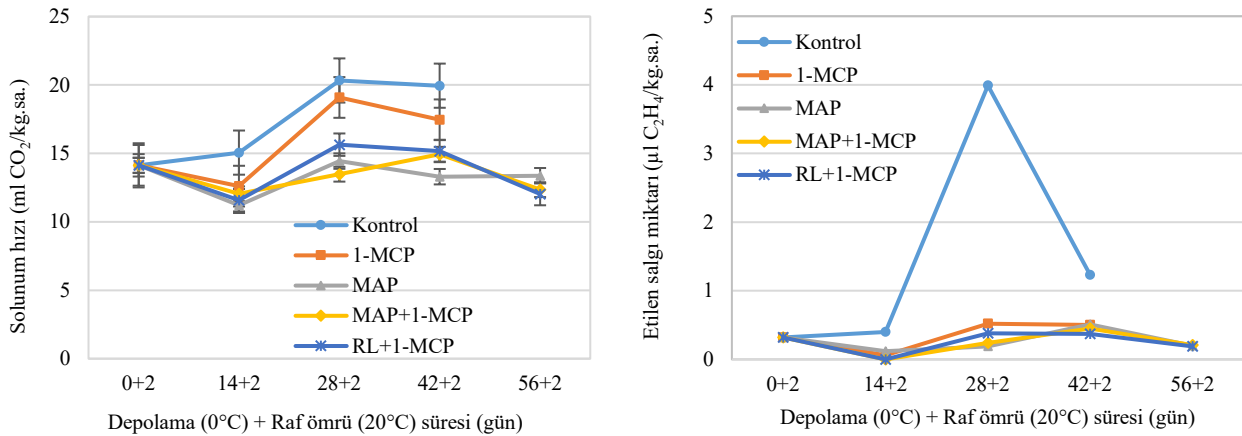
<sup>z</sup> Her sütunda bulunan ortalamaların aralarındaki farklılıklar Duncan testi ile  $P \leq 0,05$ 'e göre belirlenmiştir. <sup>ö.d.</sup> önemli değil; <sup>\*</sup> $P \leq 0,05$ 'e göre önemli.

Çizelge 3. MAP ve 1-MCP uygulamalarının depolama periyotlarına ek olarak raf ömrü sonrası kayısı meyvelerinin TF miktarı ve AA değerine etkileri

Table 3. Effects of post-harvest MAP and 1-MCP treatments on TF content and AA value of apricot fruits after shelf life in addition to storage periods

Parametreler	Uygulamalar	Depolama (0°C) + Raf ömrü (20°C) süresi (gün)				
		0+2	14+2	28+2	42+2	56+2
TF miktarı (mg GAE/100 g)	Kontrol	76,98	94,03 a <sup>z*</sup>	88,79 <sup>ö.d.</sup>	82,74 <sup>ö.d.</sup>	–
	1-MCP	76,98	91,44 a	70,56	74,85	–
	MAP	76,98	76,50 b	74,44	82,06	83,51 <sup>ö.d.</sup>
	MAP+1-MCP	76,98	83,08 ab	88,01	74,37	87,07
	RL+1-MCP	76,98	81,67 ab	72,18	77,10	85,47
AA (µmol TE/g)	Kontrol	8,72	8,60 <sup>ö.d.</sup>	8,01 a <sup>*</sup>	7,11 <sup>ö.d.</sup>	–
	1-MCP	8,72	8,46	5,33 c	5,69	–
	MAP	8,72	7,02	4,98 c	7,22	4,72 <sup>ö.d.</sup>
	MAP+1-MCP	8,72	7,49	7,44 ab	5,50	5,43
	RL+1-MCP	8,72	7,55	6,00 bc	6,01	5,48

<sup>z</sup> Her sütunda bulunan ortalamaların aralarındaki farklılıklar Duncan testi ile  $P \leq 0,05$ 'e göre belirlenmiştir. <sup>ö.d.</sup> önemli değil; <sup>\*</sup> $P \leq 0,05$ 'e göre önemli. TF; toplam fenol, AA; antioksidan aktivitesi, GAE; gallik asit eşdeğeri, TE; trolox eşdeğeri.



Şekil 2. MAP ve 1-MCP uygulamalarının depolama periyotlarına ek olarak raf ömrü sonrası kayısı meyvelerinin solunum hızı ve etilen salgı miktarına etkileri

Figure 2. Effects of post-harvest MAP and 1-MCP treatments on respiration rate and ethylene production of apricot fruits after shelf life in addition to storage periods

Çizelge 4. MAP ve 1-MCP uygulamalarının depolama periyotlarına ek olarak raf ömrü sonrasında kayısı meyvelerinin beğeni puanlarına ve çürüklük gelişimine etkileri

Table 4. Effects of post-harvest MAP and 1-MCP treatments on overall appearance scores and decay development of apricot fruits after shelf life in addition to storage periods

Parametreler	Uygulamalar	Depolama (0°C) + Raf ömrü (20°C) süresi (gün)				
		0+2	14+2	28+2	42+2	56+2
Beğeni puanları (1-9 skalası)	Kontrol	9,00	6,20 b <sup>z*</sup>	3,60 b <sup>**</sup>	2,20 b <sup>**</sup>	–
	1-MCP	9,00	7,80 a	4,60 b	2,80 b	–
	MAP	9,00	8,00 a	7,20 a	6,40 a	5,40 b <sup>*</sup>
	MAP+1-MCP	9,00	8,60 a	7,60 a	7,00 a	6,60 a
	RL+1-MCP	9,00	8,80 a	8,00 a	7,20 a	6,60 a
Çürüklük gelişimi (%)	Kontrol	0,0	1,3	0,0	0,0	–
	1-MCP	0,0	0,0	1,3	0,0	–
	MAP	0,0	0,0	0,0	0,0	0,0
	MAP+1-MCP	0,0	0,0	0,0	1,3	2,5
	RL+1-MCP	0,0	0,0	0,0	0,0	3,8

<sup>z</sup>Her sütunda bulunan ortalamaların aralarındaki farklılıklar Duncan testi ile  $P \leq 0,05$ 'e göre belirlenmiştir. <sup>ö.d</sup> önemli değil; <sup>\*</sup> $P \leq 0,05$ ; <sup>\*\*</sup> $P \leq 0,01$ 'e göre önemli.

Bu depolama dönemlerinde MAP ambalajlarının yer aldığı uygulamalar arasında solunum hızı bakımından bir farklılık görülmemiştir. Kayısı meyvelerinin etilen salınım miktarına uygulamaların etkisi 56+2 gün dışındaki depolama dönemlerinde önemli olmuş, kontroldeki meyvelerin etilen salınım miktarı uygulamalara göre daha yüksek bulunmuştur. 42+2 günde kontroldeki kayısı meyvelerinin etilen salınım miktarı 1,23  $\mu\text{l}$  C<sub>2</sub>H<sub>4</sub>/kg.sa. iken uygulamalarda bu değer 0,37-0,53  $\mu\text{l}$  C<sub>2</sub>H<sub>4</sub>/kg.sa. aralığında değişim göstermiştir.

#### Duyusal analiz

Depolama periyotlarına ek olarak raf ömrü sonrası farklı uygulamaların beğeni puanlarına etkisi önemli farklılıklar göstermiş, bu dönemlerde kontrol meyvelerinin beğeni puanları en düşük bulunurken MAP'ın yer aldığı uygulamalarda en yüksek bulunmuştur. 1-MCP'nin tek sel uygulaması 14+2'de uygulamalara benzerlik gösterirken ilerleyen depolama dönemlerinde kontrole benzerlik göstermiştir. Depolama döneminin sonunda (56+2 gün) kontrol ve tek sel 1-MCP uygulanan meyvelerde görünüşün bozulması ve aşırı yumuşamadan dolayı ölçüm ve analizler yapılmamıştır (Çizelge 4).

#### Çürüklük gelişimi

Kayısı meyvelerindeki çürüklük gelişimine farklı uygulamaların etkisi birbirine benzerlik göstermiş, farklı dönemlerde çürüklük gelişimi görülmemiş veya çok düşük oranlarda görülmüştür. Çürüklük görülen meyvelerde etmen olarak çoğunlukla monilya (*Monilinia laxa*) olduğu saptanmıştır (Çizelge 4).

#### Tartışma

Hasat sonrası dönemde meyve ve sebzelerde ortaya çıkan ağırlık kayıpları, ekonomik olarak kayıplara neden olmaktadır. Bu yüzden depolama ve raf ömrü süresince ağırlık kayıplarının en düşük seviyede olması arzulanır. Tüm depolama dönemleri ve buna ilave raf ömrü boyunca MAP ambalajlarının yer aldığı uygulamalardaki ağırlık kaybının kontrole ve 1-MCP uygulanan meyvelere göre önemli şekilde daha düşük bulunması, MAP ambalajlarının su kaybını sınırladığı etkisiyle açıklanabilir. MAP ambalajları ürünün etrafındaki nem miktarını yükselterek nem kaybını azaltmakta ve ürünlerde buruşma, kırışma,

büzüşme gibi kayıpları sınırlandırmaktadır (Özkaya ve ark., 2016). Farklı kayısı çeşitlerinin depolama sürelerinin uzamasıyla beraber ağırlık kayıplarında artışların meydana geldiği ve bu ağırlık kaybının azaltılmasında MAP'nin etkili sonuçlar verdiği bildirilmiştir (Muftuoğlu ve ark., 2012; Çalhan, 2010; Sabır ve ark., 2014). 1-MCP'nin tek sel uygulamasında ürünün ağırlık kaybını azaltmada etkili olduğunu ancak depolamanın ilerleyen döneminde bu etkinin sınırlı kaldığı rapor edilmiştir (Fan ve ark., 2000; Sabır ve ark., 2014). 1-MCP'nin olgunluğu geciktirici etkisinden dolayı, meyvelerde metabolik aktiviteyi yavaşlamasına bağlı olarak ağırlık kaybı daha düşük çıkmış olabilir (Watkins, 2006; Moradinezhad & Jahani, 2019).

'Farfia' kayısı çeşidinde tüm depolama periyotlarına ek olarak raf ömrü sonrasında MAP ve 1-MCP'nin tek sel ve birlikte uygulamalarında, meyve eti sertliği değerlerinin kontrole kıyasla daha yüksek olduğu saptanmıştır. Bu etki uygulamaların yaşlanmayı yavaşlatmasının etkisi olarak ortaya çıkmıştır. 1-MCP uygulanmasıyla meyve yumuşamasında etkili olan pektin estaraz, poligalakturonaz ve selüloz gibi meyve sertliğini etkileyen enzimlerin aktivitelerinin sınırlandırıldığından yumuşamanın geciktirildiği bilinmektedir (Feng ve ark., 2000). Benzer şekilde, MAP ambalaj atmosferindeki O<sub>2</sub> ve CO<sub>2</sub> konsantrasyonunu değiştirerek solunumu yavaşlatarak yaşlanmayı ve dolayısıyla meyve yumuşamasını geciktirmektedir (Crisosto ve ark., 2009; Karaçalı, 2016). 1-MCP uygulaması 'Perfection' ve 'Bulida' (Fan ve ark., 2000; Egea ve ark., 2010), MAP uygulaması 'Ninfa' ve 'Jumbo' (Sabır ve ark., 2014; Ezzat, 2018), MAP ve 1-MCP'nin birlikte uygulaması 'Goldrich' (Rebeaud ve ark., 2015) kayısı çeşitlerinin meyve eti sertliğinin korunmasında etkili olduğu rapor edilmiştir. Bu uygulamalar, kayısı meyvelerinin yaşlanması yanında su kaybını azaltarak meyve yumuşamasını sınırlandırmaktadır. Nitekim kayısı meyvelerinden meydana gelen su kayıplarının artışına paralel olarak meyve eti sertliğinde azalışlar meydana gelmiştir (Ezzat, 2018).

Kayısı çeşidinin kabuk rengini ifade eden  $L^*$ ,  $a^*$  ve  $b^*$  değerlerine uygulamaların etkisi sınırlı olmuştur. Bunda kayısı meyvelerinin zemin renginin homojen olmaması ve depolama sürecinde değişimlerinin sınırlı olmasının etkili olduğu düşünülmektedir. Benzer şekilde, farklı ambalaj uygulamalarının kayısı meyvelerinin renk değeri üzerine etkilerinin önemli olmadığı rapor edilmiştir (Özdoğru ve ark., 2014).

Depolamanın ilk dönemlerinde (14+2 ve 28+2 gün süresince) kontrol meyvelerinde SÇKM miktarının daha yüksek bulunmasında, MAP'ın yer aldığı uygulamalarda su kaybı ve solunumu yavaşlatıcı etkisi önemli olmuştur. Çünkü meyvelerde su kaybındaki artışlara paralel olarak meyve suyundaki suda çözünür maddelerin konsantrasyonunda da artışlar gözlenir. Nitekim bu dönemlerde su kaybının daha yüksek olduğu kontrolde SÇKM miktarı da yüksek olmuştur. MAP uygulaması depolama sürecine 'Kabaş' ve 'Roxana' kayısı çeşitlerinin SÇKM miktarındaki artışları sınırlandırdığı rapor edilmiştir (Çalhan, 2010; Muftuoğlu ve ark., 2012). 1-MCP uygulamalarının SÇKM miktarına etkisi farklılık göstermekte, 'Ceccona' kayısı çeşidinde artış, 'San Castrese' kayısı çeşidinde ise azalış gösterirken 'Canino' kayısı çeşidinde etkisiz olmuştur (Dong ve ark., 2002; Botondi ve ark., 2003). Bunda çeşit başta olmak üzere uygulama sonrasındaki depolama koşulları, uygulanan konsantrasyon gibi birçok faktörün etkili olabileceği bildirilmektedir (Dong ve ark., 2002).

Kayısı meyvelerinin depolama ve raf ömrü süresince TA miktarına MAP ve 1-MCP'nin teksele ve kombine uygulamalarının etkisinin sınırlı olması, MAP ve 1-MCP ile yapılan başka çalışmalarda da gözlenmiştir (Peano ve ark., 2014). Bunun yanında 1-MCP uygulamasının TA kaybını, kayısı (Sabir ve ark., 2014) ve erik (Dong ve ark., 2002) meyvelerinde geciktirdiği rapor edilmiştir. Kayısı meyvelerinin pH değerinin RL+1-MCP uygulamalarında en düşük bulunması meyve yaşlanması ile uyumludur. Ancak diğer uygulamaların etkisi sınırlı olmuş veya kararsızlık göstermiştir.

Fenolik bileşikler, bitkilerde doğal antioksidanlar olarak görev yapan ve oksidatif stresi azaltarak serbest radikallerin hücrelere vermiş olduğu zararları azaltan ve hastalıklarda tedavi edici rolü olan ikincil metabolitlerdir (Baek ve ark., 2021). Aynı zamanda meyvenin renk, tat ve lezzet gibi duyuşsal özelliklerinin gelişiminde, stres ve hastalıklara karşı direncinin artırılmasında rol oynamaktadır (Cevallos-Casals ve ark., 2006). Farklı uygulamaların depolama sürelerine ek olarak raf ömrü sonrası kayısı meyvelerinin TF miktarı ve AA değerlerine etkisi genellikle birbirine benzerlik göstermiştir. Benzer şekilde 1-MCP'nin elma meyvelerinde AA üzerine etkisinin olmadığını rapor etmişlerdir (Fawbush ve ark., 2009). Bunun aksini bildiren araştırma raporları da mevcuttur (Baek ve ark., 2021; Baswal ve ark., 2021). Kayısı meyvelerinin TF miktarı ve AA'nın depolama ve raf ömrü süresince, iklim faktörleri, bakım işleri, meyve olgunluğu ve bitki gelişim düzenleyicileri gibi birçok faktörün etkili olabileceği bildirilmektedir (Kalt, 2005).

MAP'ın teksele ve 1-MCP ile birlikte uygulanan kayısı meyvelerinin solunum hızının depolamanın 14., 28. ve 42. gününe ek olarak raf ömrü sonrası uygulama yapılmayan meyvelere göre daha düşük olmasında, MAP ve 1-MCP'nin solunum hızını yavaşlatıcı etkisi önemli olmuştur. Nitekim benzer şekilde birçok çalışmada kayısı gibi klimakterik yükseliş gösteren meyvelerde MAP ve 1-MCP uygulamalarının solunum hızını azalttığı rapor edilmiştir (Dong ve ark., 2002; Erkan & Eski, 2012; Erbaş & Koyuncu, 2016; Uysal ve ark., 2023). Kayısıda elde edilen bu bulguların çeşide, olgunluk seviyesine ve diğer faktörlere bağlı değişim gösterdiği vurgulanmaktadır (Fan ve ark., 2000).

Depolama süresince MAP ve 1-MCP'nin teksele ve birlikte uygulanmalarının kayısı meyvelerin etilen salınım miktarlarını kontrole göre daha düşük bulunması, bu uygulamaların etilen salınım miktarını yavaşlatıcı-geciktirici etkisi ile açıklanabilir. MAP uygulaması ambalaj içi gaz bileşimini değiştirerek (Crisosto ve ark., 2009), 1-MCP ise etilenin reseptörlere bağlanmasını engelleyerek (Blankenship & Dole, 2003) etilen salınım miktarını yavaşlatmasında etkili olmaktadır. 'Canino' ve 'Perfection' kayısı çeşitlerinde 1-MCP (Fan ve ark., 2000; Dong ve ark., 2002), 'Roxana' kayısı çeşidinde ise MAP ve 1-MCP'nin birlikte uygulanması etilen üretimini baskılamıştır (Çalhan, 2010).

Duyusal özellikler, meyve ve sebzelerde tüketici kabulü bakımından önemli rol oynar (Baswal ve ark., 2021). Kontrol ve teksele 1-MCP uygulamalarındaki meyvelerin beğeni puanlarının MAP ambalajlarının yer aldığı uygulamalara göre daha düşük olmasında, meyve kabuğunda su kaybına bağlı olarak görülen büzüşmeler ile meyve etindeki yumuşamalar etkili olmuştur. MAP ve 1-MCP'nin birlikte kullanıldığı uygulamalarda meyvelerin yaşlanmasının yavaşlaması ve su kaybının sınırlandırılması, ilerleyen depolama dönemlerinde kayısıların meyve eti sertliği ve görünüşünün korunmasında etkili olmuştur. Nitekim depolama süresince belirlenen kabuk renk değerleri ve meyve eti sertlik değerleri de bunu doğrulamaktadır.

Kayısı meyvelerinde çürüklük gelişiminin çok sınırlı olmasında, MAP ambalajlarının kullanımı, yetiştirme döneminde yapılan zirai mücadele uygulamaları ve hasadın özenli yapılmış olması etkili olmuştur. MAP ambalajının kullanılmadığı uygulamalarda su kaybının çok fazla olması çürüklük gelişimini engellemiştir.

## Sonuç

MAP ve 1-MCP uygulamalarının birlikte uygulanmasının depolamaya ilaveten raf ömrü sonrasında kayısı meyvelerinin kalite parametrelerinin birçoğunun değişimlerini sınırlandırarak kalitenin korunmasını sağlamıştır. Çalışma sonuçları, MAP'ın teksele ve 1-MCP ile birlikte uygulanmalarının 'Farfia' kayısı çeşidinin 42 güne ilaveten 2 günlük raf ömrü sonrasına kadar başarıyla saklanabileceğini göstermiştir.

## Beyanlar

### Etik Beyanı

Bu araştırma için bir etik komitesine gerek olmadığını beyan ederiz.

### Yazar Katkıları

NM: Veri toplama, araştırma, orijinal taslağın yazılması

FŞ: Proje yönetimi, denetim, metodoloji, inceleme ve düzenleme

EY: Veri toplama ve analiz

### Mali Destek

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## Effects of Different BAP, IBA, and IAA Hormone Doses Applied via Foliar Spraying on Growth and Biochemical Parameters of Lemon Balm (*Melissa officinalis* L.) Plant

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 06.08.2024 Accepted : 09.11.2024</p> <p>Keywords: Antioxidant Hormone Seedling Development Melissa officinalis L. Total Phenolic Content</p>	<p>This study investigated the effects of BAP, IBA, and IAA hormones at doses of 50 and 100 ppm on the growth and biochemical parameters of <i>Melissa officinalis</i> (Lemon Balm). The experiment was conducted under greenhouse conditions using a “Completely Randomized Design (CRD)” with three replications. The study assessed growth parameters such as seedling and root lengths, fresh and dry weights, as well as biochemical parameters including chlorophyll a and b, total carotenoids, total phenolic content, and antioxidant activities (CUPRAC and FRAP). The results indicated that hormone treatments had a significant impact on all growth parameters except root fresh weight compared to the control. The highest results for seedling length, fresh and dry weights, and root dry weight were achieved with the IBA50 dose, while the highest root length was observed with the BAP100 treatment. The highest values for chlorophyll a, b, and total carotenoids were found in the control treatments. The highest antioxidant activity (FRAP) was recorded with BAP100, and the highest total phenolic content was measured with IBA100.</p>

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## Farklı BAP, IBA ve IAA Hormon Dozlarının Oğul Otu (*Melissa officinalis* L.) Bitkisine Yaprakdan Uygulanmasının Büyüme ve Biyokimyasal Parametreler Üzerine Etkileri

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 06.08.2024 Kabul : 09.11.2024</p> <p>Anahtar Kelimeler: Antioksidan Hormon Fide gelişimi Oğul otu Toplam fenolik madde</p>	<p>Bu çalışma, BAP, IBA ve IAA hormonlarının 50 ve 100 ppm dozlarının <i>Melissa officinalis</i> L. (Oğulotu) bitkisinin büyüme ve biyokimyasal parametreleri üzerindeki etkilerini araştırmak amacıyla gerçekleştirilmiştir. Deneme, sera koşullarında “Tam Şansa Bağlı Tesadüf Parselleri” deneme desenine göre üç tekrar ile yapılmıştır. Çalışmada; fide boyu, kök uzunluğu, fide yaş ve kuru ağırlıkları, kök yaş ve kuru ağırlıkları, klorofil a ve b, toplam karotenoidler, toplam fenolik maddeler ve antioksidan aktiviteler (CUPRAC ve FRAP) değerlendirilmiştir. Çalışma sonucunda; kök yaş ağırlığı dışındaki büyüme parametrelerinde hormon uygulamalarının kontrole göre önemli etkisinin olduğu, fide boyu, yaş, kuru ağırlıkları ile kök kuru ağırlıklarında en yüksek sonuçlara IBA50 dozunda ulaşıldığı, kök uzunluğunda ise en yüksek değere BAP100 uygulamasında ulaşıldığı görülmüştür. Klorofil a, b ve total karotenoid parametrelerinde en yüksek değerler kontrol uygulamalarından, en yüksek antioksidan aktivite (FRAP) BAP100, en yüksek toplam fenolik madde miktarı ise IBA100 uygulamalarında ölçülmüştür.</p>

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## Giriş

Oğul otu, latince adıyla *Melissa officinalis* L., Lamiaceae ailesinin bir üyesidir. Ortalama 100 cm boyunda, dik veya yarı dik duruşa sahip çok yıllık bir bitkidir (Ceylan, 1997). Bu tıbbi bitki, dünya çapında geleneksel tıp uygulamalarında yaygın olarak kullanılmaktadır. Özellikle Akdeniz bölgesi ve Batı Asya'da doğal olarak yetişirken (Abdel-Naime ve ark., 2019; Ghiulai ve ark., 2020), Bulgaristan, Almanya, Romanya, İtalya, Fransa ve Kuzey Amerika'nın bazı bölgelerinde de kültürü yapılmaktadır (Katar, 2004). Oğul otu, aynı zamanda balotu, lemon balm ve balmumu nanesi gibi çeşitli yaygın adlarla da anılmaktadır (Petrisor ve ark., 2022).

Ülkemizde oğulotu bitkisinin üç farklı alt türü mevcuttur: *ssp. officinalis*, *ssp. altissima* ve *ssp. inodora*. Bu alt türler arasında sadece *Melissa officinalis ssp. officinalis* tıbbi öneme sahiptir, diğer ikisi ise kokusuz olmaları veya hoş olmayan bir koku yaymaları nedeniyle tercih edilmezler. Tıbbi amaçlar için kullanılan alt tür, ülkemizin çeşitli bölgelerinde doğal olarak bulunan dört farklı formda görülebilmektedir. (Baytop, 1984).

Ülkemizdeki oğulotu yetiştiriciliği oldukça sınırlıdır. Bu durumun en önemli nedenleri arasında bitkinin düşük uçucu yağ içeriği ve damıtma tesislerinin azlığı gösterilebilir. *Melissa officinalis* üzerine yapılan kimyasal araştırmalar, bitkinin başlıca flavonoidler, terpenoidler, fenolik asitler, tanenler ve esansiyel yağ içerdiğini göstermiştir (Zarei ve ark., 2014). *Melissa officinalis*'in başlıca aktif bileşenleri uçucu bileşikler (geranial, neral, sitronellal, geraniol), triterpenler (ursolik asit ve oleonolik asit), fenolik bileşikler (rozmarinik asit, kafeik asit ve protokateşuik asit) ve flavonoidler (kersetin, rhamnositrin, luteolin)'dir. Esansiyel yağ genellikle çoğu biyolojik aktivite için sorumlu terapötik ilke olarak kabul edilse de polifenoller de etkilidir (Shakeri ve ark., 2016; Miraj ve ark., 2017).

Oğulotu, birçok endüstriyel alanda kullanılmaktadır; özellikle ilaç, parfümeri, gıda ve kozmetik sektörleri başta gelmektedir. Temelde, bitkinin uçucu yağı yapraklarda yoğunlaşırken, gövde ve çiçeklerde bu oran daha düşüktür. Kuru oğulotu bitkisinden yaklaşık %0,01 ile %0,3 arasında değişen oranlarda uçucu yağ çıkarılabilmektedir (Zeybek, 1987; Akgül, 1993). Geleneksel tıpta ise, ateş düşürücü, uyku düzenleyici, baş ağrısı ve soğuk algınlığı tedavisi gibi çeşitli amaçlarla yaygın olarak tercih edilmektedir (Katar & Gürbüz, 2008).

*Melissa officinalis* yaprak ekstraktları, yapılan çalışmalarla; antiproliferatif, anti-anjiyojenik, antiviral, antioksidan, anksiyete karşıtı, antidepresan, alzheimer karşıtı, nöroprotektif, kardiyoprotektif, antifungal ve antibakteriyel etkilerle ilişkilendirilen fenolik profilleri barındırdığı tespit edilmiştir (Petrisor ve ark., 2022).

Bitki hormonları, bitkilerin besin alımını artırma, abiyotik stresle başa çıkma kapasitesini güçlendirme ve/veya ürün kalitesini iyileştirme amacıyla, çeşitli zamanlarda ve yöntemlerle uygulanabilen hem sentetik hem de doğal kaynaklı maddeler veya mikroorganizmalar olarak tanımlanabilmektedir (Patrick, 2015).

BAP (6-benzilaminopurin) adı verilen, ilk sentetik sitokinler arasında yer alan bu hormon, bitkilerin meristem dokularında hem hücre bölünmesine hem de protein oluşumuna önemli ölçüde katkı sağladığı, bu da hücre ve

dokuların gelişimini etkin bir şekilde hızlandırdığı (Mayerni ve ark., 2015), çeşitli bitki türleri üzerinde gerçekleştirilen araştırmalar, BAP uygulanmasının, büyüme ile ilgili ölçütleri iyileştirdiğini, özellikle yaprak sayısı ve klorofil içeriğinde belirgin artışlar sağladığını ve ayrıca hem enzimatik hem de enzimatik olmayan antioksidan düzeylerini yükselttiğini ortaya koymuştur (Rulcova & Pospisilova, 2001; Taiz & Zeiger, 2010; Nourafcan ve ark., 2014).

Indol asetik asit (IAA), bitkilerde oksin grubu içinde sınıflandırılan hormonların bir parçasıdır. Bu hormon, bitkilerin stresle başa çıkma kapasitesini güçlendirme ve onların gelişim süreçlerini destekleme açısından kritik öneme sahiptir (Yang ve ark., 2011). Araştırmalar, IAA'nın bitki tohumlarının filizlenme sürecini hızlandırdığını, hücrelerin bölünmesine katkıda bulunduğunu ve kök ile gövde gelişiminde düzenleyici rol oynadığını ortaya koymaktadır (Sevik & Guney, 2013; Kumlay & Eryiğit, 2011). Oksinler ailesinden indol bütirik asit (IBA) hormonunun, kök gelişimini teşvik etme, bitkinin morfolojik yapısını geliştirme ve özellikle yaprak boyutlarının genişlemesine yardımcı olma gibi özelliklere sahip olduğu bilinir (Sevik & Guney, 2013).

Bu çalışma, IBA, IAA ve BAP hormonlarının farklı dozlarda yapraktan uygulamalarının fide gelişim döneminde olan *Melissa officinalis* L. bitkisinin büyüme ve biyokimyasal parametreleri üzerine etkilerini belirlemek amacıyla yürütülmüştür.

## Materyal ve Yöntem

### Materyal

Bu araştırma, Sakarya Uygulamalı Bilimler Üniversitesi Ziraat Fakültesinin Tarım Bilimleri ve Teknolojileri Eğitimi Uygulama ve Araştırma Merkezi'ne ait serada gerçekleştirilmiştir. Ülkemizde çoğunlukla çelikle üretimi yapılarak kültürü yapılan oğulotu fideleri ise ticari bir firmadan tedarik edilmiştir.

### Yöntem

Deneme, Tam Şansa Bağlı Tesadüf Parselleri deneme desenine göre üç tekrarlamalı olarak gerçekleştirilmiştir. Melissa fidelerine, bitki büyümesini ve gelişimini destekleyen BAP, IAA ve IBA hormonları 50 ve 100 mg/l dozlarda uygulanmıştır. BAP ve IBA, NaOH ile, IAA ise %96 etil alkolle çözülerek saf suyla 1 litreye tamamlanmıştır. Çalışmada, her biri 2 litrelik 21 saksı kullanılmış, bu saksılara ince elenmiş bahçe toprağı (4/3) ve Klassman TS1 marka torf (4/1) karışımı eklenmiştir. Serada, saksılar 20 cm sıra üzeri ve 30 cm sıra arası olacak şekilde, zemini düzeltilmiş ve silindire sıkıştırılmış alana yerleştirilmiştir.

Saksılar seraya yerleştirildikten sonra, rastgele seçilen beş saksının her birine 500 ml su verildi. Saksıların altına, süzülen suyu toplamak için tabaklar yerleştirildi. Süzülme işlemi tamamlandıktan sonra, her bir saksıdan ortalama 215 ml su tabaklarda birikti. Saksıların su tutma kapasiteleri, her saksıya eklenen 500 ml sudan süzülen su miktarının çıkarılmasıyla hesaplandı ve 285 ml olarak ölçüldü. Daha sonra, fideler 1 Kasım 2023 tarihinde yaklaşık 3 cm derinliğe dikildi. Dikim sırasında, bitkilerin

alt iki yaprağı toprağa temas ediyorsa mantar hastalıklarını önlemek için, elle çıkarıldı. Deney boyunca her saksıya haftada bir yaklaşık 100 ml su verildi. Hazırlanan hormon çözeltileri, ışıktan korunması amacıyla alüminyum folyo ile sarılıp buzdolabında saklandı ve 05.01.2024 tarihinde, ekimden yaklaşık iki ay sonra, yapraklara ilk uygulamalar yapıldı. Kış koşulları nedeniyle büyüme yavaş ilerlediği için hormon uygulamaları geciktirildi. Her bir fideye, 10 ml hormon çözeltisi püskürtüldü ve uygulamalar 4 gün arayla üç kez tekrarlandı. Deneme sürecinde gündüz sıcaklıkları ortalama 15°C, gece sıcaklıkları ise 4°C civarında olduğu tespit edilmiştir (Anonim 2024).

Deneme 19.01.2024 tarihinde, bitki boyu ölçümlerinin ardından tamamlandı ve yaklaşık 2,5 ay sürdü. Bitkilerin kökleri suyla yumuşatılıp ayrıldı, ardından kök uzunlukları ölçüldü. Fide ve köklerin yaş ağırlıkları hassas terazide, kuru ağırlıkları ise 35°C'de 108 saat kurutulduktan sonra belirlendi. Bitkiden alınan yaprak örnekleri analizler için materyal olarak kullanıldı.

#### **Toplam Fenolik Madde Miktarı Analizi**

Toplam fenolik içerik, Waterhouse (2002)'e göre Folin-Ciocalteu yöntemi kullanılarak değerlendirildi. İlk olarak, 250 µL Folin-Ciocalteu reaktifi ve 50 µL ekstrakt çözeltisi bir tüpe eklenerek toplam hacim distile su ile 3 ml'ye tamamlandı. 5 dakika inkübasyon sonrası, tüplere 750 µL %20'lik Na<sub>2</sub>CO<sub>3</sub> çözeltisi eklendi ve karıştırıldı. Karışım, 90 dakika boyunca oda sıcaklığında karanlıkta bekletildikten sonra, absorbans 765 nm'de UV-Vis spektrofotometresi ile ölçüldü. Gallik asit standardı, 50-300 µg/mL aralığında oluşturularak, toplam fenol içeriği gallik asit eşdeğeri (mg GAE/100 g kuru ağırlık) olarak hesaplandı.

#### **FRAP İndirgeme Kapasitesi Tayini**

Öncelikle, 0,3 M sodyum asetat tamponu (pH: 3,6), 10 mM TPTZ çözeltisi, 20 mM FeCl<sub>3</sub> ve 2 mM FeSO<sub>4</sub> çözeltileri hazırlandı. Çalışma çözeltisi, tampon, TPTZ ve FeCl<sub>3</sub> çözeltilerinin 10:1:1 oranında karıştırılmasıyla elde edildi. Standart eğri, 593 nm'de 2 mM FeSO<sub>4</sub> çözeltisi kullanılarak oluşturuldu ve numunelerin absorbansı en az üç farklı konsantrasyonda ölçüldü. Sonuçlar, mg ekstrakt/µmol Fe<sup>+2</sup> cinsinden raporlandı (Sachett ve ark., 2021).

#### **CUPRAC İndirgeme Kapasitesi Tayini**

Bu çalışmada, daha önce bildirilen yöntemin kısmen değiştirilmiş bir versiyonu uygulandı. Yapraktan alınan bitki ekstraktları, 10, 20 ve 40 µg konsantrasyonlarda tüplere eklendi. Ardından, sırasıyla 0,25 mL CuCl<sub>2</sub> (0,01 M), etanolik neokuprin ve CH<sub>3</sub>COONH<sub>4</sub> tampon çözeltileri (1 M) ilave edildi. Karışımlar 30 dakika karanlıkta bekletildi ve absorbanslar 450 nm'de, kontrol çözeltilerine karşı ölçüldü (Ak ve Gülçin, 2008). Ölçüm sonuçları troloks eşdeğerleri ile karşılaştırılarak değerlendirildi.

#### **Fotosentetik Pigmentler**

Lichtenthaler (1987)'e göre belirlenen fotosentetik pigmentlerin analizinde, 0,2 g (200 mg) taze yaprak örneği 10 mL %80 asetonda ekstrakte edilip 15 dakika boyunca 4600 devir/dakikada santrifüj edilmiştir. Santrifüj sonrası alınan alikotların 663, 645 ve 470 nm dalga boylarında absorbans değerleri bir spektrofotometrede (PG T60 UV-VIS) belirlenmiş ve kaydedilmiştir. Hesaplamalar aşağıda verilen formüller yardımıyla yapılmıştır:

$$KA = 11,75 \times A_{662} - 2,350 \times A_{645}$$

$$KB = 18,61 \times A_{645} - 3,960 \times A_{662}$$

$$TK = KA + KB$$

$$TD = (1000 \times A_{470} - 2,270 \times KA) - (81,4 \times KB/227)$$

KA : Klorofil a (µg g<sup>-1</sup> FW)

KB : Klorofil b (µg g<sup>-1</sup> FW)

TK : Toplam klorofil (µg g<sup>-1</sup> FW)

TD : Toplam karotenoid (µg g<sup>-1</sup> FW)

A : absorbans değeri,

FW : taze ağırlık

#### **İstatistiksel Veriler**

Elde edilen verilerin istatistiksel analizleri COSTAT (sürüm 6.03) paket programı ile çoklu karşılaştırma testleri ise LSD testine göre yapılmıştır.

Deneme sürecine ait bazı görseller aşağıda verilmiştir.



Resim 1. Sulama  
Image 1. Irrigation



Resim 2. Hormon muamele  
Image 2. Hormone treatment



Görsel 3. Harç hazırlığı  
Image 3. Soil Mortar Preparation

## Bulgular ve Tartışma

Melissa fidelerinde, hormon uygulamalarının tüm büyüme parametreleri üzerinde %5 düzeyinde anlamlı etkisi olduğu Çizelge 1’de gösterilmiştir. Bu tabloya göre, büyüme parametrelerinde hormonlara bağlı farklılıklar gözlemlenmiştir. Fide boyu ve fide yaş ağırlığında en yüksek değerler IBA50 uygulamasında sırasıyla 8,90 cm ve 4,49 g olarak kaydedilmiş, en düşük değerler ise kontrol grubunda bulunmuştur. Kök uzunluğunda en yüksek değer 28,25 cm ile BAP100 uygulamasında, en düşük değer 21,50 cm ile kontrol grubunda görülmüştür. Kök yaş ağırlığında ise en yüksek ortalama 7,32 g ile kontrol grubunda, en düşük ortalama ise 5,01 g ile BAP50 uygulamasında tespit edilmiştir.

Fide kuru ve kök kuru ağırlıkları bakımından en yüksek değerler sırasıyla 1,12, 0,99 g olduğu ve IBA50 uygulamasından elde edildiği görülmektedir. Maş fasulyesinde yapraktan uygulanan 150 mg/l BAP hormonunun; kontrol grubuna göre, belirgin bir şekilde bitki boyunu, bitki başına yaprak sayısını, yaprak çapını, kök nodül sayısını, klorofil ve karotenoid miktarlarını arttırdığı belirlenmiştir. Bu çalışmada kök nodül sayısının ve klorofil miktarlarının artmış olması, bitkinin azot fiksasyonunun ve fotosentez aktivitesinin arttığını da göstermiştir (Sarker ve ark., 2021).

Orkide bitkisinde, 100 ppm BAP hormonunun yapraktan uygulanması sonucu koltuk altı meristemlerinden yanal sürgünlerin oluştuğu belirlenmiştir (Lee ve ark., 2021). Bu durum, bitkinin çoğalma yeteneğini arttırdığı ve yeni sürgünlerin oluşumunu teşvik ettiğini göstermektedir. Önceki çalışmalarda da BAP hormonunun bitki büyümesini etkileyerek farklı fizyolojik değişikliklerin indüksiyonunu sağladığı gösterilmiştir

(Shekhawat ve ark., 2012; Krishnan & Siril, 2015; Deng ve ark., 2015). Geçmiş yıllarda yapılan BAP ile ilgili çalışmalar, en uygun dozun bitki türüne bağlı olarak değiştiğini göstermiştir. Ayrıca, BAP hormonunun hücre büyümesini ve uzamasını teşvik ederek protein sentezini de artırarak büyüme parametrelerini artırdığı sonucu da ortaya çıkmaktadır. Bu çalışmalara bakıldığında, BAP hormonunun bitki büyümesini desteklemede etkili olduğu görülmektedir.

IBA’nın yapraktan uygulanmasının buğdayın morfolojik, biyokimyasal ve verim parametrelerini iyileştirmede etkili bir hormon olduğu (Bashir ve ark., 2021), zerdeçalda farklı hormon uygulamalarının birçok büyüme parametrelerini kontrole göre arttırdığını ancak yapraktan 200 ppm IBA uygulamasının bitki boyu ve yaprak alanını en fazla arttıran hormon olduğu (Thounaojam ve ark., 2016), çeltikte yapraktan IBA uygulamasının yaprak biyokütlesini kontrole göre en fazla arttıran hormon olduğu (Borah & Baruah, 2016), serçedili bitkisinde yapraktan 75 ppm IBA uygulamasının; bitki boyu, kök uzunluğu ve biyokütleyi kontrole göre arttırdığı bildirilmektedir (Lin ve ark., 2018).

Çizelge 2 değerlendirildiğinde; uygulanan hormonların klorofil pigmentleri üzerine istatistiksel olarak %5 düzeyinde önemli etkisinin olduğu görülmektedir. Klorofil a, b ve total klorofil parametrelerinde en yüksek değerler sırasıyla 128,88 – 144,69 – 273,57 mg/g olup BAP100 uygulamalarından ölçülmüştür. Benzil aminopurin (BAP), bitki büyümesi için kritik olan sentetik bir sitokinidir; hücre bölünmesini ve çoğalmasını uyarmakta, protein sentezini teşvik etmekte ve doku kültürü tekniklerinde yardımcı olduğu bilinmektedir (Danış ve ark., 2024).

Çizelge 1. Bazı sentetik hormonların *Melissa officinalis* bitkisinin büyüme parametrelerine etkileri

Table 1. Effects of some synthetic hormones on growth parameters of *Melissa officinalis* plant.

Sentetik Hormon	Fide Boyu (cm)	Fide Yaş Ağırlık (g)	Kök Uzunluk (cm)	Kök Yaş Ağırlık (g)	Fide Kuru Ağırlık (g)	Kök Kuru Ağırlık (g)
Kontrol	5,93 B	3,15 C	21,50 C	7,32 A	0,99 AB	0,69 B
BAP50	7,57 AB	3,46 BC	25,75 AB	5,01 B	0,74 C	0,77 AB
BAP100	6,87 AB	3,64 ABC	28,25 A	6,58 B	0,92 B	0,76 B
IBA50	8,90 A	4,49 A	25,25 AB	7,95 B	1,12 A	0,99 A
IBA100	7,40 AB	3,91 ABC	23,90 BC	6,71 B	0,95 AB	0,83 AB
IAA50	6,37 B	3,83 ABC	25,25 AB	7,48 B	0,94 AB	0,75 B
IAA100	7,70 AB	4,26 AB	25,75 AB	7,84 B	1,07 AB	0,85 AB
LSD (0.05)	2,49	0,95	3,68	1,38	0,18	0,22
CV (%)	19,69	14,2	8,38	11,35	10,8	16,08

Çizelge 2. Bazı sentetik hormonların *Melissa officinalis* bitkisinin klorofil pigmentlerine etkileri etkileri

Table 2. Effects of some synthetic hormones on chlorophyll pigments of *Melissa officinalis* plant

Sentetik Hormon	Klorofil a (mg/g)	Klorofil b (mg/g)	Total Klorofil (mg/g)
Kontrol	119,94 B	142,43 A	262,37 AB
BAP50	116,24 B	129,39 B	245,63 BC
BAP100	128,88 A	144,69 A	273,57 A
IBA50	98,04 E	120,36 B	218,40 D
IBA100	98,54 DE	118,20 B	216,74 D
IAA50	106,25 C	124,27 B	230,52 CD
IAA100	105,68 CD	122,02 B	227,70 CD
LSD (0.05)	7,40	13,01	19,35
CV (%)	3,82	5,77	4,61

Klorofil ve karotenoid içeriğindeki artışlar, sitokinlerin fotosentetik süreçleri düzenlemedeki rolüne atfedilebilir. Sitokinlerin, klorofil sentez yollarında yer alan genlerin ekspresyonunu artırarak klorofil biyosentezini artırdığı gösterilmiştir (Janečková, 2021). Karotenoidler fotosentezde yardımcı pigmentler olduğu ve fazla ışık enerjisinin dağıtılmasında ve klorofili hasardan koruyarak hayati bir rol oynadığı bilinmektedir (Maoka, 2020). Bu sonuçlar değerlendirildiğinde; bitkilerde temel üretim mekanizmasında görev alan klorofil pigmentlerinin BAP uygulamaları ile artırılabilirliği çeşitli çalışmalar eşliğinde ortaya konulmuş olup çalışma sonuçlarımız desteklenmektedir.

Oğul otu fidelerine uygulanan hormonların, CUPRAC ve FRAP yöntemleriyle ölçülen antioksidan aktiviteler, toplam fenolik madde ve karotenoid miktarları üzerinde %5 düzeyinde anlamlı bir etki oluşturduğu Çizelge 3'te gösterilmiştir. Total karotenoid miktarı bakımından en yüksek değer 1,14 mg/g ile BAP hormonlarının her iki doz uygulamalarından, CUPRAC antioksidan aktivite yöntemi bakımından görece en yüksek değer 6.89 mM/g TE ile IAA100 uygulamalarından, FRAP antioksidan aktivite yöntemi bakımından en yüksek değer 1,71 mM/g AAE ile BAP100 uygulamalarından, total fenolik madde miktarı bakımından en yüksek değer ise 0,55 mg/g GAE ile IBA100 uygulamalarından tespit edilmiştir (Çizelge 3).

Bitkiler, abiyotik stres altında artan reaktif oksijen türlerine yanıt olarak flavonoidler, karotenoidler, SOD, POD, CAT, APX gibi enzimsel ve enzimsel olmayan antioksidanları üretmektedirler (Khatun ve ark., 2021). BAP hormonunun bezelye bitkisinde yapraktan püskürtülmesi, su stresi altındaki ve stressiz koşullarda fotosentetik pigmentleri, enzimatik antioksidanları ve osmolitleri, büyüme ve verimi arttırdığı (Sultan ve ark., 2023), yapraktan BAP uygulamalarının bitkilerin antioksidan sistemini arttırdığı (Zavaleta-Mancera ve ark., 2007; Kumari & Prakash, 2018) bildirilmiştir. Çalışma sonuçlarımızda da görüldüğü gibi BAP uygulamaları, antioksidan aktiviteyi ölçmede kullanılan FRAP sonuçlarını kontrole göre artırarak literatürle paralellik göstermiştir. Çeşitli çalışmalar, IAA hormonunun bitkilerde bir antioksidan olarak görev aldığını ve dışarıdan verilen IAA'nın radikal oksijen türevlerini azaltmada etkili olduğunu göstermiştir (Simkin ve ark., 2022). Benzer şekilde, IBA hormonunun morsalkım bitkisinde antioksidan aktiviteyi kontrole göre arttırdığı bulunmuştur (Sridharan, 2015). Ayrıca, IBA'nın çeşitli sebze bitkilerinde kalite parametrelerini önemli ölçüde iyileştirdiği tespit edilmiştir. Örneğin, sarımsak ve soğan

ile nohut tohumlarına IBA'nın dışarıdan uygulanmasına yanıt olarak kalite özelliklerinden olan toplam şekerler ve toplam fenollerin arttığı çeşitli çalışmalar ile belirlenmiştir (Amin ve ark., 2007; Amin ve ark., 2013; Abd Elwahed ve ark., 2019; Waheed ve ark., 2019).

Bu bulgular, IBA ve IAA hormonlarının, farklı bitkiler üzerinde yapılan çalışmalarda enzimatik ve enzimatik olmayan antioksidanları kontrole kıyasla arttırdığına dair elde edilen sonuçlarımızı desteklemektedir. Çizelge 3 değerlendirildiğinde, antioksidan aktivite testlerinin sonuçlarının farklılık gösterdiği görülmektedir. Bu farklılıkların, örnek hazırlama yöntemleri, analiz teknikleri, kullanılan mekanizmalar (CUPRAC bakır indirgeme, FRAP demir indirgeme) ve testlerin duyarlılık seviyelerindeki değişikliklerden kaynaklanabileceği düşünülmektedir.

Çizelge 4 incelendiğinde fide boyu ile fide yaş ağırlık arasında %5 düzeyinde, fide kuru ağırlığı ile ise %1 düzeyinde olumlu bir ilişkinin olduğu görülmektedir. Fide yaş ağırlığının; fide kuru ağırlık ile arasında %1, CUPRAC antioksidan aktivite ile arasında %5 düzeyinde olumlu korelasyon varken, total karotenoid ile %5 düzeyinde olumsuz bir korelasyon olduğu görülmüştür. Kök uzunluğu ile FRAP antioksidan aktivite arasında %1 düzeyinde pozitif bir ilişkinin olduğu, kök yaş ağırlığı ile kök kuru ağırlık arasında %1 düzeyinde olumlu ilişkinin olduğu görülmüştür. Klorofil a miktarının klorofil b ve total klorofil miktarı ile arasında %1 düzeyinde pozitif ilişkinin olduğu, klorofil b'nin total klorofil ile %1, total karotenoid ile ise arasında %5 düzeyinde pozitif ilişkinin olduğu tespit edilmiştir. Total klorofil ile total karotenoid arasında %1 düzeyinde olumlu bir ilişkinin olduğu, total karotenoid miktarı ile CUPRAC antioksidan aktivite arasında %5 düzeyinde olumsuz bir ilişkinin olduğu, CUPRAC antioksidan aktivite ile total fenolik madde arasında ise %1 düzeyinde olumlu bir ilişkinin olduğu tespit edilmiştir.

## Sonuç

Hormon uygulamalarının Oğul otu bitkisinin fide gelişiminde önemli etkileri olduğu, IBA50 uygulamasının, fide boyu ve yaş ağırlığı açısından en uygun hormon olabileceği, kök uzunluğunda ise BAP100 uygulamasının öne çıktığı görülmüştür. IBA50 ve BAP100 hormon uygulamalarının bitki büyümesini olumlu yönde etkilemekte olup, bu hormonların kullanımı Melissa bitkisinin büyüme parametreleri açısından önemli olduğu söylenebilir.

Çizelge 3. Bazı sentetik hormonların *Melissa officinalis* bitkisinin biyokimyasal parametreleri üzerine etkileri  
Table 3. Effects of some synthetic hormones on biochemical parameters of *Melissa officinalis* plant.

Sentetik Hormon	Total Karotenoid (mg/g)	CUPRAC (mM/g TE)	FRAP (mM/g AAE)	Total Fenolik Madde (mg/g GAE)
Kontrol	1,10 A	2,09 B	1,08 C	0,24 C
BAP50	1,14 A	2,65 B	1,39 AB	0,27 C
BAP100	1,14 A	5,21 A	1,71 A	0,47 AB
IBA50	0,85 B	5,45 A	1,44 AB	0,40 B
IBA100	0,82 B	6,80 A	1,24 BC	0,55 A
IAA50	0,88 B	5,78 A	1,20 BC	0,49 AB
IAA100	0,92 B	6,89 A	1,35 BC	0,48 AB
LSD (0.05)	0,15	1,68	0,29	0,1
CV (%)	9,19	19,27	12,64	14,33

TE: Troloks Eşdeğeri, AAE: Askorbik Asit Eşdeğeri, GAE: Gallik Asit Eşdeğeri



Çizelge 4. Bazı sentetik hormonların *Melissa officinalis* bitkisinin incelenen parametreler arasındaki korelasyon tablosu  
Table 4. Correlation table between some synthetic hormones and the studied parameters of the plant *Melissa officinalis*

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1												
2	0,799*	1											
3	0,333	0,335	1										
4	0,098	0,560	-0,214	1									
5	0,329	0,659	-0,182	0,948**	1								
6	0,944**	0,912**	0,225	0,393	0,585	1							
7	-0,568	-0,733	0,256	-0,436	-0,478	-0,700	1						
8	-0,613	-0,741	0,059	-0,250	-0,288	-0,672	0,954**	1					
9	-0,596	-0,746	0,163	-0,351	-0,391	-0,695	0,989**	0,988**	1				
10	-0,414	-0,755*	0,168	-0,604	-0,593	-0,626	0,937**	0,866*	0,913**	1			
11	0,376	0,777*	0,379	0,478	0,481	0,523	-0,583	-0,630	-0,612	-0,758*	1		
12	0,418	0,270	0,904**	-0,221	-0,086	0,302	0,365	0,242	0,309	0,303	0,213	1	
13	0,250	0,653	0,417	0,386	0,362	0,405	-0,480	-0,521	-0,505	-0,700	0,965**	0,256	1

\* Korelasyon %5 düzeyinde önemlidir; \*\* Korelasyon %1 düzeyinde önemlidir; 1: Fide Boyu, 2: Fide Yaş Ağırlık, 3: Kök Uzunluk, 4: Kök Yaş Ağırlık, 5: Kök Kuru Ağırlık, 6: Fide Kuru Ağırlık, 7: Klorofil a, 8: Klorofil b, 9: Total Klorofil, 10: Total Karotenoid, 11: CUPRAC, 12: FRAP, 13: Total Fenolik Madde

Hormon uygulamalarının korofil a pigmentini ve toplam klorofil pigment artışına katkı sağladığı görülmüştür. *Melissa* bitkisinde fide gelişim döneminde hormon uygulamalarının antioksidan aktiviteler, toplam fenolik madde ve total karotenoid miktarları üzerinde BAP100 ve IBA100 uygulamalarının öne çıktığı görülmüştür.

## Beyan

Çalışmanın yürütülmesi için ortam sağlayan SUBÜ'ye bağlı TABTEM Kurumu çalışanlarına teşekkür ederiz.

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## Effects of Different Treatments on Germination and Seedling Development of Moringa (*Moringa oleifera* L.) Seeds

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 07.08.2024 Accepted : 07.10.2024</p> <p>Keywords: <i>Moringa oleifera</i> L. Germination GA<sub>3</sub> PEG Microbial fertilizer</p>	<p>This study consists of two phases: moringa seed germination and seedling development. Firstly, four different treatments (a: Soaking in warm water at 35 °C for 24 hours; b: 24 hours in 1000 ppm GA<sub>3</sub> solution; c: -1MPa polyethylene glycol (PEG) solution for 24 hours; d: 2500 ppm microbial fertilizer solution for 24 hours) were applied to the seeds in addition to the control treatment. In germination trials carried out under controlled conditions, the temperature was set at 25 °C and the relative humidity was above 80%. After seed sowing, germination rate, time and germination energy were determined for each treatment. After germination, the seedlings were transferred to growing medium containing peat and perlite in a 1:1 ratio. Then, NPK, NPK + 2500 ppm microbial fertilizer and NPK + 5000 ppm microbial fertilizer were applied to the seedlings six times with 15 days intervals. After 90 days, plant height, stem diameter, chlorophyll content, stem, root wet and dry weights were determined. The results of the study showed that the best results for seed germination rate, time and energy were obtained by soaking the seeds in warm water at 35 °C for 24 hours before sowing in vials, followed by soaking in 1000 ppm GA<sub>3</sub> solution for 24 hours. Considering the parameters examined in terms of seedling growth and development, the use of standard NPK together with microbial fertilizer gave better results compared to standard NPK. As a result of this study, it was recommended to keep the seeds in warm water at 35°C for 24 hours for seed germination and to use standard NPK with microbial fertilizer for seedling growth.</p>

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 12(s1): 2076-2081, 2024

## Farklı Uygulamaların Moringa (*Moringa oleifera* L.) Tohumlarının Çimlenme ve Fidan Gelişimi Üzerine Etkileri

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 07.08.2024 Kabul : 07.10.2024</p> <p>Anahtar Kelimeler: <i>Moringa oleifera</i> L. Çimlenme GA<sub>3</sub> PEG Mikrobiyal gübre</p>	<p>Bu çalışma, moringa tohum çimlenmesi ve fidan gelişimi olmak üzere iki aşamadan oluşmaktadır. Öncelikle moringa tohumlarına kontrol dışında, dört farklı uygulama (a: 35°C'deki ılık suda 24 saat bekletme; b: 1000 ppm GA<sub>3</sub> çözeltisinde 24 saat bekletme; c: -1MPa polietilen glikol (PEG) çözeltisinde 24 saat bekletme; d: 2500 ppm mikrobiyal gübre çözeltisinde 24 saat bekletme) yapılmıştır. Kontrollü koşullarda gerçekleştirilen çimlendirme denemelerinde sıcaklık, 25°C ve oransal nemi ise %80'in üzerinde olacak şekilde ayarlanmıştır. Tohum ekiminden sonra, çimlenme oranı, süresi ve çimlenme enerjisi her bir uygulama için belirlenmiştir. Çimlenmeden sonra fidanlar 1:1 oranında torf ve perlit içeren yetiştirme ortamına aktarılmıştır. Daha sonra fidanlara 15 gün ara ile altı defa NPK, NPK + 2500 ppm mikrobiyal gübre ve NPK + 5000 ppm mikrobiyal gübre uygulamaları yapılmıştır. Yetişen bitkilerde 90 gün sonra, bitki boyu, gövde çapı, klorofil miktarı, gövde, kök yaş ve kuru ağırlıkları belirlenmiştir. Araştırma bulguları, tohum çimlenme oranı, süresi ve enerjisi açısından en iyi sonucun, tohumların viyollere ekimden önce 35°C'deki ılık suda 24 saat bekletme uygulamasının verdiğini göstermiş ve bu uygulamayı 1000 ppm GA<sub>3</sub> çözeltisinde 24 saat bekletme uygulaması izlemiştir. Fidan büyüme ve gelişmesi açısından incelenen parametreler göz önüne alındığında, standart NPK'nın mikrobiyal gübre ile birlikte kullanımı, standart NPK'ya göre daha iyi sonuç vermiştir. Araştırma sonucunda, tohum çimlenmesi açısından 35 °C'deki ılık suda 24 saat bekletme ve fidan gelişimi açısından ise standart NPK'nın mikrobiyal gübre ile birlikte kullanımı tavsiye edilmiştir.</p>

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## Giriş

Anavatanı Hindistan, Pakistan, Bangladeş ve Afganistan'ın alt Himalaya bölgeleri olan moringa, Moringaceae familyası içerisinde yer almakta ve 13 adet türü bulunmaktadır (Fuglie, 1999). Multifonksiyonel bitki olarak bilinen ve aynı zamanda "mucize ağaç" ya da "yaşam ağacı" olarak tanımlanan moringa (*Moringa oleifera* L.), yüzyıllar boyunca tamamlayıcı tıpta çeşitli hastalıkların tedavisinde ve hastaların genel vücut sağlığının geliştirmesinde kullanılmıştır. Moringa, yüzyıllar boyunca farklı kullanım alanları sayesinde birçok uygarlıklarca yetiştirilmiş ve böylece dünyada tropikal ve subtropikal bölgelere yayılmıştır (Fahey, 2005). En yaygın olarak yetiştirilen tür ise *Moringa oleifera*'dır. Moringa bitkisinin tüm kısımları (kökü, kabuğu, yaprağı, çiçeği, meyvesi ve tohumu) insan ve hayvan beslenmesinden kozmetiğe, biyoyakıt üretiminden yeşil gübreye, çerez, yağ eldesi ve hatta suyun dezenfeksiyonuna kadar birçok alanda kullanılmaktadır. Meyveleri erken dönemde fasulye gibi pişirilerek tüketilmekte, ayrıca tohumu çıkartılarak farklı yemekler yapılmaktadır. Çiçekleri taze olarak ya da çay olarak tüketilmektedir (Fuglie, 1999). Çiçekleri kalsiyum ve potasyum bakımından zengin olup, arılar için nektar kaynağı olarak da kullanılmaktadır. Yapraklarının zeatin bakımından zengin olduğu ve sitokinin kaynağı olarak kullanıldığı bildirilmiştir (Fuglie, 1999). Moringanın tohumları %40 yağ içermekte, yağı çok iyi kalitede olup, parfüm sanayinde ve gaz yağı yapımında kullanılmaktadır. Odunu kâğıt endüstrisinde, ip ve paspas yapımında, kökleri ise öğütülerek salata sosu yapımında kullanılmaktadır (Barminas ve ark., 1998). Moringa bitkisinin Afrika ve Nikaragua'da yapılan çalışmalar sonucunda; insan sağlığı açısından kötü beslenmeyi önlemek ve hastalıklarla savaşmak için besin maddelerince, esansiyel (zorunlu) aminoasitler ve proteince zengin doğal bir tarım ürünü olduğu bildirilmiştir (Booth ve Wickens, 1988). Kırktan fazla antioksidan içeren moringa ayrıca askorbat, karotenoidler, fenoller, potasyum ve kalsiyum bakımından da zengindir. Moringanın 100 g kuru yaprağı; havuçtan 10 kat daha yüksek A vitamini, portakalın yarısı kadar C vitamini, süttan 17 kat daha yüksek kalsiyum, muzdan 15 kat daha yüksek potasyum, ıspanaktan 25 kat daha yüksek demir ve yoğurttan 9 kat daha yüksek protein içermektedir (Sreeramulu ve ark., 1983). Buna ilave olarak Afrika ve Hindistan'da geleneksel olarak kullanılan 539 ilacın içeriğinde yer alan moringanın, 300 hastalığın tedavisinde kullanıldığı bildirilmiştir (Foidl ve ark., 2001).

Hayvan beslenmesi açısından da önemli olan moringanın, karkas ağırlığını %32, günlük süt üretimini %43-65 oranında arttırdığı saptanmıştır. Ayrıca bitkisel gelişim açısından da yaprak gübresi olarak kullanıldığında yüksek oranlarda verimi arttırdığı bildirilmiştir (Culver ve ark., 2012; Singh ve ark., 2013; Afzal ve Iqbal, 2015). Moringa yaprak ekstraktları kullanılarak yapılan bazı ön çimlendirme uygulamalarında bitki gelişiminin teşvik edildiği belirlenmiştir (Basra ve ark., 2011).

Türkiye'de henüz yeni bir tür olan moringa, özellikle Antalya'nın Gazipaşa ve Demre ilçelerinde amatör olarak küçük ölçekli çiftçilerce yetiştirilmeye başlanmış olup, teşvikler neticesinde yeni yeni yayılım göstermektedir. Fakat subtropik koşullara özellikle kışı sert geçen

bölgelerde, fidanların her yıl yeniden dikilmesi gerekmektedir. Bu nedenle, multifonksiyonel özelliğe sahip moringa tohumlarında çimlenme ve fidan yetiştiriciliğinde optimum koşulların belirlenmesi büyük önem arz etmektedir.

Moringa tohumlarının çimlenmesi üzerinde yapılan çalışmalar, tohumlarda çimlenme oranının ön işlemlere göre değişmekle beraber %50 ile %90 oranında değiştiğini ve laboratuvar koşullarında çimlenmenin 4-10 gün arasında tamamlandığını göstermektedir (Onyekwelu ve Olabiwonnu, 2010; Mubvuma ve ark., 2013; Korsor ve ark., 2016; Hassanein ve Al-Soqeer, 2017; Nunez-Gastelum ve ark., 2023). Barraza (2017) ve Montilla-Mota ve ark. (2017), moringa tohumlarını 24 saat suda bekletmenin çimlenmeyi hızlandırdığını bildirmişlerdir. Ahmad ve ark. (2020), moringa tohumlarını iki farklı bitkiden (*Glycyrrhiza glabra* ve *Ammi majus*) elde edilen ekstraktlarda bekletmişlerdir. Araştırmacılar, kontrol uygulamasında tohumlarda çimlenme oranını %80 olarak belirlemişler, buna karşın tohumlarda en yüksek çimlenme oranı %86,6 ile 10 ppm *Ammi majus* bitkisinden elde edilen ekstraktta bekletme uygulamasından saptamışlardır. Alshoabi (2021), moringa tohumlarının çimlenmesi üzerine farklı ortam derecelerinin (15°C, 25°C ve 35°C) etkilerini araştırmıştır. Araştırmacı, tohumların çimlenmesi için en uygun sıcaklık derecesini 25°C olarak belirlemiş, ancak çimlenme sonrası fizyolojik gelişimin ise 35°C de daha iyi olduğunu bildirmiştir. Öte yandan literatürde moringa tohumlarının çimlenmesinden sonra farklı gübre uygulamaları ile fidan yetiştiriciliği yapıldığı bir çalışmaya rastlanmamıştır. Ancak Sreehari ve ark. (2022), farklı ortamlarda çimlendirilen tohumlardan elde edilen fidanların gelişmelerini incelemişlerdir. Araştırmacılar 11 farklı yetiştirme ortamının moringada fidan gelişimi üzerine etkilerini araştırmışlar ve %50 toprak+%25 vermikülit+%25 perlitten oluşan yetiştirme ortamının incelenen parametreler açısından en iyi sonucu verdiğini bildirmişlerdir.

Ülkemiz için henüz çok yeni bir tür olan moringanın yaygınlaştırılması, ancak türün çoğaltılması ve fidan gelişimi için en uygun şartların belirlenmesi ile mümkündür. Bilindiği üzere günümüzde meyve ve sebze yetiştiriciliğinde kültürel işlemlerden gübreleme (organik veya kimyasal) rutin olarak yapılmaktadır. Bunlara ilave olarak mikrobiyal gübreleme son zamanlarda önem kazanmış olup özellikle yapılan organik veya kimyasal gübrelerin bitki tarafından alınabilirliğini arttırmaktadır (Javorekova ve ark. 2015; Altunlu 2021).

Bu çalışmada, moringa tohumlarına ekimden önce yapılan bazı uygulamalar ile çimlenmeden sonra rutin gübrelemenin yanı sıra, farklı dozda mikrobiyal gübre uygulamalarının, rutin gübreleme ile birlikte kullanımının fidanlarda büyüme ve gelişmesi üzerine etkilerinin belirlenmesi amaçlanmıştır.

## Materyal ve Metod

Çalışmada Güney Amerika menşeli, araştırma amaçlı gönderilen *Moringa oleifera* L. türüne ait tohumlar kullanılmıştır. Araştırma, tohum çimlendirmesi ve fidan yetiştiriciliği olmak üzere iki aşamadan oluşmakta olup ilgili çalışmalar, 2021-2022 yılları arasında Akdeniz Üniversitesi Ziraat Fakültesi Araştırma ve Uygulama

Arazisinde yürütülmüştür. Çalışmada kullanılan mikrobiyal gübre sıvı formda olup (toplam canlı mikroorganizma sayısı  $1 \times 10^7$  kob/ml) *Bacillus megaterium*, *Paenibacillus polymyxa*, *Pantoea agglomerans*, *Pseudomonas fluorescens* bakterilerini içermektedir.

### Tohum Çimlendirmesi

Tohum çimlendirme aşamasında, tohumlara ekimden önce aşağıda belirtilen ön işlemler uygulanmıştır;

- Kontrol
- 35°C'deki ılık suda 24 saat bekletme
- 1000 ppm giberellik asit (GA<sub>3</sub>) çözeltisinde 24 saat bekletme
- -1MPa polietilen glikol (PEG) çözeltisinde 24 saat bekletme
- 2500 ppm (mikrobiyal gübre) çözeltisinde 24 saat bekletme

Tohum çimlendirmesinde ortam olarak 2:1 oranında torf:perlit karışımı kullanılmıştır. Ön işlem uygulanan tohumlar, 45'lik plastik viyollere ekilmişlerdir. Tohumlarda çimlendirme çalışmaları, iklimlendirme odasında kontrollü koşullarda yürütülmüştür. İklimlendirme odasının sıcaklığı 25°C ve oransal nemi ise %80'in üzerinde olacak şekilde ayarlanmıştır.

Tohumlarda çimlenme, 20 gün boyunca günlük takip edilmiş ve çimlenme süresince aşağıda bildirilen gözlemler yapılmıştır.

**Çimlenme oranı (%):** Çimlenen tohum sayısı, toplam ekilen tohum sayısına oranlanarak hesaplanmıştır. (Güneş ve ark., 2013).

**Ortalama çimlenme süresi (gün):** Ellis ve Roberts (1981)'e göre aşağıdaki şekilde hesaplanmıştır.

- $MGT = \frac{\sum TiNi}{\sum N}$
- MGT: Ortalama çimlenme süresi
- Ti : Ekimden sonra kaçınıcı günde gözlem yapıldığını belirtir
- Ni : Gözlem yapıldığı gün çimlenen tohum sayısını belirtir
- N : Toplam çimlenen tohum sayısı

**Çimlenme enerjisi:** Tohum ekiminden çimlenme sonuna kadar geçen sürenin yarısına kadar çimlenen tohum sayısı, toplam çimlenen tohum sayısına oranlanarak (%) hesaplanmıştır (Karaguzel ve ark., 2002).

### Fidan Yetiştiriciliği

Tohum çimlenmesi tamamlandıktan sonra fidanlar 1:1 oranında torf ve perlit içeren, 15x35 cm boyutlu plastik torbalara aktarılmıştır ve deneme cam fidan yetiştirme serasında yürütülmüştür. Fidan yetiştiriciliği aşamasında üç farklı uygulama denemeye alınmış, bu uygulamalara aşağıda yer verilmiştir.

- NPK
- NPK + 2500 ppm (mikrobiyal gübre)
- NPK + 5000 ppm (mikrobiyal gübre)

Çalışmada, her fidan tüpüne 3 g olacak şekilde 20:20:20 oranında NPK içeren gübre ilave edilmiştir. Fidanların plastik torbalara aktarılmasından 20 gün sonra her 15 günde bir NPK ve uygulamalara göre değişmekle beraber yukarıda belirtilen dozlarda hazırlanan çözeltiden 100 ml mikrobiyal gübre (MG) ilave edilmiştir. Ayrıca bitkilerde toprak nem durumuna göre

eşit miktarda sulama yapılmıştır. Çalışma 75 gün boyunca devam ettirilmiş ve araştırma sonucunda aşağıda bildirilen gözlem ve ölçümler yapılmıştır.

**Gövde çapı (cm):** Toprak yüzeyinin 5 cm üzerinden dijital bir kumpas yardımı ile ölçülerek belirlenmiştir.

**Bitki boyu (cm):** Gövdenin kök boğazı kısmı ile en uç büyüme noktası arasındaki mesafe, şerit metre ile ölçülerek belirlenmiştir.

**Klorofil miktarı (SPAD):** Bitkilerde klorofil miktarı SPAD 502 marka klorofil ölçer cihaz ile ölçülerek belirlenmiştir.

**Gövde yaş ağırlık (g/bitki):** Her bitkinin toprak üstü kısmı hassas terazide tartılarak g ( $\pm 0,1$ ) yaş ağırlıkları belirlenmiştir.

**Kök yaş ağırlık (g/bitki):** Her bitkinin toprak altı kısmı hassas terazide tartılarak g ( $\pm 0,1$ ) yaş ağırlıkları belirlenmiştir.

**Gövde kuru ağırlık (g/bitki):** Yaş ağırlıkları belirlenen toprak üstü kısmı 65°C etüvde sabit ağırlığa ulaşmaya kadar kurutulduktan sonra kuru ağırlık g olarak belirlenmiştir.

**Kök kuru ağırlık (g/bitki):** Yaş ağırlıkları belirlenen toprak altı kısmı, etüvde 65°C'de sabit ağırlığa ulaşmaya kadar kurutulmuş ve kuru ağırlık g olarak belirlenmiştir.

### İstatistiksel Analiz

Araştırma üç tekerrürlü olarak, tesadüf parselleri deneme desenine göre planlanmıştır. Tohum çimlenmesinde her tekerrürde 15 tohum, fidan yetiştiriciliğinde ise her tekerrürde 10 bitki olacak şekilde planlanmıştır. Araştırmada toplam 225 adet (5 uygulama  $\times$  3 tekerrür  $\times$  15 tohum) tohum ile toplam 90 adet (3 uygulama  $\times$  3 tekerrür  $\times$  10 fidan) fidan kullanılmıştır. Çalışma sonucunda elde edilen tüm verilerin varyans analizi XLSTAT programında yapılmış ve ortalamaların karşılaştırılmasında Asgari Önemli Fark (AÖF) testi kullanılmıştır. Ayrıca fidan yetiştirme aşamasında elde edilen sonuçlarda temel bileşen analizleri yapılmış olup bu analiz için de XLSTAT programı kullanılmıştır.

### Bulgular ve Tartışma

Tohumlara yapılan uygulamaların, tohumların çimlenme oranı (%), ortalama çimlenme süresi (gün) ve çimlenme enerjisi (%) üzerine etkileri Çizelge 1'de verilmiştir. Çizelge 1'de, tohumlarda en yüksek çimlenme oranı %86,66 ile 35 °C ılık suda bekletme uygulamasından belirlenmiştir. Çimlenme oranı üzerine, 35°C ılık su uygulaması dışındaki kontrol dahil tüm uygulamalar istatistiksel olarak aynı grup içerisinde yer almıştır. Uygulamaların ortalama çimlenme süresi üzerine etkileri incelendiğinde, kontrol dışındaki uygulamaların tohumlarda ortalama çimlenme süresini kısalttığı saptanmıştır. Ortalama en kısa çimlenme süresi 9,12 gün ile çimlenme oranında olduğu gibi 35°C ılık suda bekletme uygulamasında saptanmıştır. Ortalama en uzun çimlenme süresi ise 11,10 gün ile kontrol uygulamasında belirlenmiştir (Çizelge 1). Çimlenme enerjisi üzerine uygulamaların etkisi incelendiğinde ise diğer kriterlerde olduğu gibi en yüksek çimlenme enerjisi (%77,93), incelenen diğer iki kriterde olduğu gibi 35°C ılık suda bekletme uygulamasında saptanmıştır.

Çizelge 1. Moringa tohumlarında farklı uygulamaların tohumlarda çimlenme oranı, ortalama çimlenme süresi ve çimlenme enerjisi üzerine etkileri

Table 1. Effects of different treatments on germination rate, mean germination time and germination energy in Moringa seeds

Uygulamalar	Çimlenme Oranı (%)	Ortalama Çimlenme Süresi (gün)	Çimlenme Enerjisi (%)
Kontrol	77,77b	11,10a	47,8d
Ilık Su (35 °C)	86,66a	9,12d	77,93a
GA <sub>3</sub> (1000 ppm)	77,77b	10,40c	62,47b
PEG (-1MPa)	75,55b	10,75b	39,21e
Mikrobiyal Gübre (2500 ppm)	73,33b	10,53bc	51,88c
AÖF <sub>%5</sub>	4,973	0,301	2,738

Çizelge 2. Farklı gübre uygulamalarının moringa fidanlarda gövde çapı ve bitki boyu ile klorofil miktarı üzerine etkileri

Table 2. Effects of different fertilizer applications on stem diameter, plant height and chlorophyll content in moringa seedlings

Uygulamalar	Gövde Çapı (mm)	Bitki Boyu (cm)	Klorofil (SPAD)
NPK	4,31b	89,42a	32,42a
NPK + 2500ppm MG	4,57ab	84,80b	30,67b
NPK + 5000 ppm MG	4,66a	74,62c	29,39c
AÖF <sub>%5</sub>	0,273	2,969	0,652

Çizelge 3. Farklı gübre uygulamalarının fidanlarda gövde ve kök yaş ağırlığı ile kuru ağırlıkları üzerine etkileri

Table 3. Effects of different fertilizer applications on stem and root wet weight and dry weight of seedlings

Uygulamalar	Gövde Yaş Ağırlığı (g)	Kök Yaş Ağırlığı (g)	Gövde Kuru Ağırlığı (g)	Kök Kuru Ağırlığı (g)
NPK	130,39	48,15c	20,13b	8,72c
NPK + 2500ppm MG	133,97	73,99b	20,85b	15,11b
NPK + 5000 ppm MG	136,37	134,39a	23,47a	17,50a
AÖF <sub>%5</sub>	Ö,D,	9,922	0,913	1,959

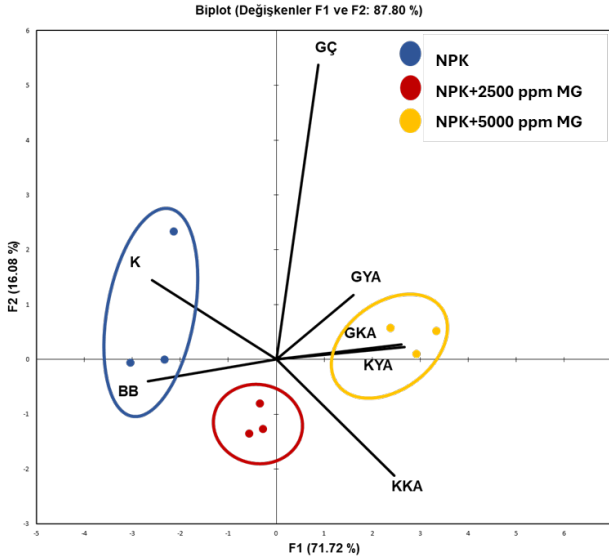
Farklı gübre uygulamalarının fidanlarda gövde çapı (mm), bitki boyu (cm) ve klorofil miktarı (SPAD) üzerine etkileri Çizelge 2’de verilmiştir. Uygulamaların fidanlarda gövde çapı üzerine etkileri incelendiğinde, istatistiksel olarak iki ana ve bir ara grubun olduğu görülmektedir. En yüksek gövde çapı 4,66 mm ile NPK + 5000 ppm MG uygulamasından ve en düşük gövde çapı ise 4,31 mm ile NPK uygulamasından elde edilmiştir. Bitki boyu üzerine uygulamaların etkisi incelendiğinde ise gövde çapının aksine en yüksek bitki boyu 89,42 cm ile NPK uygulamasından ve en düşük bitki boyu ise 74,62 cm ile NPK + 5000 ppm MG uygulamasından elde edilmiştir. Farklı dozlarda gübre uygulamalarının bitki yapraklarındaki klorofil miktarı üzerine etkisi incelendiğinde, bitki boyunda olduğu gibi en yüksek klorofil miktarı 32,42 SPAD değeri ile NPK uygulamasında, en düşük klorofil miktarı ise 29,39 SPAD değeri ile NPK + 5000 ppm MG uygulamasından elde edilmiştir.

Farklı gübre uygulamalarının fidanlarda gövde ve kök yaş ağırlığı (g) ile her iki kısmın kuru ağırlıkları (g) üzerine etkileri Çizelge 3’te verilmiştir. Bu çizelgede de görüldüğü gibi uygulamaların gövde yaş ağırlığı üzerine etkileri istatistiksel olarak önemsiz bulunmuştur. Ancak uygulamalar arasında doz ve gövde yaş ağırlığının doğrusal oranda artış gösterdiği tespit edilmiştir. Gövde yaş ağırlığının aksine, uygulamaların kök yaş ağırlığı üzerine etkisi istatistiksel olarak önemli bulunmuştur. Uygulamaların kök yaş ağırlığı üzerine etkileri incelendiğinde, en yüksek ağırlık 134,39 g ile NPK + 5000 ppm MG uygulamasından ve en düşük ise 48,15 g ile NPK’nın bağımsız uygulamasından elde edilmiştir. Farklı gübre uygulamalarının gövde kuru ağırlığı üzerine etkisi incelendiğinde, NPK ve NPK + 2500

ppm MG uygulamaları aynı istatistik grup içerisinde yer almış ve değerler sırası ile 20,13 ve 20,85 olarak kaydedilmiştir. En yüksek gövde kuru ağırlığı ise 23,47 g ile NPK + 5000 ppm MG uygulamasından saptanmıştır. Kök kuru ağırlığı üzerine uygulamaların etkisi incelendiğinde, Çizelge 3’te verilen diğer kriterlerde olduğu gibi dozlar ve değerler arasında doğrusal ilişki kaydedilmiş, en düşük kök kuru ağırlık 8,72 g ile NPK uygulamasında ve en yüksek kök kuru ağırlığı ise 17,50 g ile NPK + 5000 ppm MG uygulamasında saptanmıştır.

Fidan yetiştiriciliği aşamasında incelenen tüm kriterler dikkate alınarak belirlenen temel bileşen analizi Şekil 1’de verilmiştir. Şekil 1 incelendiğinde NPK uygulamasının bitki boyu ve klorofil miktarı üzerine olumlu etkisinin olduğu izlenebilmektedir. Fidanlara NPK gübresi yanında uygulanan mikrobiyal gübre dozlarındaki artış gövde çevresi ile gövde ve kök yaş ağırlığının yanı sıra kuru ağırlıklarında da artış meydana getirdiği yine Şekil 1’den izlenebilmektedir. Yapılan temel bileşen analizi ile incelenen kriterler arasında da ilişkiler belirlenmiş olup özellikle bitki boyu ve klorofil içeriği arasında pozitif bir ilişkinin olduğu belirlenmiştir. Bununla beraber gövde ve köke ait yaş ve kuru ağırlıklar arasında pozitif yönde ilişki tespit edilmiş ve özellikle gövde kuru ağırlığı ile kök yaş ağırlığı arasında kuvvetli pozitif ilişki belirlenmiştir. Ayrıca bitki boyu ile gövde kuru ve kök yaş ağırlığı arasında kuvvetli negatif ilişki olduğu tespit edilmiştir.

Yürütülen bu araştırmanın ilk aşamasında, farklı uygulamaların tohum çimlenmesi üzerine etkileri incelenmiş, uygulamalara göre değişimle birlikte çimlenme oranı %73,33 ile %86,66, ortalama çimlenme süresi 9,12 gün ile 11,10 gün ve çimlenme enerjisi ise %39,21 ile 77,93 arasında değişim göstermiştir.



Şekil 1. Farklı gübre uygulamalarına bağlı olarak moringa fidanlarında incelenen parametrelere ait temel bileşen analizi sonuçları

GÇ: Gövde Çapı, BB: Bitki Boyu, K: Klorofil, GYA: Gövde Yaş Ağırlığı, KYA: Kök Yaş Ağırlığı, GKA: Gövde Kuru Ağırlığı, KKA: Kök Kuru Ağırlığı

Figure 1. Results of principal component analysis of the parameters examined in moringa seedlings based on different fertilizer applications

Moringa tohumlarının çimlenmesi üzerine yapılan çalışmalarda, çimlenme oranının %50-90 arasında, çimlenme süresinin ise 4-10 gün arasında değiştiği bildirilmiştir (Onyekwelu ve Olabiwonnu, 2010; Mubvuma ve ark., 2013; Korsor ve ark., 2016; Hassanein ve Al-Soqeer, 2017; Nunez-Gastelum ve ark., 2023). Bulgularımızın, bu çalışmalar ile uyum içinde olduğu tespit edilmiştir. Bununla birlikte, bulgularımız sonucu saptanan en düşük çimlenme süresi, diğer çalışmalardan biraz yüksek, en yüksek çimlenme oranı ise diğer çalışmalara yakın saptanmıştır. Nitekim en yüksek çimlenme oranı, en düşük ortalama çimlenme süresi ve en yüksek çimlenme enerjisi 35°C ılık suda 24 saat bekletme uygulamasından elde edilmiştir. Çalışmanın ikinci basamağını oluşturan fidan yetiştirme aşamasında, fidanlara standart NPK gübresi uygulanmasının yanı sıra, 2 farklı dozda mikrobiyal gübre uygulaması yapılmıştır. Mikrobiyal gübre dozu arttıkça gövde çapında artış belirlenirken, bitki boyu ve klorofil miktarında düşüş saptanmıştır. Ancak bitki boyu ve klorofil miktarının aksine, mikrobiyal gübre dozları arttıkça gövde ile kök yaş ve kuru ağırlıklarında artışlar saptanmıştır. Fidan yetiştiriciliğinde, bitki boyundan ziyade, gövde çapının da önemli olduğu göz önüne alınır, mikrobiyal gübrenin standart NPK ile birlikte kullanımının daha avantajlı olduğu düşünülmektedir. Katoriya ve ark. (2021), moringa tohumlarında farklı GA<sub>3</sub> dozlarının fidan gelişimi üzerine etkisini araştırmışlar ve çimlenmeden 45 gün sonra fidanlarda gövde yaş ağırlığının uygulamalara göre değişmekle beraber 11,6 g ile 18,33 g, gövde kuru ağırlığının 2,97 g ile 5,65 g arasında olduğunu belirlemişlerdir. Aynı araştırmacılar kök yaş ağırlığının 3,87 g ile 6,01 g, kök kuru ağırlığının ise 0,77 ile 1,94 g arasında olduğunu bildirmişlerdir. Bulgularımız, Katoriya ve ark. (2021)'nin bulguları ile farklılık göstermektedir.

Çalışmamızda elde edilen yaş ve kuru ağırlıkların Katoriya ve ark. (2021)'nin bulgularına göre çok yüksek olmasının nedeni, gübre uygulamaları ile ilişkilendirilebilir. Bulgularımız ayrıca Shreehari ve ark. (2022) tarafından yürütülen çalışma ile de farklılık göstermiştir. Nitekim Shreehari ve ark. (2022), farklı yetiştirme ortamlarına ekilen tohumların, çimlenmeden sonra fidan gelişimi incelemişlerdir. Araştırmacılar, 90 günün sonunda gövde ve köke ait yaş ile kuru ağırlıkları tespit etmişlerdir. Uygulamalara göre değişmekle beraber gövde yaş ağırlığının 12,43 g ile 21,48 g, gövde kuru ağırlığının 5,46 g ile 10,50 g arasında, kök yaş ağırlığının 6,09 g ile 8,54 g, kök kuru ağırlığının 2,37 g ile 3,90 g arasında değiştiğini bildirmişlerdir. Bulgularımız sonucu saptanan yaş ve kuru ağırlığın, her iki çalışmaya göre yüksek saptanması, moringada gübre kullanımının fidan yetiştiriciliğine olumlu yönde yansması olarak gösterilebilir.

## Sonuç

Araştırma sonuçları; tohum çimlenme oranı, süresi ve enerjisi açısından denenen uygulamalar arasında en iyi sonucun, tohumların ekimden önce 35 °C ılık suda 24 saat bekletme uygulaması olduğunu göstermiştir. Bitkisel özellikleri ile ön plana çıkan, yaprakları ve kökleri ilaç sanayinde tercih edilen moringanın yetiştiriciliğinde standart gübreleme ile mikrobiyal gübre uygulamalarının birlikte kullanılması tavsiye edilmiştir.

## Teşekkür

Bu çalışma, TÜBİTAK 2209-B “Sanayiye Yönelik Lisans Araştırma Projeleri Destekleme Programı” kapsamında desteklenmiştir. Yazarlar TÜBİTAK’a teşekkür etmektedir. Ayrıca yazarlar, destekleme programı kapsamında sanayi danışmanı Onur ÇALIK (Naturmed İlaç Kimya ve Kozmetik)’a da teşekkür etmektedir.

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## Effects of Some Insecticides on *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psyllidae)

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Insecticides

### ABSTRACT

Chemical control is the most common method used to control *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psyllidae). This study compared the application of the five most widely used insecticides in the management of *A. pistaciae* which has resulted in significant yield losses, in Siirt (Kurtalan) and Şanlıurfa (Birecik) in 2023. Active ingredients spinetoram, lambda-cyhalothrin, deltamethrin, sulfoxaflo, and spirotetramat were examined in the study. The study was designed with four replications, and its characters were formed by the insecticides registered concentration and the control. The plots measured 3 x 3 = 9 trees. Nymph and adult counts were conducted from the middle trees before, as well as 7, 14, and 21 days after application following the application of the spray. The Henderson-Tilton formula was used to analyze the counting data for nymph or adult counts in each plot of leaf samples. All registered active ingredients effected the *A. pistaciae* above %80 all count days. Among the active ingredients the biological efficacy of spirotetramat was the highest compared to other insecticides. These active ingredients can be used to control *A. pistaciae*, with consideration given to the pest's potential resistance to insecticides and the impact of the ingredients on natural enemies.

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## Introduction

Pistachio psyllid, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psyllidae) is one of the most important pests in pistachio-growing regions in Türkiye. For half a century, *A. pistaciae* has been causing great economic losses in pistachio orchards in the world. The pistachio psyllid is found in many pistachio-growing areas around the borders of Türkiye, including Armenia, Iraq, Turkmenistan, and Iran, as well as in Mediterranean regions such as Greece and Syria (Burckhardt & Lauterer, 1989, Burckhardt & Lauterer, 1993, Mart et al., 1995; Lauterer et al., 1998). According to Tokmakoğlu (1973), *A. pistaciae* was detected for the first time in Türkiye by *A. targionii* Lich in pistachio orchards in Gaziantep province. Additionally, in previous studies, many researchers identified the psyllid species as *A. succincta* Heeg. (Çelik, 1975; Klimaszewski & Lodos, 1977; Günaydın, 1978). Burckhardt & Önuçar (1993) reported that the species found in pistachio fields in the Southeastern Anatolia Region was *A. pistaciae*, not *A. targionii* or *A. succincta*. Both nymphs and adults of the pistachio psyllid are harmful by sucking leaf sap. They also produce large amounts of honeydew, leading to the formation of sooty

mold. By feeding directly on tree leaves, *A. pistaciae* causes the trees to lose vitality, become stunted, bud drop, leaf shedding, and yield decrease (Samih et al., 2005). Therefore, the control of *A. pistaciae* is very important.

While it has been reported that psyllids are difficult to control using only pesticides, it has been reported that as a result of chemical control, psyllids develop morphological, physiological, and behavioral changes and are active throughout most of the year (Horton, 1999). For years, farmers have been unconsciously spraying pesticides against this pest, which is important in pistachio fields in Türkiye. As a result of this, negativities such as residue on the plant, destruction of natural enemies, negative effects on the environment and human health, as well as resistance to the pest in a short time, occur, and as a result of these, the control becomes more complex. In addition, some of these pesticides have been reported to use biological control agents used to control the pistachio psyllid by negatively affecting their populations, which can then emerge more easily and more intensely with the pressure of a natural enemy that does not remain above the pest (Mehrnejad, 2003). *Psyllaephagus pistaciae* Ferrière

(Hymenoptera: Encyrtidae) and *Oenopia conglobata* (Linnaeus) (Coleoptera: Coccinellidae) have been reported to be the main biological control agents for pistachio psyllid (Mehrnejad, 2001; Bolu, 2004). It has been reported that among natural enemies, especially *O. conglobata*, can be seen on pistachio psyllid populations all year round, that both adult and larval stages can predator, and that it can feed on all stages of the pest, and that this predator has a better potential in biological control than other predators and parasitoids (Bolu, 2004). However, natural enemies alone cannot keep the *A. pistaciae* population below the economic damage threshold. Therefore, the use of chemical control becomes inevitable. Active ingredients used within the scope of integrated control should also have low negative effects on the natural enemies of *A. pistaciae*.

Some studies are on the detection, population development, and control of *A. pistaciae* on pistachio trees in Türkiye. Most of these studies (Tokmakoğlu, 1973; Günaydın, 1978; Çelik, 1981; Bolu & Kornoşor, 1995; Mart et al., 1995; Kaplan & Çınar, 2000; Şimşek & Bolu, 2017; Kaplan & Çiftçi, 2020; Özgen et al., 2022) are in the Southeastern Anatolia Region. The aim of this study was to determine whether the insecticides used in the control of *A. pistaciae* in field conditions continue to be effective in field conditions.

## Material and Method

### Insecticides

The insecticides used in the trial were determined as a result of interviews with consultants and producers working in the region. In the interviews, it was found that spirotetramat was the most widely used insecticide, with other active ingredients being used less frequently. To assess the differences in effectiveness, other active ingredients were favored for comparison against spirotetramat, which has been in use for is more commonly applied (Table 1).

### Experimental Design

While selecting the pistachio orchards where the trials will be established, it was paid attention that the age, variety, and planting intervals of the trees were homogeneous. Biological efficacy trials were conducted according to the method of the Ministry of Agriculture of the Republic of Türkiye. The trials were carried out in 4 different locations in Arslanlı and Bağlarbaşı neighborhoods in Birecik district of Şanlıurfa province and Yunuslar and Avcılar neighborhoods in Kurtalan district of Siirt province, where the pest is above the economic damage threshold of 20-30 nymphs per compound leaf. The trials were established in Birecik district on 07.05.2023 and in Kurtalan district on 03.06.2023. The

trials were carried out according to the randomize blocks design with 3x3=9 trees as one plot. In addition, a control plot without spraying was established. The number of replications was set as 4. Information about the insecticides used in the study is given in Table 1. For the counts, 10 old and 10 young compound leaf samples were taken from the trees in the middle of each plot on 10 labelled shoots. Counts were made once in the field before application. After the spraying, counts were made four times on days 3, 7, 14 and 21. The count results were evaluated according to the Henderson-Tilton formula based on the number of live insects counted in each plot in leaf samples.

### Data Analysis

The effect rates of insecticides tested under field conditions on *A. pistaciae* populations were calculated using the Henderson-Tilton formula (1955).

$$\text{Corrected \%} = \left(1 - \frac{n \text{ in CBT} \times n \text{ in TAT}}{n \text{ in CAT} * n \text{ in TBT}}\right) * 100$$

CBT : Control before treatment

CAT : Control after treatment

TAT : Treated after treatment

TBT : Treated before treatment

\*n : Insect population

The normality of the data obtained from the experiment on the effects of different pesticide applications on *A. pistaciae* and the Henderson-Tilton corrected data was checked with the Shapiro Wilk test. The homogeneity of the data was checked with the Levene's test. Since the data showed normal and homogeneity distribution, one-way ANOVA was applied to detect differences between the applications and Henderson-Tilton data. Tukey HSD test was used in multiple comparison tests to detect the difference between means of treatments. (IBM SPSS 20.0 Statistical package; SPSS Inc., Armonk, NY). General Linear Model (GLM) analysis was applied to the data to assess the effects of weeks, treatments, and their interactions (GLM, Univariate;  $\alpha=0.05$ ).

## Results

The results of the trials conducted in 4 different locations in Şanlıurfa and Siirt are given in Tables 2, 3, 4, and 5. In the trial conducted in Arslanlı neighborhood of Birecik district of Şanlıurfa, 3 days after the application (daa), the most effective active ingredients were determined to be spirotetramat with 93.46% effectiveness and deltamethrin with 92.96% effectiveness ( $F_{4, 15}=11.54$ ;  $P < 0.001$ ; Table 2).

Table 1. Information on insecticides used in the studies.

Company	Trade name	Active ingredient	Formulation type	Concentration
Corteva	Delegate 250 WG	%25 Spinetoram	WG	30 g/100 lt
Koruma	Kung-fu 5 EC	50 g/l Lambda-cyhalothrin	EC	20 ml /100 lt
Bayer	Decis 2.5 EC	25 g/l Deltamethrin	EC	30 ml /100 lt
Corteva	Transform 500 WG	%50 Sulfoxaflor	WG	15 g/100 lt
Bayer	Movento SC 100	100 g/l Spirotetramat	SC	100 ml/100 lt

Table 2. Results of the biological activity trial established in the Arslanlı neighborhood of Şanlıurfa/Birecik district.

Active ingredient	R	Live individuals					Henderson-Tilton Efficacy (%)				
		BA	3 DAA	7 DAA	14 DAA	21 DAA	3 DAA	7 DAA	14 DAA	21 DAA	
Spinetoram	1	1220	125	101	56	88	90.17	93.01	96.48	95.03	
	2	1093	79	89	81	103	93.28	92.89	93.84	92.88	
	3	517	112	101	93	141	80.29	83.61	85.88	79.81	
	4	794	104	87	79	87	87.62	90.19	91.83	91.54	
	Mean	906	105±2.77	94.5±2.2	77.25±2.25	104.75±3.41	87.84 ba <sup>a</sup>	89.92 bc	92.01 bc	89.91 bc	
Lambda-cyhalothrin	1	1341	259	241	207	211	81.48	84.84	88.15	89.15	
	2	1081	191	182	197	208	83.57	85.30	84.86	85.45	
	3	1127	218	231	180	194	82.40	82.80	87.47	87.26	
	4	1042	225	199	162	172	79.58	82.89	87.23	87.26	
	Mean	1147.75	223.25±3.84	213.25±3.65	186.5±3.72	196.25±3.76	81.76 b	83.96 d	86.93 d	87.28 c	
Deltamethrin	1	1313	51	48	37	50	96.27	96.92	97.84	97.37	
	2	1172	99	81	71	96	92.15	93.96	94.97	93.81	
	3	1018	89	79	68	77	92.04	93.49	94.76	94.40	
	4	1009	92	59	37	82	91.38	94.76	96.99	93.73	
	Mean	1128	82.75±1.12	66.75±0.76	53.25±0.76	76.25±0.86	92.96 a	94.78 ab	96.14 ba	94.83 ab	
Sulfoxaflor	1	981	153	131	121	125	85.04	88.73	90.53	91.21	
	2	1091	149	140	135	142	87.30	88.79	89.72	90.16	
	3	1037	155	151	133	138	86.40	87.78	89.93	90.15	
	4	1108	162	156	122	147	86.18	87.39	90.96	89.76	
	Mean	1054.25	154.75±2.46	144.5±2.35	127.75±2.26	138±2.31	86.23 b	88.17 cd	90.29 cd	90.32 bc	
Spirotetramat	1	871	64	41	28	28	92.95	96.03	97.53	97.78	
	2	967	84	39	27	21	91.92	96.48	97.68	98.36	
	3	981	55	37	27	23	94.90	96.83	97.84	98.26	
	4	1007	63	29	22	17	94.08	97.42	98.21	98.70	
	Mean	956.5	66.5±0.65	36.5±0.29	26±0.15	22.25±0.19	93.46 a	96.69 a	97.81 a	98.28 a	
Control	1	751	783	890	978	1089	0.00	0.00	0.00	0.00	
	2	951	1023	1089	1145	1258	0.00	0.00	0.00	0.00	
	3	981	1078	1169	1250	1325	0.00	0.00	0.00	0.00	
	4	850	899	949	1035	1101	0.00	0.00	0.00	0.00	
	Mean <sup>a</sup>	883.25	945.75 a	1024.25 a	1102 a	1193.25 a					

R: Replication; BA: Before App.; <sup>a</sup>means followed by different letters in the same column are significantly different according to Tukey HSD tests (P<0.001)

Table 3. Results of the biological activity trial established in the Bağlarbaşı neighborhood of Şanlıurfa/Birecik district.

Active ingredient	R	Live individuals					Henderson-Tilton Efficacy (%)				
		BA	3 DAA	7 DAA	14 DAA	21 DAA	3 DAA	7 DAA	14 DAA	21DAA	
Spinetoram	1	1346	256	201	81	151	81.43	86.33	94.88	91.22	
	2	989	69	55	88	117	93.29	94.92	92.31	91.11	
	3	1213	198	133	113	149	84.87	90.86	92.98	91.68	
	4	1411	238	178	142	111	84.03	88.62	91.68	93.92	
	Mean	1239.75	190.25±2.17	141.75±1.23	106±0.79	132±1.31	85.90 c <sup>a</sup>	90.18 b	92.96 b	91.98 b	
Lambda-cyhalothrin	1	851	166	140	135	127	80.95	84.94	86.50	88.32	
	2	1123	147	154	128	181	87.42	87.48	90.15	87.89	
	3	1284	221	187	161	152	84.04	87.85	90.55	91.98	
	4	1349	187	190	158	182	86.87	87.30	90.32	89.57	
	Mean	1151.75	180.25±3.34	167.75±3.91	145.5±3.54	160.5±3.82	84.82 c	86.89 b	89.38 c	89.44 b	
Deltamethrin	1	1250	58	45	34	43	95.47	96.70	97.69	97.31	
	2	1078	71	65	58	65	93.67	94.50	95.35	95.47	
	3	1207	82	72	51	62	93.70	95.02	96.82	96.52	
	4	1155	87	75	31	45	92.87	94.14	97.78	96.99	
	Mean	1172.5	74.5±0.39	64.25±0.21	43.5±0.13	53.75±0.74	93.93 ab	95.09 a	96.91 a	96.57 a	
Sulfoxaflor	1	894	123	111	101	127	86.56	88.63	90.39	88.88	
	2	982	107	99	85	109	89.53	90.80	92.52	91.66	
	3	1018	118	108	121	132	89.25	91.15	91.04	91.22	
	4	964	138	127	110	118	86.45	88.12	90.57	90.53	
	Mean	964.5	121.5±1.11	111.25±1.55	104.25±1.01	121.5±1.21	87.95 bc	89.67 b	91.13 bc	90.57 b	
Spirotetramat	1	1278	55	45	33	35	95.80	96.78	97.80	97.86	
	2	1078	78	64	56	56	93.04	94.58	95.51	96.10	
	3	1353	69	46	28	29	95.27	97.16	98.44	98.55	
	4	1289	59	42	34	17	95.67	97.06	97.82	98.98	
	Mean	1249.5	65.25±0.45	49.25±0.57	37.75±0.33	34.25±0.15	94.94 a	96.40 a	97.39 a	97.87 a	
Control	1	792	811	865	931	1012	0.00	0.00	0.00	0.00	
	2	818	851	896	947	1089	0.00	0.00	0.00	0.00	
	3	789	851	946	1047	1165	0.00	0.00	0.00	0.00	
	4	891	941	988	1078	1152	0.00	0.00	0.00	0.00	
	Mean	822.5	863.5	923.75	1000.75	1104.5					

R: Replication; BA: BA: Before App.; <sup>a</sup>means followed by different letters in the same column are significantly different according to Tukey HSD tests (P<0.001)

Table 4. Results of the biological activity trial established in the Yunuslar neighborhood of Siirt/Kurtalan district.

Active ingredient	R	Live individuals					Henderson-Tilton Efficacy (%)				
		BA	3 DAA	7 DAA	14 DAA	21 DAA	3 DAA	7 DAA	14 DAA	21 DAA	
Spinetoram	1	954	112	91	82	96	88.64	91.10	92.24	91.39	
	2	857	90	86	74	101	89.65	90.62	92.26	89.89	
	3	893	95	89	94	123	89.60	90.85	90.78	88.34	
	4	1009	97	91	87	94	90.85	91.68	92.38	92.24	
	Mean	928.25	98.5±0.23	89.25±0.6	84.25±0.8	103.5±0.93	89.69 b <sup>a</sup>	91.06 b	91.92 bc	90.46 b	
Lambda-cyhalothrin	1	1171	184	155	143	161	84.80	87.65	88.97	88.23	
	2	1001	134	126	103	134	86.81	88.24	90.77	88.52	
	3	988	167	161	164	162	83.47	85.04	85.47	86.12	
	4	931	169	141	111	121	82.72	86.02	89.47	89.17	
	Mean	1022.75	163.5±1.46	145.75±1.89	130.25±1.56	144.5±1.68	84.45 c	86.74 c	88.67 d	88.01 b	
Deltamethrin	1	979	71	57	58	34	92.98	94.57	94.65	97.03	
	2	897	56	55	46	48	93.85	94.27	95.40	95.41	
	3	1008	78	67	69	75	92.43	93.90	94.01	93.70	
	4	1093	84	82	85	89	92.68	93.08	93.13	93.22	
	Mean	994.25	72.25±0.45	65.25±0.21	64.5±0.32	61.5±0.36	92.99 a	93.95 a	94.30 ab	94.84 a	
Sulfoxaflor	1	1071	120	125	110	145	89.16	89.11	90.73	88.41	
	2	1093	115	123	89	131	89.63	89.48	92.70	89.72	
	3	921	89	95	95	110	90.55	90.53	90.97	89.89	
	4	847	97	86	87	114	89.10	90.63	90.93	88.79	
	Mean	983	105.25±1.23	107.25±1.67	95.25±1.79	125±1.91	89.61 b	89.94 b	91.33 cd	89.20 b	
Spirotetramat	1	1102	61	57	51	66	94.64	95.17	95.82	94.87	
	2	1037	74	66	45	47	92.97	94.05	96.11	96.11	
	3	1055	59	59	46	51	94.53	94.87	96.18	95.91	
	4	1123	64	65	36	55	94.58	94.66	97.17	95.92	
	Mean	1079.25	64.5±0.68	61.75±0.29	44.5±0.13	54.75±0.45	94.18 a	94.69 a	96.32 a	95.70 a	
Control	1	1218	1259	1305	1349	1423	0.00	0.00	0.00	0.00	
	2	1128	1145	1207	1258	1315	0.00	0.00	0.00	0.00	
	3	1098	1123	1196	1254	1297	0.00	0.00	0.00	0.00	
	4	1067	1121	1156	1208	1281	0.00	0.00	0.00	0.00	
	Mean	1127.75	1162	1216	1267.25	1329					

R: Replication; BA: BA: Before App; \*means followed by different letters in the same column are significantly different according to Tukey HSD tests (P<0.001)

In the counts made 21 days after the application, spirotetramat showed a high effect with 98.28% and was statistically separated from other active ingredients ( $F_{4,15}=6.975$ ;  $P = 0.002$ ; Table 2). In the trial conducted in the Bağlarbaşı neighborhood of Birecik district of Şanlıurfa, an effect of over 90% was observed in deltamethrin and spirotetramat applications 3 days after the application. Spirotetramat was in a different group compared to other applications ( $F_{4,15}=10.34$ ;  $P<0.001$ ; Table 3). 21 days after the application, an effect of over 90% was calculated for deltamethrin, spinetoram, sulfoxaflor, and spirotetramat applications. Spirotetramat and deltamethrin were statistically separated from other active ingredients ( $F_{4,15}=31.00$ ;  $P<0.001$ ; Table 3).

In Siirt, another province where the trials were conducted, in the Yunuslar neighborhood of Kurtalan district, the effectiveness rates for lambda-cyhalothrin, sulfoxaflor, spinetoram, deltamethrin, and spirotetramat were determined as 84.45, 89.61, 89.69, 92.99 and 94.18%, respectively, 3 days after the application (Table 4). Again, spirotetramat and deltamethrin were in a statistically separate group ( $F_{4,15}=50.93$ ;  $P<0.001$ ; Table 4). In the counts made 21 days after the application, an effect of over 90% was observed in spinetoram, deltamethrin, and spirotetramat applications. In the trial conducted in the Avcılar neighborhood of Kurtalan, it was determined that spinetoram, deltamethrin, and spirotetramat had an effect of over 90% 3 days after the application, and spirotetramat was statistically in a separate group ( $F_{4,15}=16.29$ ;  $P<0.001$ ; Table 5). After 21 days of application, active ingredients except lambda-cyhalothrin showed an effect of over 90%.

Spirotetramat, deltamethrin, and spinetoram were statistically separated from the others ( $F_{4,15}=30.07$ ;  $P<0.001$ ; Table 5).

As a result of the General Linear Model analysis, treatments, counts, and their interactions were found to be statistically significant at all experimental locations (Table 6).

## Discussion

Insecticides are the most important component of current control methods to reduce the damage of *A. pistaciae*. In the biological effectiveness trials conducted in Şanlıurfa and Siirt provinces in the Southeastern Anatolia Region of Türkiye, it was revealed that the insecticides registered for *A. pistaciae* and included in the trial were highly effective on the nymphs of this pest and could be used in pest control. Among the insecticides used in the trials, spirotetramat, which is among the Tetric and Tetric Acid Derivatives according to the IRAC MoA classification and has ambimobile systemic properties, was determined to be highly toxic to *A. pistaciae*. In their study, Gheibi & Taheri (2017) determined that after spirotetramat was administered with irrigation water, the highest mortality occurred on the 20<sup>th</sup> day in nymphs (99.44%) and on the 30<sup>th</sup> day in adults (98.23%). As can be seen from this study, spirotetramat is quite successful in the control against *A. pistaciae*. Spirotetramat may be safer than other active ingredients for *O. conglobata*, which is an important predator of *A. pistaciae* in the pistachio ecosystem, and its use in integrated pest management can be recommended to maintain the natural balance (Bemani et al., 2018).

Table 5. Results of the biological activity trial established in the Avcılar neighborhood of Siirt/Kurtalan district.

Active ingredient	R	Live individuals					Henderson-Tilton Efficacy (%)			
		BA	3 DAA	7 DAA	14 DAA	21 DAA	3 DAA	7 DAA	14 DAA	21 DAA
Spinetoram	1	1126	91	92	76	101	92.10	92.20	93.67	92.53
	2	1030	86	81	67	69	91.96	92.78	94.23	94.31
	3	1091	95	92	96	84	92.01	92.91	92.92	93.99
	4	1165	79	85	82	88	93.27	93.24	93.66	93.45
	Mean	1103 a <sup>a</sup>	87.75±0.63	87.5±0.60	80.25±0.57	85.5±0.74	92.33 ab	92.78 a	93.62 ab	93.57 a
Lambda-cyhalothrin	1	856	178	189	152	167	79.67	78.92	83.36	83.76
	2	981	142	138	132	133	86.06	87.09	88.06	88.48
	3	1021	129	146	141	155	88.41	87.97	88.89	88.15
	4	941	145	146	134	148	84.70	85.63	87.17	86.36
	Mean	949.75	148.5±1.60	154.75±1.52	139.75±1.23	150.75±1.49	84.71 c	84.91 b	86.87 b	86.69 b
Deltamethrin	1	1055	65	65	57	55	93.98	94.12	94.94	95.66
	2	1002	71	68	63	70	93.18	93.77	94.42	94.07
	3	1104	85	72	42	53	92.94	94.52	96.94	96.25
	4	1265	91	82	63	89	92.86	94.00	95.51	93.90
	Mean	1106.5	78±0.45	71.75±0.45	56.25±0.29	66.75±0.79	93.24 ab	94.10 a	95.45 a	94.97 a
Sulfoxaflor	1	939	105	94	94	101	89.07	90.44	90.62	91.05
	2	981	113	119	103	116	88.91	88.87	90.68	89.95
	3	893	99	95	95	101	89.83	91.05	91.44	91.17
	4	796	88	85	73	90	89.02	90.11	91.74	90.19
	Mean	902.25	101.25±1.24	98.25±1.13	91.25±1.56	102 ±1.89	89.21 b	90.12 a	91.12 b	90.59 b
Spirotetramat	1	1067	76	71	71	87	93.03	93.65	93.76	93.21
	2	955	64	61	59	65	93.55	94.14	94.52	94.22
	3	1099	63	63	71	71	94.74	95.18	94.80	94.96
	4	917	75	68	69	55	91.88	93.13	93.22	94.80
	Mean	1009.5	69.5±0.56	65.75±0.91	67.5±0.21	69.5±0.58	93.30 a	94.02 a	94.08 a	94.30 a
Control	1	1238	1266	1297	1321	1487	0.00	0.00	0.00	0.00
	2	1095	1137	1193	1234	1289	0.00	0.00	0.00	0.00
	3	1079	1176	1283	1341	1382	0.00	0.00	0.00	0.00
	4	1163	1171	1256	1291	1341	0.00	0.00	0.00	0.00
	Mean	1143.75	1187.5	1257.25	1296.75	1374.75				

R: Replication; BA: BA: Before App; <sup>a</sup>means followed by different letters in the same column are significantly different according to Tukey HSD tests (P<0.001)

Table 6. The results of the General Linear Model (GLM) analysis to determine the effects of deltamethrin, lambda cyhalothrin, spirotetramat, spinetoram and sulfoxaflor applications and different periods on the density of *Agonosceca pistaciae*.

Location	Source of variation	df	Mean square	F value	P value
Şanlıurfa/Birecik/Arsıslanlı	Applications	5	1866634.50	251.30	<0.001
	Weeks	4	2655699.41	357.54	<0.001
	Applications*Weeks	20	176633.96	23.78	<0.001
Şanlıurfa/Birecik/Bağlarbaşı	Applications	5	1341831.95	284.10	<0.001
	Weeks	4	3472363.89	735.19	<0.001
	Applications*Weeks	20	211138.61	44.70	<0.001
Siirt/Kurtalan/Yunuslar	Applications	5	2988459.48	1442.86	<0.001
	Weeks	4	2608938.01	1259.62	<0.001
	Applications*Weeks	20	145853.54	70.42	<0.001
Siirt/Kurtalan/Avcılar	Applications	5	3171514.37	1644.74	<0.001
	Weeks	4	2663944.59	1381.52	<0.001
	Applications*Weeks	20	160117.68	83.04	<0.001

The studies carried out to date on the biological effects of pesticides on natural enemies show the great importance of this problem. The study conducted by Mohammadkhani et al. (2021), reported that the results showed lower side effects of spirotetramat than thiamethoxam, lambda-cyhalothrin, and acetamiprid on *O. conglobata*. Although the effect of spirotetramat on natural enemies was not evaluated in this study, it is predicted that its side effects may be low according to previous studies. In this study, all active ingredients used in the results of biological efficacy trials against pistachio psyllid can be used in chemical control. However, when implementing these applications, the presence of natural enemies should also be taken into consideration. In weekly observations to be made in

pistachio orchards starting from April-May, if an average of 20-30 nymphs are seen in 100 compound leaves of the pest with a licensed plant protection product success in its struggle (Mart et al., 1995). In the study conducted by Hassani et al. (2009) in Iran, they stated that the damage threshold of pistachio psylla depends on many variables such as temperature, humidity, precipitation. Farmers spray in spring even though there are no pests in nature. These unnecessary practices disrupt the natural balance and cause extra costs to producers in economic terms. Therefore, in the control against pests, it is necessary not to control before reaching the economic damage threshold. In addition to all these, it is recommended to reduce the use of chemicals in the control against pests and to use

environmentally friendly chemicals if they are to be used. Because there are natural enemies in nature that put pressure on pests. Özgen et al. (2022) reported that the larvae or adults of *O. conlobata* were highly effective on *A. pistaciae* when 100 were released per tree, and the *A. pistaciae* population remained below the economic damage threshold. In order to protect natural enemies that can be successful like this and are found in Türkiye's natural fauna, it is important to use pesticides that have side effects on natural enemies.

In conclusion, it was determined that the licensed insecticides used in the study were still effective in controlling *A. pistaciae*. Among insecticides, spirotetramat can be recommended for use as it is both more effective and has a lower impact on natural enemies. In order to prevent or slow down the development of resistance in the pest, active ingredients in different groups according to the IRAC MoA classification should be preferred in sequential sprayings.

## Declarations

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### Declaration of Competing Interests

The authors declare they have no financial interests.

### Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Adnan Tusun, Çağlar Kalkan, and Serdar Satar. The first draft of the manuscript was written by Adnan Tusun, Çağlar Kalkan, and Serdar Satar, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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## Evaluation of Physiological Changes in Important Dried Apricot Varieties Under Drought Stress

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### ABSTRACT

Nearly all of the apricot varieties grown in Malatya are dried apricots and the plantation areas in this region are expanding daily. Due to the impact of climate change, producers are growing apricots mostly under limited irrigation or even dry conditions. Therefore, it is essential to determine the drought resistance characteristics of the varieties commonly cultivated in this region. In this study, different irrigation levels of 100%, 75%, 50% and 25% of available water were applied to Hacıhaliloğlu, Kabaası, Çataloğlu, Hasanbey and Soğancı apricot varieties. To evaluate the resistance of the varieties to drought stress and its relationship with physiological changes, chlorophyll a and b, carotenoids, total sugar, total starch and abscisic acid contents in the leaves were analyzed. A decrease in chlorophyll a and b, carotenoids, total starch values and an increase in total sugar and ABA values were determined due to the decrease in irrigation rates. In Kabaası and Hasanbey varieties, which were observed as the most resistant to water shortage, chlorophyll a and b, carotenoids, total starch values were higher and total sugar content was lower at decreased irrigation levels. No difference was detected between varieties in ABA values. As a result of the observations in the drought resistance tests and physiological analyses, it was concluded that the most resistant varieties were Kabaası and Hasanbey. Unfortunately, the most sensitive variety was the most widespread Hacıhaliloğlu. In addition, analyzing and evaluating the physiological changes occurring in apricot under drought stress will be useful in developing the most appropriate irrigation strategies for each variety and increasing water use efficiency. It may also be useful in cross-breeding studies to develop new drought-resistant varieties.

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### Introduction

Malatya province in Türkiye is the global leader in dried apricot production and export. Approximately 90-95% of the apricots grown in Malatya are dried and exported. 2022, the province exported 76.4 thousand tons of dried apricots, generating \$402 million in revenue (Hasdemir, 2023).

The interest in dried apricot cultivation in the Malatya region is constantly increasing yearly; more and more areas are being planted each year. This situation causes irrigation problems, especially in years when summers are dry. In recent years, producers have been trying to cultivate with little or no irrigation due to limited water resources.

Drought stress is becoming increasingly important in plant cultivation. Plants are affected by drought depending on species, age, growth stage and development period, drought level and duration, and environmental factors. They have developed different morphological, physiological and biochemical responses to avoid or

overcome stress (Ghahremani et al., 2023; Liu et al., 2023). Especially, when vegetative development accelerates, regular limited water applications to trees are recommended. Thus, the efficiency of using of existing water resources will increase along with the increase in yield (Chalmers et al., 1986; Mitchell et al., 1989).

Physiological parameters such as chlorophyll, carotenoid, total sugar, total starch and abscisic acid content play an important role in resistance to drought stress. Chlorophyll content is affected by drought stress and decreases significantly compared to normal irrigation conditions (Aghanejad et al., 2015). In contrast, carotenoid levels are increased due to drought and ROS is neutralized to reduce cellular damage (Niyogi, 1999; Fang & Xiong, 2015). Carbohydrate metabolism is also affected under drought stress conditions. Concentrations of sugars such as fructose and glucose change. This change is effective in photosynthetic metabolism. It is stated that sugar molecules activate the antioxidant defense mechanism

(Keunen et al., 2013). Drought is also a factor affecting starch content (Bing et al., 2014). On the other hand, it is known that water shortage perceived by the roots is transmitted to the stem by abscisic acid (ABA). It has been determined that ABA ensures the closure of stomata in drought stress and enables it to resist adverse conditions (Anjum et al., 2011).

In this study, it was aimed to determine and compare the drought resistance of the most essential dried apricot varieties such as Hacihaliloglu, Cataloglu, Hasanbey, Kabaasi and Soganci, by examining the physiological changes in their leaves that develop as a result of drought stress.

## Materials and Methods

### Materials

This study was carried out in Malatya Fruit Research Institute between 2001-2002. The research area is located at 38° 21' north latitude and 38° 17' east longitude, with an altitude of 980 m.

In the study, 2-year-old saplings of Kabaasi, Hacihaliloglu, Hasanbey, Cataloglu, Soganci apricot varieties grown in the region were planted in 8-liter pots in a glass greenhouse.

The soil mortar was prepared from sand: loamy soil: peat: farm fertilizer in the ratio of 1:2:1:0.5 (Hartmann and Kester, 1974). The mortar sample was found to have a field capacity of 23.77%, a wilting point of 12.20%, a texture of 54.26% sand, 23.63% silt and 22.11% clay; a texture class of SCL, a specific gravity of 2.63 g/cm<sup>3</sup> and a bulk density of 1.12 g/cm<sup>3</sup>.

### Drought Resistance Test

In drought resistance test, methods defined by Ozbek et al. (1976) and Tuzuner (1983) were used to determine the amount of water given to the plants. Accordingly, four different irrigation programs were applied to the plants, 100%, 75%, 50% and 25% of the available water, calculated using the field capacity (usable moisture capacity) and wilting point values of the mortar mixture. At the beginning of the tests, the irrigation level given to the plants was cut off, and the determined irrigation program was applied.

This test, which aims to determine the resistance levels of saplings to drought conditions created by reducing the amount of usable water at specific rates, started on June 12 in 2001 and June 20 in 2002, as the most suitable period for drought tests in outdoor conditions. Irrigation provided to the saplings at sufficient levels and evenly until these dates, was cut off and the usable water in the plant root zone was expected to decrease to 75%, 50% and 25%. In 100% applications, sufficient irrigation was continued. During the trial, saplings were kept at the targeted irrigation levels and re-irrigation was performed when 20% of each irrigation level was consumed (Gunbatili, 1979). For irrigation, the amount of water to be given was determined by weighing the pots in the morning, and then the missing water was completed to the desired level (Steinberg et al., 1990). During the test period, no fertilization or pesticide applications were made to ensure homogenization. The yellowing and shedding of the first few leaves were taken as the beginning of stress (Proebsting and Middleton, 1980).

The trial was organized according to the randomized plot design, with three replications for each application and three saplings in each replication, based on varieties.

### Chlorophyll And Carotenoid Contents

Chlorophyll and carotenoid contents were determined by taking three leaves that completed their development in the middle part of young shoots of each sapling at 15-day intervals throughout the experiment. For this purpose, 1 g of leaf sample ground in the dark was taken and subjected to extraction with 90% acetone. Since chlorophyll can quickly decompose in light, it is essential to have a light-free environment and to cover the laboratory equipment used with aluminum foil during the analyses. Then, the absorbance values of the samples were read with the help of a spectrophotometer (Shimatsu brand, UV-120-01 model) at 662, 645 and 470 nm wavelengths. According to Lichtenthaler and Welburn (1983), chlorophyll-a (KL-a), chlorophyll-b (KL-b). Carotenoid contents of the leaves were calculated from the following formulas and the results were given in g/g fresh weight.

$$\text{Chlorophyll-a} = 11.75 \times A_{662} - 2.35 \times A_{645}$$

$$\text{Chlorophyll-b} = 18.61 \times A_{645} - 3.96 \times A_{662}$$

$$\text{Carotenoid} = [1000 (A_{470} - 2.27(Cl-a) - 81.4(Cl-b))] / (227 \times 1000)$$

### Total Sugar

Total sugar content of the samples was determined by the spectrophotometric method described by Dimler et al. (1952), which was developed for dried leaf samples. Based on the repetitions of the applications, 20 leaf samples taken from previously selected saplings at 15-day intervals were dried in the oven at 60°C for 48 hours and then ground in a mortar to make powder. 0.1 g of this sample was extracted with 25 ml of 80% ethyl alcohol. Absorbance values were created at 620 nm wavelength and evaluated with a standard curve drawn using pure glucose before the study. The results are given in mg/100 g dry weight and presented by combining two years of data.

### Total Starch

Starch contents of leaf samples of the applications were determined by the spectrophotometric method described by Dimler et al. (1952). 0.1 g of the samples prepared for total sugar analysis were taken, hydrolyzed with concentrated sulfuric acid (5 ml) and diluted with distilled water (1:10 v). Absorbance values were made at 620 nm wavelength and evaluated with a standard curve drawn using pure starch before the study. The results are given in mg/100 g dry weight and presented by combining two years of data.

### Abscisic Acid

For determining abscisic acid (ABA) content in leaves, extraction, purification, and analysis processes were carried out according to the method described by Yurekli et al. (1974). Depending on the replications formed in the irrigation applications in the drought resistance tests, one plant was marked in each replication before the study. Accordingly, 20 leaf samples that completed their development in the middle part of the shoots were used between 12.00-13.00 with 15-day intervals. For thin layer chromatography, 20x20 cm prepared plates coated with silica gel containing an inorganic fluorescent compound (Merck) were used. To determine the ABA regions, the plates were examined in a dark room under UV light at 254 nm wavelength. The ABA region belonging to the extract gave a purple fluorescence color, which showed a purple fluorescence color on the plate and was compared with the standard synthetic-ABA region. ABA amounts was made

by ABA amounts were determined using 5 different concentrations of standard synthetic ABA and a Desaga CD60 scanning densitometer.

**Statistical Analysis**

In the statistical evaluation of the obtained data, two-way variance analysis and LSD test were used to determine the differences between the means. Statistical evaluations were carried out using the “SPSS 10.0 for Windows” program.

**Results and Discussion**

**Amounts of Water**

Statistically significant differences were determined between varieties in terms of the amount of water given. For 25% and 50% irrigation rates, the least irrigation water was given to Hasanbey, Kabaasi and Cataloglu varieties, while the amount of water given to Hacıhaliloglu and Soganci varieties was higher than the other varieties. Among the irrigation rates, the difference in the amounts of irrigation water used for 25% and 50% compared to 75% and 100% irrigation water rates was found to be statistically significant. It is noteworthy that the amount of irrigation water applied for each irrigation rate in the Hacıhaliloglu variety did not show a statistically significant difference (Table 1).

When the varieties were examined in general in the trial, plant water consumption was also the least in the varieties that observed as having the highest resistance. For example, the amounts of water given to the Kabaasi variety which showed almost no signs of stress at 25% irrigation rate, were found to be significantly lower than the Hacıhaliloglu variety which showed the most signs of stress.

This situation suggested that the Kabaasi variety may have a higher ability to benefit from the available water.

In the study of irrigation practices and internal water balance, it is recommended to work primarily with young trees. It is emphasized that this period is a more critical period in drought. Similarly, it has been stated that early drought resistance performance affects the future performance of the plant stress (Jackson et al., 1986; Proebsting et al., 1977).

**Chlorophyll Contents**

Chlorophyll a content was significantly higher in Kabaasi and Hasanbey varieties than in other varieties in both years. The lowest chlorophyll values were usually seen in the Hacıhaliloglu and Cataloglu varieties. The chlorophyll a contents of Kabaasi variety were found to be significantly higher in 2001 at 25% irrigation rate and in 2002 at 50% irrigation rate than in all other varieties. In addition, an increase in chlorophyll a content was observed in both years parallel to the increase in irrigation rate and this was especially evident at 75% and 100% irrigation rates (Table 2).

Generally, Kabaasi and Hasanbey varieties, which were observed to be least affected by stress, were analyzed to contain more chlorophyll-a than other varieties. This situation was primarily mainly determined for Kabaasi variety at 25% and 50% irrigation rates, where they were most stressed. Chlorophyll b content, similar to chlorophyll a values, was found to be higher in Kabaasi and Hasanbey varieties than in other varieties in both years. This difference was more pronounced and statistically significant, especially in 2002. An increase in chlorophyll-b content was also observed with the increasing-rise in irrigation rate, which was especially evident in 2002 at 75% and 100% irrigation rates (Table 3).

Table 1. Effect of different irrigation levels on the amount of water (kg) replaced in the saplings of different apricot varieties. When 20% of each irrigation level was consumed, the lost water was completed to the desired level.

Variety	Irrigation rate (%)				Mean
	25	50	75	100	
Hacıhaliloglu	13.26 cd	13.17 d	13.28 cd	13.25 cd	13.24 a
Cataloglu	12.68 f	12.78 e	13.53 ab	13.29 cd	13.07 b
Hasanbey	12.50 g	12.66 f	13.36 bcd	13.31 cd	12.96 c
Kabaasi	12.74 f	12.93 e	13.21 d	13.06 d	12.99 bc
Soganci	13.11 d	13.07 d	13.43 bc	13.63 a	13.31 a
Mean	12.86 b	12.92 b	13.36 a	13.31 a	

LSD<sub>5%</sub> variety: 0.091\*\*, irrigation rate: 0.081\*\*, variety\*irrigation rate: 0.181\*\*

Table 2. Effect of different irrigation levels on chlorophyll-a content (µg/g fresh weight) in the leaves of different apricot varieties

Year	Variety	Irrigation rate (%)				Mean
		25	50	75	100	
2001	Hacıhaliloglu	1.109 c	1.094 c	1.229 ac	1.123 c	1.139 b
	Cataloglu	1.087 c	1.189 c	1.147 c	1.164 bc	1.147 b
	Hasanbey	1.120 c	1.269 ac	1.395 a	1.363 a	1.287 a
	Kabaasi	1.389 a	1.379 a	1.242 ac	1.331 ab	1.336 a
	Soganci	1.073 c	1.140 c	1.254 ac	1.245 abc	1.177 b
	Mean	1.156 b	1.214 ab	1.254 a	1.246 a	
2002	Hacıhaliloglu	1.523 e	1.957 cd	2.081 bc	2.317 ab	1.969 c
	Cataloglu	1.768 de	1.711 d	2.159 b	2.427 a	2.016 c
	Hasanbey	1.979 cd	1.881 cd	2.520 a	2.417 a	2.199 b
	Kabaasi	2.015 cd	2.426 a	2.558 a	2.482 a	2.375 a
	Soganci	1.819 d	1.824 d	1.836 cd	2.113 b	1.894 c
	Mean	1.821 d	1.960 c	2.231 b	2.352 a	

Year 2001: LSD<sub>5%</sub> variety: 0.091\*\*, irrigation rate: 0.081\*\*, variety\*irrigation rate: 0.182\*\*; Year 2002: LSD<sub>5%</sub> variety: 0.124\*\*, irrigation rate: 0.111\*\*, variety\*irrigation rate: 0.248\*\*

Table 3. Effect of different irrigation levels on chlorophyll-b content ( $\mu\text{g/g}$  fresh weight) in the leaves of different apricot varieties

Year	Variety	Irrigation rate (%)				Mean
		25	50	75	100	
2001	Hacihaliloglu	1.839	1.880	2.090	1.906	1.929 b
	Cataloglu	1.777	1.856	2.766	2.000	2.100 ab
	Hasanbey	2.236	2.017	2.271	2.138	2.166 ab
	Kabaasi	2.316	2.177	2.229	3.196	2.480 a
	Soganci	2.041	1.749	1.914	1.914	1.905 b
	Mean	2.042	1.936	2.254	2.231	
2002	Hacihaliloglu	1.543 d	1.842 cd	2.508 a	2.166 bc	2.015 b
	Cataloglu	1.641 d	1.698 d	1.892 cd	2.088 bc	1.830 bc
	Hasanbey	2.064 bc	1.936 cd	2.659 a	2.509 a	2.292 a
	Kabaasi	1.905 cd	2.326 a	2.470 a	2.451 ab	2.288 a
	Soganci	1.617 d	1.653 d	1.858 cd	2.083 bc	1.802 c
	Mean	1.754 b	1.891 b	2.278 a	2.260 a	

Year 2001: LSD<sub>5%</sub> variety: 0.560\*\*, irrigation rate: ns, variety\*irrigation rate: ns ; Year 2002: LSD<sub>5%</sub> variety: 0.198\*\*, irrigation rate: 0.178\*\*, variety\*irrigation rate: 0.397\*\*

Table 4. Effect of different irrigation levels on carotenoid content ( $\mu\text{g/g}$  fresh weight) in the leaves of different apricot varieties

Year	Variety	Irrigation rate (%)				Mean
		25	50	75	100	
2001	Hacihaliloglu	0.920	1.186	0.842	0.939	0.972 ab
	Cataloglu	0.901	1.004	0.618	0.950	0.868 b
	Hasanbey	0.976	1.180	1.029	1.006	1.048 a
	Kabaasi	1.052	1.113	0.919	0.781	0.966 ab
	Soganci	1.069	0.925	0.974	0.919	0.972 ab
	Mean	0.984 ab	1.081 a	0.877 b	0.919 ab	
2002	Hacihaliloglu	1.171 d	1.236 cd	1.232 cd	1.553 ab	1.291 b
	Cataloglu	1.177 d	1.323 cd	1.254 cd	1.454 bc	1.302 b
	Hasanbey	1.219 d	1.282 cd	1.548 ab	1.498 bc	1.387 b
	Kabaasi	1.490 bc	1.630 ab	1.742 a	1.761 a	1.656 a
	Soganci	1.392 cd	1.291 c	1.416 bc	1.018 e	1.279 b
	Mean	1.284 c	1.352 bc	1.439 ab	1.457 a	

Year 2001: LSD<sub>5%</sub> variety: 0.178\*\*, irrigation rate: 0.196\*, variety\*irrigation rate: ns; Year 2002: LSD<sub>5%</sub> variety: 0.111\*\*, irrigation rate: 0.099\*\*, variety\*irrigation rate: 0.221\*\*

Various studies have shown that the chlorophyll content in plants decreases as a result of its breakdown due to stress caused by water limitation (Marler et al., 1994; Eris et al., 1998; Kumar et al., 2023). Studies on cherry (Kırnak and Demirtas, 2002), plum (Kaynas and Kaynas, 1999; Laita et al., 2024) and apricot (Ali and Nazar, 2023) have also reported that leaf chlorophyll content varies depending on the amount of water given to the plant. Consistent with these findings, it was determined that there was a decrease in leaf chlorophyll-a and chlorophyll-b contents at low irrigation rates in apricots in our study.

Plants with low chlorophyll reduction rates have been reported to be more tolerant to drought stress (Arunyanark et al., 2008; Talebi, 2011; Nofrizal, 2022). Similarly, in our study, the change in chlorophyll content was at the lowest rate in Kabaasi and Hasanbey varieties, which are thought to be most resistant to drought stress. The decrease in chlorophyll content under drought conditions may reflect the decrease in photosynthetic capacity and thus the weakening of drought resistance of plants. On the other hand, studies indicate that there is no relationship between the change in chlorophyll content and drought resistance (Buxton et al., 1985; Tsiupka et al., 2023).

#### Carotenoid Contents

In 2001, the values in the Hasanbey variety were found to be significantly higher than the Cataloglu variety in terms of carotenoid content (Table 4).

In 2002, the carotenoid content of the Kabaasi variety was detected to be statistically significantly higher than the other varieties. An increase in carotenoid content was also observed due to increased irrigation rates. However, the highest values for each irrigation rate were measured in the Kabaasi variety (Table 4).

Carotenoids are terpenoid pigments synthesized via the isoprenoid pathway and play a critical role in reducing oxidative stress. Drought stress negatively affects cellular functions by reducing water potential in plants. Under drought stress, ROS production increases in plants and these molecules can damage cellular components. Carotenoids reduce cellular damage by neutralizing ROS and increase the resistance of plants to drought stress (Niyogi, 1999; Fang and Xiong, 2015). Stonefruit species have high carotenoid content. These fruits increase carotenoid levels when exposed to drought stress, keep ROS accumulation under control and protect the integrity of cell membranes. In this process, plant growth regulators (e.g., abscisic acid) also support the drought response by regulating carotenoid metabolism (Qin and Zeevaart, 1999; McQuinn and Waters, 2024). In our study, supporting these findings, carotenoid contents were generally found to be high in Kabaasi and Hasanbey varieties, which were observed to have high drought tolerance. In particular, the highest carotenoid value was measured in Kabaasi variety at 25% irrigation rate in the 2nd year, indicating that carotenoid content may be critical-essential physiological parameter in drought resistance.

Table 5. Effect of different irrigation levels on total sugar content (mg/100 g dry weight) in the leaves of different apricot varieties

Variety	Irrigation rate (%)				Mean
	25	50	75	100	
Hacihaliloglu	4.140	4.083	4.227	3.915	4.091 a
Cataloglu	3.697	3.710	3.673	3.400	3.620 b
Hasanbey	3.936	3.918	3.600	3.620	3.768 ab
Kabaasi	3.672	3.596	3.421	3.476	3.541 b
Soganci	3.840	3.811	3.627	3.721	3.750 ab
Mean	3.857	3.824	3.710	3.626	

LSD<sub>5%</sub> variety: 0.461\*\*, irrigation rate: ns, variety\*irrigation rate: ns

Table 6. Effect of different irrigation levels on total starch content (mg/100 g dry weight) in the leaves of different apricot varieties

Variety	Irrigation rate (%)				Mean
	25	50	75	100	
Hacihaliloglu	2.878	3.446	3.803	3.531	3.415
Cataloglu	3.600	3.773	3.711	3.777	3.715
Hasanbey	3.081	3.615	3.573	4.026	3.574
Kabaasi	3.587	3.905	4.777	4.047	4.079
Soganci	3.506	3.926	3.822	3.917	3.793
Mean	3.330	3.733	3.937	3.860	

LSD<sub>5%</sub> variety: ns, irrigation rate: ns, variety\*irrigation rate: ns

Table 7. Effect of different irrigation levels on abscisic acid content (µg/100 g) in the leaves of different apricot varieties

Year	Variety	Irrigation rate (%)				Mean
		25	50	75	100	
2001	Hacihaliloglu	475.95	254.83	188.65	125.38	261.20
	Cataloglu	363.73	377.71	256.51	291.10	322.26
	Hasanbey	313.45	346.56	296.58	297.91	313.62
	Kabaasi	478.41	297.75	237.95	241.26	313.84
	Soganci	262.43	256.78	163.71	187.50	217.60
	Mean	378.79 a	306.72 ab	228.68 b	228.63 b	
2002	Hacihaliloglu	421.48	451.30	95.63	69.96	259.59
	Cataloglu	359.43	300.41	183.23	242.32	271.35
	Hasanbey	440.93	207.90	226.50	187.85	265.79
	Kabaasi	407.77	315.41	179.88	199.60	275.66
	Soganci	411.23	249.32	208.03	176.05	261.16
	Mean	408.16 a	304.86 ab	178.65 b	175.15 b	

Year 2001: LSD<sub>5%</sub> variety: ns, irrigation rate: 146.12\*, variety\*irrigation rate: ns; Year 2002: LSD<sub>5%</sub> variety: ns, irrigation rate: 175.30\*, variety\*irrigation rate: ns

### Total sugar Contents

The highest total sugar content was found in Hacihaliloglu variety, while the lowest total sugar content was found in Kabaasi and Cataloglu varieties (Table 5). According to irrigation practices, the highest total sugar content was found at 25% irrigation rate, indicating that the plants were under stress and a decrease in total sugar content was observed as the irrigation rate increased. However, these differences did not occur at the level of statistical significance (Table 5).

In various studies, consistent with our findings, it has been shown that as the amount of irrigation water given to the plants decreases, the total amount of sugar in the leaves increases (Balasimha et al., 1987; Pomper and Breen, 1997; Shinozaki and Yamaguchi-Shinozaki, 2007). In some studies on apricots, it has been reported that water restriction can improve fruit quality by increasing the total sugar amount in the fruit without significantly affecting tree yield, and the importance of irrigation strategies appropriate to varieties has been emphasized (Falchi et al., 2020).

### Total starch Contents

Although there was no statistically significant difference between the varieties, the highest starch content was analyzed in the Kabaasi variety and the lowest in the Hacihaliloglu variety. The increase in starch content with the rise in irrigation rate was evident starting from 50% irrigation rate in the varieties. However, these findings showed no statistically significant difference (Table 6).

Plants under drought stress meet their energy needs by increasing starch degradation and regulating cellular osmotic pressure. Starch degradation can facilitate plant adaptation to water stress; however, excessive starch degradation can have adverse effects in the long term by depleting plant energy reserves (Chaves et al., 2003). Due to the alternating change between carbohydrate forms in plants with water restriction, the amount of leaf starch has a trend opposite to the shift in sugar (Munns and Weir, 1981; Fuhr and Lenz, 1989). Similarly, in our study, there was a decrease in the starch content of the leaves as the irrigation rate decreased; however, this difference was not found to be statistically significant.

Although it has been reported that starch content decreases in response to drought in general, this situation does not always occur. The degree of changes in starch content depends on the plant species, the duration of exposure to stress and environmental factors (Hasan et al., 2023). Our study found the lowest values in the Hacihaliloglu variety, especially at 25% irrigation rate, where the plants were under the most stress.

#### ***Absciscic acid Contents***

In both years, no statistically significant difference was found between the varieties in terms of ABA contents; however, in 2001, the lowest ABA contents were observed in Hacihaliloglu and Soganci varieties, which generally showed the most stress symptoms (Table 7). On the other hand, with the increase in irrigation rate, a decrease was observed in the ABA content of the varieties in both years. It was determined that the ABA amounts at 25% irrigation rate were significantly higher than the values at 75% and 100% irrigation rates. In particular, a significant decrease was detected in the ABA content of Hacihaliloglu variety starting from 75% irrigation rate (Table 7).

Stonefruits use a variety of physiological and physiological strategies to cope with drought, and ABA is central to these processes. Under water stress, ABA levels in plants increase, triggering a variety of physiological responses such as stomatal closure. In this way, plants reduce water loss through transpiration (Zhang et al., 2006). In our previous study, we demonstrated that the Kabaasi variety, identified as the most drought-resistant, exhibited stomatal closure at varying degrees depending on irrigation levels. Microscopic analysis revealed that even at a 75% irrigation rate, which was a minimal stress, the stomata of the Kabaasi variety were partially closed; whereas in the Hacihaliloglu variety, the stomata remained largely open under the same irrigation conditions (Olmez et al., 2010). ABA also helps plants adapt to low water conditions by increasing the expression of drought-related genes, such as promoting the synthesis of antioxidant enzymes that protect cells from damage caused by oxidative stress (Zhang et al., 2009; Cutler et al., 2010; Wilkinson and Davies, 2010). Increased ABA levels in plants stressed by reduced water supply or under drought conditions have been explained in many studies (Hiron and Wright, 1973; Eriş and Kaynaş, 1995; Jalili et al., 2023). Parallel to this information, in our research, ABA values, which were relatively high at 25% irrigation rate, showed a significant decrease especially starting from 75% irrigation rate. These decreases were detected most clearly in Hacihaliloglu variety, which was observed to be most affected by water stress. This may suggest that ABA mechanism is more activated in sensitive varieties under water limitation.

#### **Conclusion**

The current study was conducted to determine the physiological changes at different irrigation rates in essential varieties grown in the Malatya region, where apricot production is intensive, and their relationship with the drought resistance of the varieties.

The primary purpose of the irrigation programs is to keep the trees at minimum stress in order to get the maximum product. Regular limited water applications are recommended to the trees, especially during the period when vegetative development accelerates.

In physiological evaluations, chlorophyll values in the leaves decreased depending on the water deficit rates. The change in chlorophyll content was at the lowest rate in Kabaasi and Hasanbey varieties, which are thought to be most resistant to water deficit. In Hacihaliloglu, Soganci and Cataloglu varieties, chlorophyll contents were lower, especially at 25% irrigation. In order to increase the resistance to drought stress in fruit trees, monitoring of chlorophyll content and implementation of appropriate genetic improvement strategies can be recommended. In terms of carotenoid content, the highest values were determined in Hasanbey and Kabaasi varieties. Many studies have confirmed that carotenoids to resist drought stress in stone fruits. The antioxidant functions of carotenoids and their interactions with plant growth regulators these fruits' survival and productivity capacities under drought conditions.

Total sugar content was the highest in Hacihaliloglu, the lowest in Kabaasi and Hasanbey varieties. The highest values of total starch content, which is expected to have an inverse interaction with total sugar and stress, were determined in Kabaasi variety, especially at 25% and 50% irrigation rates, and the lowest in Hacihaliloglu variety. It provides crucial-essential information in understanding the relationship between drought resistance and total sugar in stone fruit species. These findings confirmed that plants with high drought resistance generally have lower sugar content.

Absciscic acid is critical in regulating drought resistance in stone fruit species. ABA is central in controlling water use, strengthening stress response mechanisms, and increasing plant resistance in water-limited conditions. The Hacihaliloglu variety, which was found to be sensitive in terms of ABA content, which is expected to increase due to stress, had high values under restricted irrigation conditions, while it showed the most significant decrease at a 75% irrigation rate.

Consequently, it was concluded that Kabaasi was the most drought-resistant variety, followed by Hasanbey, while Hacihaliloglu, the most common variety in the region, was the most susceptible to drought. Although all varieties exhibited signs of stress at 25% irrigation, they generally managed to tolerate stress at 50% irrigation. Due to global warming and the related drought problem that has increasingly threatened our country in recent years; developing new, variety-specific irrigation strategies that consider the periods during which varieties can endure drought and their minimum irrigation water requirements is crucial for enhancing water use efficiency. Additionally, understanding the drought resistance of these important varieties will offer valuable information for cross-breeding programs focused on developing new drought-resistant candidate varieties.

#### **Declarations**

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

Not necessary

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

H.O.: Project administration, data collection, investigation, writing the original draft

B.C.: Data collection and investigation, methodology

A.M.: Supervision, review and editing

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### Conflict of Interest

The authors declare no conflict of interest.

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## Determination of Zucchini Yellow Mosaic Virus and Watermelon Mosaic Virus Infections in Cucurbit Production Areas of Çanakkale Province from Türkiye

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### ABSTRACT

Viral diseases are among the most significant challenges in protecting plants of the *Cucurbitaceae* family, with viruses from the Potyvirus genus, such as zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV), causing up to 100% yield losses under favorable conditions. Despite the importance of these viruses, there have been no previous studies investigating potyvirus diseases in *Cucurbitaceae* production areas in Çanakkale province. Consequently, the status of these diseases in the region remains unknown. This study aims to address this gap by analyzing the presence of potyviruses in *Cucurbitaceae* production areas in Çanakkale. In the 2021 production year, a total of 137 samples exhibiting virus and virus-like symptoms were collected from various *Cucurbitaceae* production sites in Çanakkale province and its districts. The samples were tested using RT-PCR with primer pairs specific to WMV and ZYMV. From the infected samples, seven isolates were selected for further analysis, and the coat protein (CP) genes were amplified and sequenced. The results revealed that WMV was detected as a single infection in 78 samples, ZYMV in one sample, and mixed infections of ZYMV+WMV were found in 39 samples, indicating that WMV is notably prevalent in Çanakkale. Bioinformatics analyses demonstrated that the Turkish WMV and ZYMV isolates share more than 90% similarity with other isolates in both the local samples and the GenBank database. Phylogenetic analysis further revealed that Turkish WMV and ZYMV isolates are closely related to each other. This is the first study to reveal the presence and phylogenetic relationships of ZYMV and WMV in *cucurbitaceous* plants in Çanakkale province of Türkiye.

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## Introduction

Viruses that negatively impact plant growth and fruit production pose a significant threat to yields in *Cucurbitaceae* cultivation. *Cucurbitaceae* species are known to host over 90 viruses, with approximately 15 belonging to the Potyvirus genus within the *Potyviridae* family. Among the most widespread potyviruses affecting cucurbit production globally are watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) (Sharma, 2023).

Potyvirus which comprises the largest number of species among plant-infecting viruses, is responsible for substantial economic losses in cultivated plants worldwide. The genus includes 167 pathogenic species that infect a broad range of many plants. Symptoms caused by potyviruses in plants changeable based on the virulence, species, and strain of the virus. Common symptoms include mosaic or streak patterns on leaves, chlorosis, leaf deformation, stunting, and even plant death (Revers and García, 2015; Abd El-Aziz, 2020).

Potyviruses are primarily transmitted by aphids in a non-persistent manner with over 200 vector aphids. Some

potyviruses are also known to be seed-transmitted (Nigam et al., 2019). Potyviruses are characterized by their filamentous, non-enveloped, helical symmetry, measuring 680–900 nm and 11–13 nm in length and in width, respectively. They possess a single-stranded, positive-sense RNA genome, ranging from 9.300 to 10.800 nucleotides (Inoue-Nagata et al., 2022).

Numerous studies on cucurbit viruses in Türkiye have been reported that these infections are widespread and significantly diminish the market value of affected crops (Özer et al., 2012; Kamberoğlu et al., 2016; Kamberoğlu and Keçe, 2016; Güller and Usta, 2019; Topkaya et al., 2019; Güller and Usta, 2020; Yeşil, 2021; Karanfil, 2022; Karanfil et al., 2023; Güller et al., 2024a). Similar studies conducted in various provinces of the Marmara region have indicated that potyviruses are prevalent in *Cucurbitaceae*, causing severe mosaic symptoms on leaves and fruit deformations such as blistering or warts, which render the fruits unmarketable (Köklü and Yılmaz, 2006; Kaya and Erkan, 2011; Karakurt, 2015; Altınay, 2017; Karabulut, 2020; Sari, 2023; Güller et al., 2024b). Despite their

economic impact, no comprehensive study has been conducted on the diagnosis and molecular characterization of cucurbit viruses in Çanakkale province. Therefore, this study aims to identify and molecularly characterize the economically important potyviruses, ZYMV and WMV, in cucurbit-growing regions across Çanakkale and its districts.

## Materials and Methods

### Sampling

Field studies for sample collection were conducted during the summer and autumn seasons of 2021. The sampling covered various locations in Çanakkale province and its districts, including open fields, greenhouses, and home gardens. Samples were collected from several *Cucurbitaceae* species, including pumpkin (*Cucurbita pepo*), squash (*Cucurbita* spp.), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), melon (*C. melo*), bottle gourd (*Lagenaria siceraria*), and snake melon (*C. melo* var. *flexuosus*). During the sampling, plants with symptoms such as yellow mosaic patterns, leaf deformation, blistering, excessive growth or stunting, fruit deformation, and discoloration; indicative of viral and virus-like infections were specifically targeted. In fields where multiple plants appeared infected, a maximum of three samples were collected to represent the area adequately. The samplings were carried to the plant virology laboratory in ice bag to ensure their integrity. As a result of the field studies, samples were collected from eight districts of Çanakkale province.

### Testing

To determine the infection of WMV and ZYMV in the samplings from Çanakkale province and its districts, the RT-PCR method was employed. Initially, total RNA was isolated using the CTAB (cetyltrimethyl-ammonium bromide) procedure (Li et al., 2008). The isolated RNA samples were then subjected to denaturation, reverse transcription (RT), and PCR (amplification) steps sequentially. For the RT-PCR tests, WMV-specific primer pairs from Ali et al. (2012) and ZYMV-specific primers from Khanal et al. (2021) were used (Table 1). The RT-PCR test conditions were established according to the protocols of the respective primer references (Ali et al., 2012; Khanal et al., 2021).

### Molecular Characterization Studies

The molecular characterization of WMV and ZYMV isolates were conducted based on their coat protein (CP) gene regions. For molecular characterization, a total of seven isolates were selected from those identified as infected with WMV and ZYMV, considering different districts and host species (Table 2). The selected isolates of CP gene were amplified using PCR, and the resulting PCR products were addressed to sequencing. The nucleotide sequencing of the WMV and ZYMV isolates was carried out by a commercial service provider (BMLabosis, Ankara). Amino acid sequences of the ZYMV and WMV isolates were derived from the obtained sequence data. These data were subsequently used to perform multiple sequence alignments and phylogenetic analyses, comparing the similarities of the selected isolates with each other and with other global isolates.

### Sequence Analysis and Phylogenetic Studies

The nucleotide and amino acid sequences obtained from WMV and ZYMV isolates in Çanakkale were aligned using Clustal W in the CLC Main Workbench V.20 software. Comparisons were performed between the nucleotide and amino acid sequences of the isolates themselves and with other global isolates (Table 3 and 4) to assess similarity percentages. The similarities between the selected isolates and other global isolates were also analyzed using Clustal W in the CLC software.

To further determine the percentage similarity of WMV and ZYMV isolates, the Sequence Demarcation Tool Version 1.2 (SDTv1.2) was used to create a similarity matrix. This matrix was color-coded according to the percentage similarity values obtained (Muhire et al., 2014).

Phylogenetic analyses of the WMV and ZYMV isolates were conducted using CP gene sequences at the nucleotide level, utilizing the multiple sequence alignment files generated by Clustal W. Phylogenetic relationships between global isolates and the WMV and ZYMV isolates were examined using the neighbor-joining method to construct phylogenetic trees. A 1000-replicate bootstrap analysis was performed to statistically validate the accuracy of the constructed phylogenetic trees. ZYMV was used as an outgroup to determine the phylogenetic relationships of WMV isolates and ZYMV was used as an outgroup to determine the phylogenetic relationships of WMV isolates.

Table 1. Primer pairs specific to coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus.

Primer Direction	Primer Sequence	Amplificon Length	Reference
Forward	GAACAAGGAGACACTGTGAT	902	Khanal et al., 2021
Reverse	GCAGCGAAACAATAACCTAG		
Forward	AACACACAACCAAGTGAATT	979	Ali et al., 2012
Reverse	TAACGACCCGAAATGCTAACT		

Table 2. Information for watermelon mosaic virus and zucchini yellow mosaic virus isolates used in molecular characterization studies

Species	Isolate Code	Districts	Host
WMV	WVM10	Çan	Watermelon
	WMV47	Eceabat	Melon
	WMV78	Bozcaada	Pumpkin
	WMV88	Merkez	Bottle gourd
ZYMV	ZYMV9	Çan	Melon
	ZYMV10	Çan	Watermelon
	ZYMV29	Gelibolu	Melon

Table 3. Information for watermelon mosaic virus isolates retrieved from genbank

Accession Number	Host	Origin	Isolate Code
MG952635.1	<i>Cucumis melo</i>	Türkiye	Alakoy 2
MG952634.1	<i>Cucumis melo</i>	Türkiye	Alakoy 1
LC434453.1	<i>Panax ginseng</i>	South Korea	AS
LC434452.1	<i>Panax ginseng</i>	South Korea	SJ
MN814408.1	<i>Bromus sp.</i>	Spain	E1P_87
MN814378.1	<i>Cucumis melo</i>	Spain	M3V_6
KC447295.1	<i>Citrullus lanatus</i>	Saudi Arabia	WMV-SA
KF021299.1	<i>Cucurbita pepo</i>	Türkiye	W26
KF021300.1	<i>Cucurbita pepo</i>	Türkiye	W59
KF021298.1	<i>Cucumis sativus</i>	Türkiye	W2
MZ130405.1	<i>Cucumis melo</i>	Türkiye	Igdir 7
GQ421158.1	<i>Cucurbita pepo</i>	İran	Meşhed
MN966673.1	<i>Cucurbita pepo</i>	Egypt	WMV-Egy1
MH992141.1	<i>Cucurbita moschata</i>	Poland	D2
MT437295.1	<i>Cucumis melo</i>	Türkiye	Bingol W4
MT413451.1	<i>Cucumis melo</i>	Türkiye	Bingol W2
MZ055421.1	<i>Citrullus lanatus</i>	Türkiye	Igdir 6
MW962978.1	<i>Cucurbita pepo</i>	Türkiye	Diyarbakir D3
AY464948.1	-	China	WMV-HLJ
MG021273.1	<i>Citrullus lanatus</i>	USA	KY-1
MG021301.1	<i>Citrullus lanatus</i>	USA	TX-20
MG021250.1	<i>Citrullus lanatus</i>	USA	OK-4
AB001994.1	<i>Habenaria radiata</i>	Japan	Habenaria
MG021268.1	<i>Cucurbita pepo</i>	USA	MS-3
L22907.1	<i>Vanilla fragrans</i>	Australia	Tonga

Table 4. Information on other zucchini yellow mosaic virus isolates retrieved from genbank

Accession Number	Host	Origin	Isolate Code
KP872543.1	<i>Cucurbita pepo</i>	Türkiye	ER6-8
KP872575.1	<i>Cucurbita pepo</i>	Türkiye	G3
KP872574.1	<i>Cucurbita pepo</i>	Türkiye	G2
KP872573.1	<i>Cucurbita pepo</i>	Türkiye	G1
KP872550.1	<i>Cucurbita pepo</i>	Türkiye	AS6
KP872581.1	<i>Cucumis melo</i>	Türkiye	S5
KP872578.1	<i>Cucurbita pepo</i>	Türkiye	E-7
KP872576.1	<i>Cucurbita pepo</i>	Türkiye	K3
KP872546.1	<i>Cucurbita pepo</i>	Türkiye	KAR12-4
KP872541.1	<i>Cucurbita pepo</i>	Türkiye	AKS5-7
KP872572.1	<i>Cucurbita pepo</i>	Türkiye	AYS7
MK689858.1	<i>Cucurbita pepo</i>	Türkiye	ZYMV- Bingol
JF317296.1	<i>Cucumis sativus</i>	Türkiye	ZYMV-Adana
KP872577.1	<i>Cucurbita pepo</i>	Türkiye	K17
KP872571.1	<i>Cucumis sativus</i>	Türkiye	D14
AJ420019.1	-	Germany	Berlin 1
AJ420015.1	-	Austria	Austria 10
AJ420017.1	-	Austria	Austria 12
KP872561.1	<i>Cucurbita pepo</i>	Türkiye	BE26
AJ251527.1	<i>Cucumis sativus</i>	Hungary	10
AJ420018.1	-	Slovenia	Slovenia 1
AJ459956.1	-	Hungary	H272-8
KP872565.1	<i>Cucurbita moschata</i>	Türkiye	BRD4
JF317297.1	<i>Cucumis melo</i>	Türkiye	ZYMV-Ahlat
JF795797.1	<i>Mukia maderaspatana</i>	Austria	Cvn-13
KP872580.1	<i>Cucurbita pepo</i>	Türkiye	Y4
KP872579.1	<i>Cucurbita pepo</i>	Türkiye	Y23

## Results

The distribution of these samples by district is presented in Figure 1. As shown in Figure 1, 41 samples were collected from the Çanakkale Central district, 32 from Eceabat, 24 from Çan, 18 from Gelibolu, 9 from Bozcaada, 7 from Lapseki, 4 from Biga, and 2 from Bayramiç (Figure 1).

When the sampled cucurbit plants were analyzed for species and potyvirus presence, 45 samples were collected from squash, of which 40 showed single or mixed infections with watermelon mosaic virus (WMV), and 14 were infected with zucchini yellow mosaic virus (ZYMV). Additionally, 5 samples tested negative for virus presence. In pumpkin, 35 samples were collected, with 19 showing single WMV infections and 11 infected with both viruses, while 5 samples were negative for any virus. For melon and watermelon plants, 23 and 19 samples were collected, respectively; 15 melon samples were infected with WMV, 5 with mixed infections, and 12 watermelon samples were infected with WMV, with 3 showing mixed infections. Additionally, 3 melon and 4 watermelon samples were negative for virus presence. Seven cucumber samples were collected, with 4 infected with WMV and 1 with ZYMV, but no mixed infections were detected. Due to limited cultivation, 4 samples were collected from both Armenian cucumber and bottle gourd. One WMV infection was detected in each species, and 3 samples from each exhibited mixed infection. Of all collected samples, single infections were determined 78 and 1 plants infected with WMV and ZYMV, respectively. Furthermore, mixed infections were determined in 39 plants. Potyvirus presence was not detected in 19 samples (Table 5).

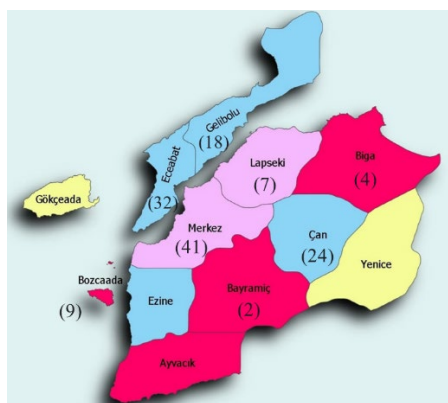


Figure 1. Districts of Çanakkale where field studies were conducted (The number of samples collected is given in parentheses).



Figure 2. Mosaic symptoms in melon (A) and gourd (B) plants mixed infected with watermelon mosaic virus and zucchini yellow mosaic virus

Table 5. Number of infected and collected samples based on hosts as a result of field and virus identification studies

Host	Number of WMV		Number of ZYMV		Number of Samples	
	Infected/Collected	Samples	Infected/Collected	Samples	WMV+ZYMV	Infected/Collected
Melon	15	23	0	23	5	23
Watermelon	12	19	0	19	3	19
Squash	26	45	0	45	14	45
Pumpkin	19	35	0	35	11	35
Snake melon	1	4	0	4	3	4
Cucumber	4	7	1	7	0	7
Bottle gourd	1	4	0	4	3	4
Total	78	137	1	137	39	137

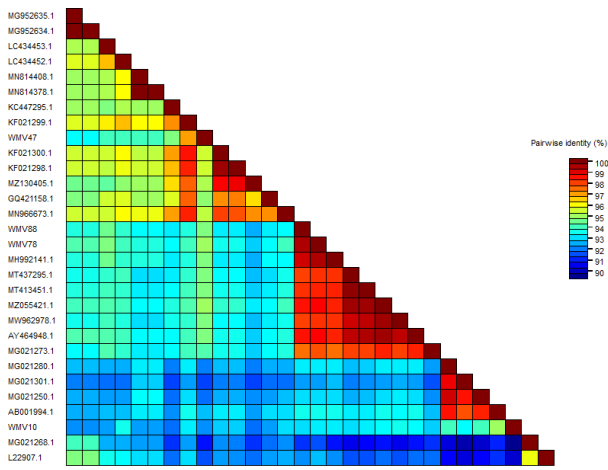


Figure 3. Similarity rates of coat protein nucleotide sequences between Çanakkale and global watermelon mosaic virus isolates

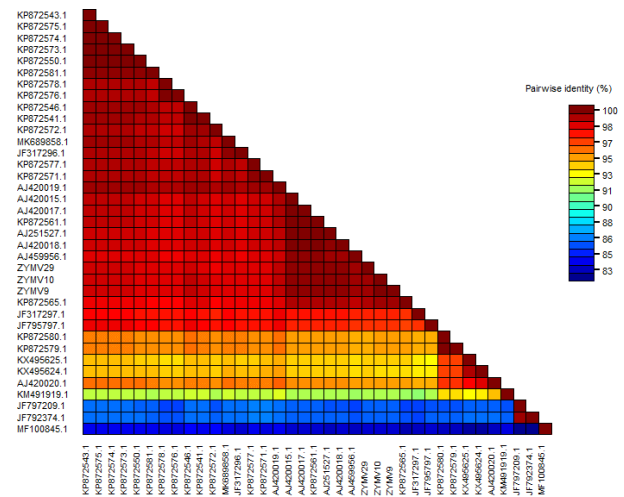


Figure 4. Similarity rates of coat protein nucleotide sequences between Çanakkale and global zucchini yellow mosaic virus isolates

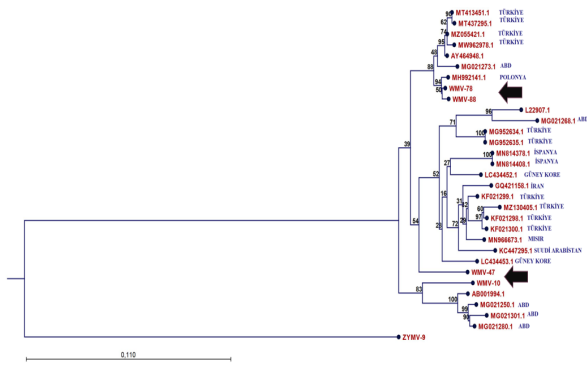


Figure 5. Phylogenetic family tree of Çanakkale watermelon mosaic virus isolates based on nucleotide sequences

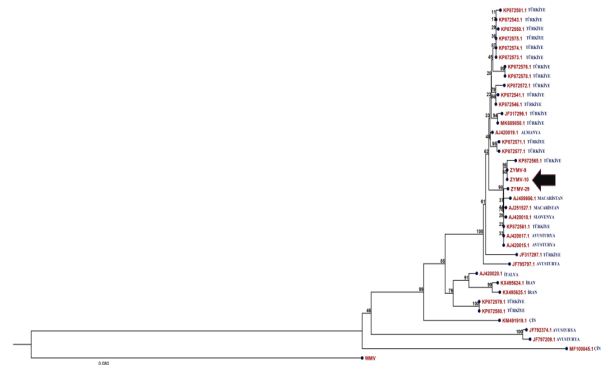


Figure 6. Phylogenetic family tree of Çanakkale zucchini yellow mosaic virus isolates based on nucleotide sequences

The phylogenetic tree of ZYMV isolates is shown in Figure 6. The tree comprised four main groups. Phylogenetic analyses indicated that, unlike the Çanakkale WMV78, WMV88, WMV47, and WMV10 isolates, the ZYMV9, ZYMV10, and ZYMV29 isolates were clustered in the same main group, with ZYMV9 and ZYMV10 being closely related. Notably, the ZYMV29 isolate showed a close resemblance to isolates from Hungary, Slovenia, Turkey, and Austria.

**Discussion**

The study investigated the prevalence of two major potyviruses affecting cucurbit crops, revealing that WMV is significantly more prevalent than ZYMV. In Türkiye, previous studies have documented the widespread occurrence of both viruses in cucurbits. For instance, Köklü and Yılmaz (2006) reported ZYMV infection rates of 45.5% in watermelon and 40.3% in melon, while WMV infection rates were 34.3% in watermelon and 31.2% in melon out of 502 samples collected from melon and watermelon fields in the Thrace region. Similarly, a study conducted in Tokat by Korkmaz et al. (2018) found that 12% of the 571 leaf samples from pumpkin plants were infected with ZYMV, while 37% were infected with

WMV. These findings indicate that both viruses are prevalent and have high infection rates in regions neighboring our study area, such as Thrace and Central Anatolia, consistent with the results of our research.

In the study, potyvirus infection was not detected in some samples showing typical viral symptoms, which is probably due to other viruses affecting cucurbits. Karanfil and Korkmaz (2021) identified the presence of Cucumber mosaic virus (CMV) in 10 out of 72 cucurbit samples from the Balıkesir, Çanakkale and Bursa provinces. Numerous studies in Turkey have also identified other viruses in cucurbits besides WMV and ZYMV, such as CMV, papaya ringspot virus (PRSV), squash mosaic virus (SqMV), and melon necrotic spot virus (MNSV) (Köklü and Yılmaz, 2006; Korkmaz et al., 2018). These studies highlight the diversity of viruses, beyond potyviruses, that cause infections in cucurbits in Türkiye.

The infection rates of WMV and ZYMV in Çanakkale vary across districts and crop types. These variations may be influenced by factors such as the transmission capacity and population density of virus-carrying vectors, differences in sample types and varieties, cultivation techniques, and environmental factors.

In terms of sequence similarity, Gara et al. (1997) reported that the WMV-Habenaria isolate (AB001994.1)



from Japan showed sequence similarities ranging from 78% to 96% with other isolates in GenBank. The nucleotide sequence similarity between the WMV isolates from Çanakkale and those from Japan ranged from 95% to 96%. In Iran, Sharifi et al. (2008) analyzed the amino acid sequence similarity of the coat protein gene region of 14 WMV isolates, finding a similarity range from 97.4% to 100%. When compared to two Iranian isolates in the GenBank, the similarity rates ranged from 91.6% to 93.4%. These findings indicate a high degree of similarity in the amino acids encoded by the coat protein gene region of WMV isolates. In Türkiye, Yeşil (2013) reported sequence similarity rates of 99% based on CP gene sequence analyses of positive samples among 652 collected in 2009 and 2010. Studies conducted globally and in Türkiye consistently show that WMV isolates coat protein genes exhibit over 90% similarity, aligning with the results of our study.

Vučurović et al. (2012) conducted a study in Serbia in 2011, where they found that 6 out of 26 watermelon samples were infected with ZYMV. They sequenced one of these isolates using RT-PCR, finding nucleotide similarity rates between the Serbian isolate and other global isolates ranging from 93.7% to 99.9%. In a study conducted in Türkiye, Topkaya et al. (2019) identified the presence of WMV, ZYMV, CMV, PRSV, and CGMMV viruses in the Ankara and Antalya provinces between 2009 and 2014. They sequenced the CP nucleotide sequences of 45 ZYMV isolates and compared them with global isolates from GenBank, finding similarity rates ranging from 96% to 99%. The similarity between isolate BE26 (accession number KP872561, Ankara) and the Çanakkale isolates (ZYMV9, ZYMV10, and ZYMV29) was 100%, demonstrating high similarity between isolates from the same geographical regions.

Nematollahi et al. (2021) collected 305 samples showing virus symptoms from watermelon fields in northern Iran, finding that 80 samples were infected with WMV. Their sequence and phylogenetic analyses of the CP and P1 regions identified three main groups (CL, EM, and G2) formed by WMV isolates, each further subdivided into several branches. Our study also identified three main clusters of WMV-infected samples based on CP gene phylogenetic analysis, demonstrating parallel results between the two studies.

The phylogenetic relationships of ZYMV isolates have been extensively studied in Türkiye, consistently showing that Turkish isolates are closely related (Kamberoğlu et al., 2016; Yeşil, 2021). These findings align with our study, confirming the consistency and mutual support among various studies conducted in Turkey.

## Conclusion

This study provides insights into the infection rates of WMV and ZYMV species in Çanakkale province. Additionally, the molecular characterization of seven isolates based on the CP gene region was conducted. Future research should include sequencing other genes beyond the CP gene or even the entire viral genome to generate comprehensive genomic information on isolates specific to Türkiye, contributing valuable data to the scientific community.

## Declarations

### Author Contribution Statement

M.S. conducted the experiments; A.K. contributed to the writing of the manuscript and the analyses; S.K. designed the study, contributed to the writing of the manuscript and the analyses. All authors have made significant contributions to the final manuscript and have approved its content.

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### Conflict of Interest

The authors declare no conflict of interest.

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## Assessment of Field Performance and Nutritional Quality of Mung Bean (*Vigna radiata* L.) for Food Diversification

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### ABSTRACT

This research was carried out to evaluate the field performance of some mung bean accessions and their nutritional composition for inclusion in household diet. Twenty-one (21) accessions of mung bean were evaluated in the early and late season of 2022 at Ile-Ife, and in the early season at Kishi out-station of the Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Nigeria. The experiment was established according to randomized complete block design with three replications. Agronomic and yield data were collected. Six of the mung bean accessions were randomly selected and analyzed for proximate, mineral composition and sensory properties along with two cowpea varieties as standard checks. MB-3, 6, 14 and 15 produced the highest seed yield across the locations (Ile-Ife early season, Ile-Ife late season, and Kishi early season). However, the performance was generally better in the early season than in the late season. The mung bean samples had slightly lower protein values than the standard checks. The mung bean samples had considerably higher mineral levels ( $p \leq 0.05$ ) than the control samples. Moin-moin (processed bean cake) made from some of the mung bean samples compared favorably with that from Ife brown cowpea (standard check). This work revealed good adaptability of the mung bean accession to southwest agro-ecology of Nigeria. It also revealed better nutritional quality of mung bean relative to cowpea for inclusion in household meals.

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## Introduction

On a global scale, there were more than 700 million people who experienced extreme food insecurity in 2018. (FAO, 2019). Because of this, many have resolved to taking cheap diet mainly carbohydrate. This has accounted for the high level of malnutrition most importantly in children and women in the reproductive age. Malnutrition in all its forms is thought to cost society up to US\$3.5 trillion annually (UNICEF, 2018). Cowpea, often referred to as “the meat for the poor” in Nigeria is now beyond the reach of the masses. National Bureau of Statistics (NBS) (2017) data showed a price increase of 44.15% for cowpea between May 2016 and May 2017. Between 2020 and 2021, the price has increased by 116.7% and there is no sign of fall in price. Animal protein that could be an alternative source is by far beyond the reach of the

populace due to its outrageous price. The possible strategy for attaining nutrition and food security in African is through exploitation of underutilized legumes such as mung bean, lima bean and bambara groundnut.

Mung bean, [*Vigna radiata* (L.) Wilczek], is an underutilized legume in the same family and genus with cowpea. The self-pollinating mung bean crop has a diploid chromosome number of  $2x = 22$ . The seeds are free from anti-nutritional factors, but rich in essential amino acids particularly lysine (504 mg/g) (Minh, 2014); minerals such as iron, zinc, phosphorus and magnesium; and vitamins including selenium. Mung bean is easier to cook unlike other less commonly consumed legumes, with easy digestibility and less flatulence. Its protein content (21-31.32 %) is higher than that of soybean (18-22 %) and

kidney bean (20-30 %) (Shevkani et al. 2015). Previous research findings revealed that flavonoids concentration in mung bean seeds ranged between 125 – 352 mg QE / 100g seed (Wang et al. 2021), while that of cowpea ranged between 7.46- 23.95 mg QE / 100g seed (Sombie et al. 2018). Flavonoids are secondary metabolites that are in charge of the qualities of flavor, color, and aroma in food of plant origin (Dias et al. 2021).

The distribution of mung bean all over the world is due to its short growth cycle, wide adaptability and its ability to fix nitrogen to the soil (Yi-Shen et al. 2018). Mung beans thrive in temperatures between 25 and 35 °C and 400 to 500 mm of rain spread evenly over 60 to 90 days of growth. It survives in a variety of soil types, such as red laterite soils and sandy loams with a pH range of 6.3 to 7.2. For optimal growth, the soil should have a slight acidity. With up to 41% of the world's production, India is the leading producer of mung beans, followed by China, Bangladesh, Pakistan, and Myanmar (Pataczek et al. 2018).

Despite its potential, the small seed size, inadequate farming practices and restricted availability of better cultivars limits using of mung bean (Mbeyagala et al. 2016). Also, there is little research on the agronomic potential and nutritional properties of different mung bean accessions especially in Nigeria. Unraveling the potential of mung bean as a substitute to cowpea and other prominent legumes through research is an important strategy to improve the adoption of mung bean in household diets in Nigeria and other African countries, thereby tackling the challenges of food and nutrition insecurity and food diversity.

The goal of this research therefore is to (1) examine the field performance of some accessions of mung bean, (2) determine the nutritional quality of the crop for possible inclusion in household diets nationwide.

## Materials and Methods

Twenty-one (21) mung bean accessions were evaluated in the early and late cropping season at Ile-Ife (rain forest ecology), and only in the early season at Kishi (savanna ecology) out-station of the Institute of Agricultural Research and Training (IAR&T), Nigeria in 2022. The early season planting was between May- August, while late season planting was between August-November, 2022. The two locations with the seasons (Ile-Ife early season, Ile-Ife late season and Kishi early season) are to be referred to as “Environment” in this study. Soils in Ile-Ife are generally classified as ultisols with low nutrient reserves and high gravel content. They are slightly to strongly acidic with low available phosphorus and high exchangeable bases. The average temperature and rainfall at Ile-Ife in 2022 were 25.3°C and 1509mm, respectively. The wettest month was September with average rainfall of 225mm. Kishi soils are classified as Arenic kandiodults according to USDA taxonomy. The soil is sandy with low cation exchange capacity (CEC) and potassium deficiency. The average temperature and rainfall at Kishi in 2022 were 28°C and 1050 mm, respectively. The wettest month was June with average rainfall of 150mm. The accessions were obtained from the International Institute of Tropical Agriculture (IITA), Nigeria. Three replicates in a randomized complete block comprised the experimental

design. The plot measured 2.4 by 2.4 meters making 5.76m<sup>2</sup>, with 0.6 meters separating rows and within rows, and two seedlings per stand were maintained at field establishment. Metolachlor 960g E.C. at 1.44kg ai/ha was the pre-emergence herbicide that was sprayed at planting while manual weeding was carried out on the field as necessary. Magic Force (Lambdacyhalothrin 15% + Dimethoate 300 g L-1) was used to suppress pest insects in fields throughout both their vegetative and reproductive phases.

Data were gathered on plant height, days to first and 50% flowering, days to first and 50% pod formation, pod length, days to maturity and seed yield. Using the meter rule, the height of the plant was measured in centimeters, from the ground surface to the tip where the plant branched. Days to first and 50% flowering was determined by keeping track of the days following planting to the day the first flower was noticed and when the flowering reached 50% respectively, per plot. The BBCH growth stage code is presented in Table 1. Days to first and 50% pod formation was recorded by counting the number of days from planting to the day the first pod was noticed and when the pod formation reached 50% per plot, respectively. Pod length (in centimeter) was taken as the average length of ten randomly selected pods per plot. Days to maturity is regarded as the number of days from the time of sowing until when 75% of the pods are dry. Seed yield was taken after threshing the dry harvested pods per plot. The chaff was removed and weight of the seeds was recorded per plot as seed yield in kg/m<sup>2</sup>.

To assess the nutritional qualities, six of the mung bean accessions were randomly selected based on seed color, pod length and yield performance on the field for proximate analysis and mineral composition test, together with two cowpea genotypes namely- *Ife brown* (improved variety) and *Cotonou* (land race) as control. Seeds of the mung bean and cowpea samples are presented in Figure 1. The mung bean along with the cowpea samples were also processed into Moin moin, a popular bean cake in Nigeria, for sensory evaluation. Proximate analyses of mung bean and cowpea seeds were done using the guidelines provided by AOAC (2005).

## Data analyses

Mean, standard error, coefficient of variation and range were estimated. Combined analysis of variance (ANOVA) for the Ile-Ife early season, Ile-Ife late season and Kishi early season (here referred to as “Environment”) was performed using Proc GLM of SAS. Duncan Multiple Range test was utilized for means separation.

Genotypic and phenotypic coefficient of variation were computed according to Singh and Chaudhary (1979) as:

$$PCV = \sqrt{\sigma^2_{ph}/\bar{x}} \times 100$$

$$GCV = \sqrt{\sigma^2_g/\bar{x}} \times 100$$

where

PCV : Phenotypic coefficient of variation

GCV: Genotypic coefficient of variation

$\sigma^2_{ph}$  : Phenotypic variance;

$\sigma^2_g$  : Genotypic variance;

$\bar{x}$  : Mean

Table 1. Phenological growth stages and BBCH- identification keys of bean

Growth stage	Code	Description
0. Germination	00	Dry seed
	01	Beginning of seed imbibition
	03	Imbibition completed
	05	Radicle emergence
	07	Hypocotyl with cotyledons breaking through seed coat.
	08	Hypocotyle arch visible
	09	Emergence; (cracking stage)
1. Leaf development	10	Cotyledon completely unfold
	12	2 full leaves
	13	3rd true leaf (first trifoliolate leaf) unfolded
	19	9 or more leaves unfolded
2. Formation of side shoots	21	First side shoot visible
	22	2nd side shoots (and this continues)
	29	9 or more side shoots visible
5. Inflorescence emergence	51	First flower buds visible
	55	First flower buds enlarged
	59	First petals visible, flowers still closed.
6. Flowering	60	First flowers opened
	61	10% of flower open
	62	20% of flower open
	63	30% of flower open ( and this continues)
	65	50% of flower open
	67	Majority of petals fallen or dry
	69	End of flowering: first pods visible
7: Development of fruit	71	10% of pods have reached typical length
	72	20% of pods have reached typical length
	73	30% of pods have reached typical length
	75	50% of pods have reached typical length ( and this continues)
	79	Pods: individual beans easily visible
8: Ripening of fruit and seed	81	10% of pods ripe Seeds beginning to mature
	82	20% of pods ripe
	83	30% of pods ripe (beans hard)
	85	50% of pods ripe (beans hard)
	86	60% of pods ripe (beans hard)
	89	Fully ripe: pods ripe (bean hard)
	9: Senescence	97
99		Harvest product

Source: [https://en.m.wikipedia.org/wiki/BBCH-scale\\_\(bean\)](https://en.m.wikipedia.org/wiki/BBCH-scale_(bean))

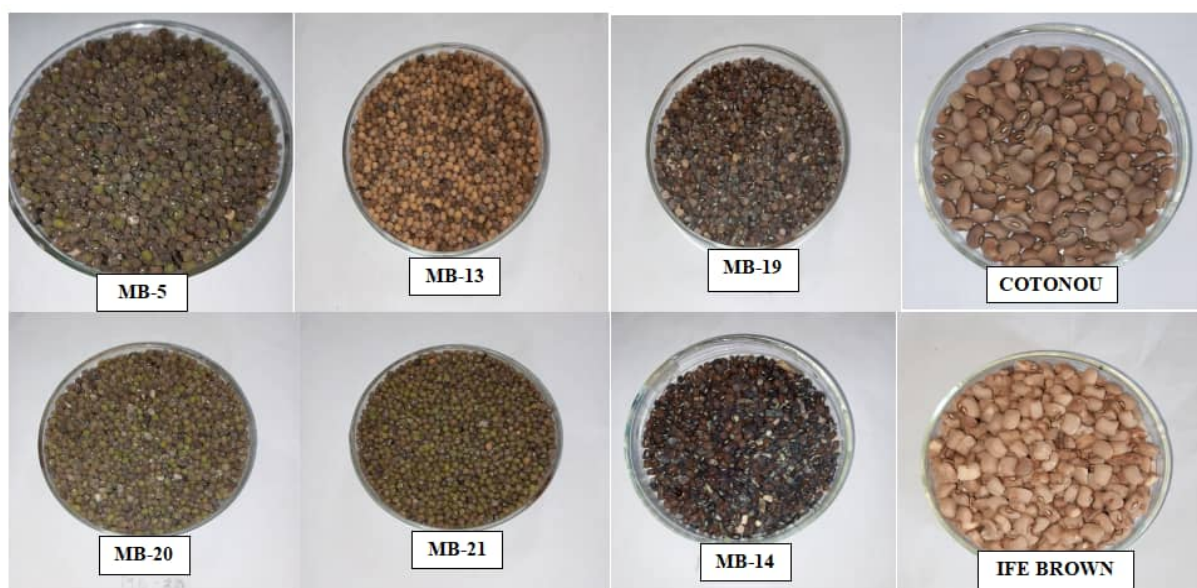


Figure 1. Seeds of the mung bean and cowpea samples

## Results and Discussion

Mean square of environment was highly significant for all attributes under investigation (Table 2), this indicates the environment's influence on the traits expression. Belay et al. (2019) also observed significant environmental (year) variation for the traits they studied aside for number of pods per plant. Mean square of genotype was significant for most of the traits except days to 50% podding, days to maturity and seed yield. Significant mean square of genotype for plant height, number of days to flowering, number of days to first pod and pod length. Similar findings were reported on genotypes of mung beans by Kassa et al. (2018), Belay et al. (2019) and Yoseph et al. (2022). Mean square due to genotype by environment interaction was not significant for all the traits measure aside for number of days to 50% pod formation, implying that the accessions behaved differently across the environment for this trait.

The accessions exhibited wide ranges for all the traits studied. Mean value for plant height was observed to be 88.14 cm (Table 3). MB-8, 10, 11 and 19 were the tallest

(> 100cm). The mean days to first flowering was (41.63) days, with MB-21 flowering and setting pod earliest, while MB-5 and MB-8 flowered and set pod latest. The mean number of days to 50% flowering and pod formation was 46 and 51 days respectively meaning that pod formation takes average of 5 days after flowering. Mean pod length was 8.53 cm with MB-9, MB-4 and MB-17 having the longest pods (9.50 cm, 9.51 cm and 9.61 cm respectively) and MB-21 having the shortest pods (4.6 cm). Mean seed yield was 0.51kg/m<sup>2</sup> with MB-3, 6, 14 and 15 having the highest seed yield ranging between 0.74 - 0.94kg/m<sup>2</sup> MB-21 recoded the lowest seed yield (0.15kg/m<sup>2</sup>). The shortest pod length produced by accession MB-21 could have contributed to its low seed yield value. Similar findings was reported by Aysun (2004) on longer pods of cowpea producing better yield than those with shorter pods. Mean days to maturity for the mung bean accessions was 73 days, with MB-7 reaching maturity earliest in 68 days and MB-10 had the highest mean days to maturity (78.50 days) (Table 3).

Table 2. Mean squares from combined analysis of variance for agronomic traits in the mung bean accessions at Ile-Ife early, Ile-Ife late season and Kishi early season of 2022

Source	df	PH	DFP	D50F	DFP	D50P	PL	DTM	SYD
Env	2	5349.12**	661.64**	273.06**	793.08**	211.83**	48.86**	752.89**	22.25**
Rep (Env)	6	727.77	8.25	24.28	9.41	17.84	0.31	38.87	0.58
Gen	20	515.98**	71.98**	77.53**	61.78*	37.50	16.88**	39.86	0.36
Env x Gen	40	179.93	9.02	13.51	29.61	25.42**	0.30	35.89	0.3
Error	120	108.89	7.52	11.42	30.74	9.62	0.34	37.07	0.16

PH: Plant height (cm); DFP: Days to first flowering; D50F: Days to 50% flowering; DFP: Days to first podding; D50P: Days to 50% podding; PL: Pod length (cm); DTM: Days to maturity; SYD: Seed yield (kg/m<sup>2</sup>); Env: Environment (Ile-Ife early season, Ile-Ife late season and Kishi early season); Gen: Genotype; Rep.: Replicate; cm: centimeter \*\*: significant at p=0.01, \*: significant at P=0.05

Table 3. Mean performance of the 21 mung bean accessions for agronomic traits at Ile-Ife early season, Ile-Ife late season and Kishi early season of 2022

Accession	PH	DFP	D50F	DFP	D50P	PL	SYD	DTM
MB-1	78.56	40.89	45.89	44.56	51.00	9.21	0.26	70.50
MB-2	92.89	42.00	48.33	45.78	51.22	8.89	0.53	74.33
MB-3	80.56	41.22	45.56	44.33	49.00	8.91	0.74	71.83
MB-4	84.50	41.89	47.11	45.33	50.89	9.51	0.60	72.17
MB-5	99.40	47.89	53.57	49.44	56.83	8.60	0.48	69.50
MB-6	98.22	42.11	48.14	45.33	50.43	9.12	0.94	70.33
MB-7	80.78	41.56	46.44	43.75	50.56	8.89	0.69	67.50
MB-8	102.61	48.22	52.67	50.38	56.00	8.91	0.39	74.67
MB-9	78.11	40.67	44.89	43.00	50.75	9.50	0.47	74.83
MB-10	110.00	40.11	46.56	44.78	52.22	5.09	0.08	78.50
MB-11	103.50	42.63	48.50	45.88	52.00	9.25	0.48	76.17
MB-12	85.00	41.11	44.43	49.11	48.57	8.83	0.57	74.17
MB-13	80.56	40.33	44.33	41.56	50.22	9.33	0.34	73.00
MB-14	88.72	41.00	45.88	41.50	49.88	8.60	0.88	74.33
MB-15	86.72	43.33	48.78	45.22	51.63	9.07	0.88	75.83
MB-16	86.89	40.33	44.00	42.38	47.89	9.22	0.63	72.50
MB-17	83.06	40.67	44.56	43.00	50.44	9.61	0.36	72.00
MB-18	75.56	41.75	46.38	45.13	51.13	9.14	0.43	74.00
MB-19	102.50	41.11	46.67	45.22	51.63	4.99	0.20	76.83
MB-20	82.06	41.78	48.11	45.44	51.13	8.72	0.56	74.00
MB-21	88.33	33.67	38.56	39.11	46.67	4.60	0.15	73.67
Mean	88.14	41.63	46.46	44.77	50.78	8.53	0.51	73.37
SE	1.50	0.34	0.36	0.48	0.33	0.13	0.05	0.58
CV%	11.84	6.58	7.27	12.38	6.11	6.88	78.18	8.30
Range	38.33-116	28-56	34- 60	29- 70	40- 49	3.92- 11.4	0- 3.1	39- 87
PCV	81	43	43	39	29	47	28	25
GCV	65	41	39	28	16	46	11	8

PH: Plant height (cm); DFP: Days to first flowering; D50F: Days to 50% flowering; DFP: Days to first podding; D50P: Days to 50% podding; PL: Pod length (cm); DTM: Days to maturity; SYD: Seed yield (kg/m<sup>2</sup>); SE: Standard error; CV: Coefficient of variation; cm: centimeter; kg: kilogram

The extent of environmental impact on any given character is reflected in the magnitude of the difference between PCV and GCV values. Large difference indicates high environmental influence, while small difference indicates high genetic influence. In this study, values of PCV were marginally greater than GCV for most traits, this implies that the expression of those traits has less of environmental influence, however traits like number of days to maturity and seed yield/plot which had high difference between the two parameters indicates a higher environmental effects. PCV and GCV values were categorized by Deshmukh et al. (1986) as above 20% were considered high, between 10-20% as moderate and less than 10% as low. PCV and GCV values were high for most of the traits, while number of days to 50% podding and seed yield had high PCV and moderate GCV values this indicates that those accessions exhibit much variation with respect to these traits and selection on them will be effective. High PCV and low GCV values was observed for days to maturity, this implies that selection based on their performance will be misleading. The greater influence of environment observed for number of days to maturity was also reported by Meena et al. (2017) in pea plants. The results obtained in this study was however contrary to the findings of Sharma et al. (2018) who reported low PCV and GCV scores for plant height, number of days to first flowering, number of days to 50% flowering and pod length.

An analysis of the mung bean accessions' yield performance in each environment is shown in Table 3. All the accessions performed best in the early season at Ile-Ife, a rain forest zone, with mean seed yield of 1.20 kg/m<sup>2</sup> followed by early season at Kishi, a savannah zone, with mean seed yield of 0.25 kg/m<sup>2</sup>. The yield performance was however poor in the late season at Ile-Ife with mean seed yield of 0.01 kg/m<sup>2</sup>. The high seed yield obtained in early season at Ile-Ife could be attributed to the moderate to high rainfall during the season which seems to favour mung bean production, coupled with the slightly acidic soil. The poor performance in the Ile-Ife late season could however be due to excessive rainfall around the time (225mm in September, 2022 alone). Asfaw et al. (2012) reported similar finding in a mung bean multi-locational trial.

The accessions vary significantly in their mean performance. MB-6, 14 and 15 performed best in the early season at Ile-Ife, with seed yield of 2.27, 2.01 and 2.28 kg/m<sup>2</sup>, respectively, followed by MB-3 and MB-7. MB-4, 8, 16 and 17 performed best at Kishi with mean seed yield ranging between 0.41 - 0.56 kg/m<sup>2</sup> followed by MB-3, 7, 14 and 15. Despite the poor performance of the accessions in the late season at Ile-Ife, MB-3 and MB-4 had the highest seed yield of 0.04 and 0.03 kg/m<sup>2</sup> respectively. Across all the three environments, MB-3, 6, 14 and 15 had the highest seed yield with mean yield ranging between 0.74 - 0.88 kg/m<sup>2</sup>, these accessions were also characterized with moderate plant height, therefore they are recommended as candidate accessions to be considered for improvement. Accessions 6, 14 and 15 were also identified as the best performers at Ile-Ife in the early season, each producing seed yield greater than 2 kg/m<sup>2</sup>. These

accessions could be recommended to farmers for improved yield.

The result of the proximate analyses and mineral composition of selected mung bean and cowpea seeds (control) is presented in Table 4. The proximate analyses of food reveal the basic nutrients and the specific amount of such nutrients in the food. The result revealed that mung bean seeds were higher in carbohydrates than the standard checks (Cotonou and Ife brown). Also, the mung bean seeds had lower values for protein in comparison to the cowpea samples. The report of USDHHS (2019) stated that a diet that will support growth comprise of 45-65 % carbohydrate, 10-35 % protein and 20-35 % fat. All the mung bean samples had carbohydrate and protein values within the USDHHS specified range hence it implies that it is sufficient to support the growth of the consumers. The selected mung bean accessions also had significantly lower values ( $p \leq 0.05$ ) for fat and fibre when compared with the cowpea samples. The percentage fat in the mung bean seeds and the control cowpea seeds was far below the standard value of 20-35% fat (USDHHS, 2019). Fat content in the mung bean seeds ranged between 3.18 - 3.48 %, while COT and IFbr had 4.16 % and 4.27 % fat content respectively. Generally, legumes are low in fat, therefore, they are not abundant in vitamins that dissolve in fat, such as A, D, E, and K (Albrahani & Griavis, 2016). The only exception is soybean with 30.31 % fat and groundnut with 47.8 % fat (Erbersdoblar, et al. 2017). To balance the requirement for fat intake in mung bean and other legume diets, consumers will need to add consumable oil during food processing. Fibre content of mung bean seeds ranged between 4.15 - 4.27 %. This value will provide between 16.6 - 17.1 % and between 10.92 - 11.2 % of the Recommended Daily Allowance (RDA) for fibre in women and men respectively (Soliman, 2019). The proximate values obtained for ash were significantly higher ( $p \leq 0.05$ ) in the mung bean than in the cowpea varieties. This result was reiterated in the result of mineral analysis. The ash content of a food is an indication of the mineral contents of that food. For most of the minerals analyzed in this study, the mung bean seeds had significantly higher values ( $p \leq 0.05$ ) than the control cowpea samples (COT and IFbr), particularly for Mg, S, and Na, which showed significantly lower values ( $p \leq 0.05$ ) in the control samples compared to the mung bean samples. Among the mung bean accessions, MB-20 had highest values for Fe (4842.50 mg/kg). The values for Fe in MB-20, MB-21 and MB-13 were significantly higher ( $p \leq 0.05$ ) than value obtained for the two cowpea varieties, whereas all the mung bean accessions had better values than Ife brown cowpea variety (3100 mg/kg). The highest values for Zn were found in MB-5 and MB-13 (460.75mg/kg, 448.00mg/kg), while the least value was found in MB-21 (357.25mg/kg). The concentrations of zinc and iron in the mung bean seeds showed that consumption of 100 g of mung bean seeds will meet more than 100 % of the RDA for zinc and iron. The RDA for zinc is 6 mg/day, 8 mg/day and 11 mg/day for children, women and men respectively (Nazanin et al. 2013), whereas the value for iron is 10 mg/day, 8-15 mg/day, and 10-12 mg/day for children, women and men respectively (CDCP, 1998).

Table 4. Seed yield (kg/m<sup>2</sup>) of the twenty one (21) mung bean accessions at Ile-Ife early season, Ile-Ife late season and Kishi early season of 2022

Accession	Ile-Ife early season	Ile-Ife late season	Kishi early season	Across
MB-1	0.67def	0.012bcd	0.10b	0.26bcd
MB-2	1.38abcdef	0.008bcd	0.21ab	0.53abcd
MB-3	1.80abcd	0.040a	0.37ab	0.74ab
MB-4	1.07abcdef	0.030ab	0.45ab	0.52abcd
MB-5	1.15abcdef	0.003d	0.20ab	0.45abcd
MB-6	2.27ab	0.002d	0.22ab	0.83a
MB-7	1.63abcde	0.002d	0.30ab	0.65abc
MB-8	0.76cdef	0.001d	0.41ab	0.39bcd
MB-9	1.18abcdef	0.001d	0.24ab	0.47abcd
MB-10	0.16f	0.028abc	0.09b	0.09d
MB-11	1.07abcdef	0.004d	0.21ab	0.43abcd
MB-12	1.44abcdef	0.012bcd	0.27ab	0.57abc
MB-13	0.85cdef	0.005d	0.09b	0.32bcd
MB-14	2.01abc	0.018bcd	0.32ab	0.78ab
MB-15	2.28a	0.010bcd	0.34ab	0.88a
MB-16	1.32abcdef	0.005d	0.56a	0.63abc
MB-17	0.64def	0.006d	0.43ab	0.36bcd
MB-18	0.99bcdef	0.007cd	0.15ab	0.38bcd
MB-19	0.43ef	0.004d	0.15ab	0.19cd
MB-20	1.27abcdef	0.005d	0.11b	0.46abcd
MB-21	0.25f	0.014bcd	0.18ab	0.15cd
Mean±SE	1.20±0.10	0.010±0.002	0.25±0.03	0.49±0.04

Means with different alphabets within the same column are significantly different at  $p < 0.05$

Table 5. Proximate analyses and minerals composition of the selected mung bean and cowpea samples

Sample	Proximate analysis				
	CHO %	Protein %	Fat %	Fibre %	Ash %
MB-5	56.14±0.21a	19.57±0.23c	3.21±0.12b	4.25±0.11c	4.89±0.13a
MB-13	56.29±0.34a	19.64±0.31c	3.18±0.31b	4.17±0.31c	4.86±0.22a
MB-14	56.25±0.22a	19.29±0.22c	3.23±0.23b	4.27±0.61c	4.67±0.16b
MB-19	56.40±0.41a	19.48±0.11c	3.27±0.19b	4.23±0.42c	4.79±0.19a
MB-20	55.87±0.21b	20.16±0.14b	3.48±0.11b	4.15±0.16c	4.53±0.22b
MB-21	56.16±0.32a	19.79±0.24c	3.34±0.23b	4.21±0.29c	4.72±0.13b
COT	48.79±0.36c	24.49±0.23a	4.16±0.22a	6.81±0.19a	4.17±0.21c
IFbr	49.33±0.22c	24.78±0.22a	4.27±0.18a	5.69±0.11b	4.28±0.14c
Sample	Minerals composition				
	Mg (mgkg <sup>-1</sup> )	S (mgkg <sup>-1</sup> )	Na (mgkg <sup>-1</sup> )	Zn (mgkg <sup>-1</sup> )	Fe (mgkg <sup>-1</sup> )
MB-5	427.25±2.74b	556.37± 7.24d	1041.25±4.52c	460.75±4.27a	3622.50± 3.36c
MB-13	435.50±5.05a	651.36± 5.00c	1105.00±7.95a	448.00±4.12b	4695.00±16.47a
MB-14	405.25±4.77c	664.93± 5.70c	977.50 ± 7.79e	374.00±5.04d	3057.50±13.37f
MB-19	410.00±3.11c	712.43± 3.11b	1020.00±4.62d	360.75±3.65e	3252.50±10.43d
MB-20	402.75±4.26c	658.15 ± 1.62c	1062.50±7.45b	383.75±2.94d	4842.50±11.67a
MB-21	385.75±2.32e	1126.31±7.58a	998.75 ± 8.37d	357.25±6.11e	4737.50±12.42a
COT	395.00±2.12d	312.11 ± 5.75e	913.75 ± 7.39f	415.00±5.23c	4090.00±15.31b
IFbr	399.00±4.39d	271.14 ± 7.24f	807.50 ± 6.17g	415.75±4.32c	3100.00±13.34e

Values are means of three determinations ± SEM; Means with different alphabets within the same column are significantly different at  $p < 0.05$ ; COT = Cotonou; IFbr= Ife brown; CHO = Carbohydrate; SEM: Standard error of mean; Mg = Magnesium, S = Sulphur, Na = Sodium, Zn = Zinc, Fe = Iron

The sensory evaluation of Moin-moin (bean cake) samples prepared from the selected mung bean revealed that consumers preferred Moin-moin made from MB-20 over other mung bean samples, citing its superior color, appearance, flavor, texture, and taste (Table 5). The flavor, texture, and taste scores for most mung bean samples were significantly better ( $p \leq 0.05$ ) than the control samples (COT and IFbr). Notably, Moin-moin from MB-19 and MB-21 were the least accepted, while Moin-moin from other mung bean samples were well accepted and comparable to Ife brown. However, Moin-moin from

Cotonou (COT) was not well accepted. The overall acceptability of Moin-moin from MB-20 was higher, although not significantly different ( $p \leq 0.05$ ), from Ife-brown Moin-moin. The preference for Moin-moin made from mung bean seeds, particularly MB-20, can be attributed to their high flavonoid content, which is responsible for their desirable color, fragrance, and flavor characteristics. This finding supports earlier research by Ibeogu et al. (2021), who stated that Moin-moin from mung bean seeds scored higher than those from lablab and cowpea seeds due to their high flavonoid content.



Table 6. Sensory evaluation of Moin-moin (bean cake) prepared from the mung bean and cowpea samples

Sample	Colour (1-9)	Appearance (1-9)	Flavour (1-9)	Texture (1-9)	Taste (1-9)	Overall acceptability (1-9)
MB-5	6.86 ±0.1c	7.00 ±0.1b	6.86 ±0.3a	7.14 ±0.1a	6.57 ±0.2b	6.80 ±0.2b
MB-13	7.13 ±0.1b	7.00 ±0.1b	6.71 ±0.2b	7.00 ±0.1b	6.71 ±0.1a	6.80 ±0.2b
MB-14	6.71 ±0.1d	7.14 ±0.2a	6.42 ±0.2c	6.14 ±0.2d	6.57 ±0.2b	6.60 ±0.3b
MB-19	5.33 ±0.2f	5.00 ±0.1d	5.14 ±0.1d	4.86 ±0.3f	4.71 ±0.1c	4.00 ±0.1d
MB-20	7.43 ±0.1a	7.14 ±0.2a	6.86 ±0.1a	7.14 ±0.2a	6.71 ±0.2a	7.50 ±0.1a
MB-21	3.57 ±0.2g	3.57 ±0.1e	4.00 ±0.2f	4.50 ±0.3g	3.52 ±0.2e	4.20 ±0.2d
COT	5.86 ±0.2e	5.86 ±0.1c	5.00 ±0.1e	5.43 ±0.1e	4.57 ±0.1d	5.00 ±0.2c
IFbr	7.43 ±0.1a	7.14 ±0.2a	6.43 ±0.3c	6.71 ±0.2c	6.57 ±0.1b	7.20 ±0.2a

Values are means of 20 determinations ± SEM; Means with different alphabets within the same column are significantly different at  $p < 0.05$ ; COT = Cotonou; IFbr= Ife brown; SEM = Standard error of mean; (1-9): 1 for least and 9 for highest.

Previous research findings revealed that flavonoids concentration in mung bean seeds ranged between 125 – 352 mg QE / 100g seed (Wang et al. 2021), while that of cowpea ranged between 7.46- 23.95 mg QE / 100g seed (Sombie et al. 2018), which may explain the consumer preference for Moin-moin made from mung bean seeds.

## Conclusion

Adaptability and nutrient assessment of any introduced crop is vital in crop improvement. This research revealed that mung bean is well adapted to the southwest ecology of Nigeria, however, excessive rainfall limits its yield. Seed yield was better at Ile-Ife early season, which may be attributed to the slightly acidic nature of the soils and the moderate to high rainfall at the period which favored its growth. Mung bean has protein content level comparable to that of cowpea and is richer in some major mineral elements such as iron and zinc, and could therefore be included in household diets. MB-5, 13 and 20 are high in nutritional contents, while MB-3, 6, 14 and 15 had good yield performance across the study locations. These accessions could be selected for further breeding work.

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## Zinc and Phosphate Solubilizing by Rhizobacteria Promotes Lettuce (*Lactuca sativa* L.) Growth in Salty Conditions

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### ABSTRACT

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Due to climate change, the world is negatively affected by drought, temperature, salinity, and flood stress, leading to a significant decline in crop production. Lettuce is particularly significant when considering salt stress. To increase plant tolerance to salinity, various strategies are employed to support the development of agriculture. Rhizobacteria play a key role in regulating phosphorus (P) and zinc (Zn) homeostasis in plants. According to the study results, Rhizobium bacteria supported plant growth by improving the solubility of zinc and phosphate. These findings highlight the beneficial effects of plant growth-promoting rhizobacteria (PGPR) on the antioxidant system, which helps detoxify reactive oxygen species. The relationship between proline accumulation and antioxidant enzyme activities showed that PGPR inoculation enhanced the plant's defense mechanism against salt stress. In establishing this tolerance, increases in chlorophyll content, repair of membrane repair, and higher leaf relative humidity under salt stress were observed. PGPR also improved seedling height, diameter, and fresh and dry weight under stress by 70%, 51.4%, 55%, and 109%, respectively, due to the stress-mitigating effects of P and Zn. In conclusion, it is predicted that there will be a need to develop fertilization programs containing different rhizobacteria and Zn+P combinations. These programs would activate the antioxidant mechanism in saline soils, stabilize physiological processes, and positively impact plant growth.

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## Introduction

Lettuce (*Lactuca sativa* L.), a member of the *Asteraceae* (= *Compositae*) family, is cultivated worldwide and its leaves are commonly consumed as salad vegetables. Lettuce, which is an important food source for health, constitutes 3.41% of the total agricultural land in Türkiye and represents of 1.19% in vegetable production areas (TÜİK, 2019). According to TÜİK data, Türkiye produced 577,773 tons of lettuce in 2022. This highlights the significant contribution of lettuce to the overall vegetable production in the country.

Global warming, driven by climate change, represents a severe threat to the Earth's ecosystems (Borjas-Ventura et al., 2020). The main factor influencing agricultural productivity is the climate (Adams, 1998). One of the major global issues negatively affecting agricultural productivity in arid and semi-arid regions is soil salinity (El hasini et al., 2019). According to Oster and Jayawardane

(1998), soil salinization can impact surface water runoff, soil erosion, and seedling emergence. Salinity, for instance, can adversely effect on plant growth by hindering root penetration, reducing the plant's water-holding capacity, and disrupting the circulation of water and air in the soil. Soil osmotic stress initially increases as a result of salinity stress (Munns et al., 2008). A few minutes after salt accumulates in the root zone, the osmotic phase begins. In this phase, stomatal closure, an increase in leaf temperature, and restricted shoot elongation are the primary indicators in plants, due to the thick inner wall of the guard cells and the low soil water potential (Mukhopadhyay et al. 2021). Salt stress also induces oxidative stress by increasing reactive oxygen species (ROS), such as superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (OH<sup>\*</sup>) (Alscher et al., 1997; Mittler, 2002; Neill et al., 2002).

Plants have developed various defense mechanisms against ROS, with the antioxidant defense system being crucial. This system includes enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and ascorbate peroxidase (APX) activities which are effective in mitigating oxidative stress (Farooq et al., 2008). An increase in the activity of antioxidant enzymes in response to salt stress has also been reported in various studies (Unlukara et al., 2008; Hela et al., 2011; Sardar et al., 2023)

To improve plant tolerance to salinity, various strategies are employed for sustainable agriculture. One promising approach is microbial technology, specifically the use of plant growth-promoting rhizobacteria (PGPR). Historically, the concept of the rhizosphere and the role of PGPR in mitigating salt stress and enhancing plant health were highlighted by Hilter in 1904 (Al-Barakah et al., 2019; Zhang et al., 2019). The most researched PGPR species are *Bacillus* and *Pseudomonas*, which are well-known for their ability to enhance plant health by solubilizing zinc and phosphate (Ku et al., 2019; Desoy et al., 2020). Lettuce, in particular, benefits from phosphate fertilizers, which significantly increase yield and improve quality (Johnstone et al., 2005; Hoque et al., 2010). The application of phosphate-solubilizing PGPR has been shown to increase soil phosphate availability and plant yield (Chabot et al., 1996).

Zinc is also crucial for various physiological functions, including photosynthesis, chlorophyll content, phytohormone synthesis, protein synthesis, and growth rate, antioxidant activity (Mousavi et al., 2013). Zinc deficiency can result in stunted development, chlorosis, and an increased vulnerability to diseases and other stressors (Tavallali et al., 2010). Ensuring adequate zinc supply can enhance growth, chlorophyll content, photosynthesis, and stress tolerance. The use of zinc-solubilizing PGPR has been proposed as a means of enhancing crop development and bioenrichment (Vaid et al., 2014; Mumtaz et al., 2017; Hussain et al., 2020).

Additionally, photosynthesis, the synthesis of phytohormones, growth rate, chlorophyll content, protein synthesis, and antioxidant activity are all dependent on zinc (Mousavi et al., 2013). Tropical areas are known for their high prevalence of zinc deficiency, which can lead to chlorosis, stunted growth, and heightened vulnerability to diseases and other stressors (Tavallali et al., 2010). Ensuring an adequate supply of zinc can improve growth, chlorophyll content, photosynthesis, and stress tolerance. According to several studies (Vaid et al., 2014; Mumtaz et al., 2017; Hussain et al., 2020), applying zinc-solubilizing PGPR can enhance crop development and bioenrichment.

Phosphorus (P) is one of the key plant nutrients whose absorption, transport, and distribution in plants are negatively impacted by salinity-stress (Dey et al., 2021). Crop productivity is limited by low P availability mediated by salinity stress. It is often recommended to apply more P fertilizer to saline soils in order to control P deficits; however, in salt-affected soils, the low efficiency of available P fertilizer use limits P availability and P fertilizers can pose significant environmental risks (Dey et al., 2021). One of the most important PGPR in the salt-tolerant microbial communities is phosphate-solubilizing bacteria (PSB), which can produce a variety of metabolites that promote plant growth in saline conditions, such as phytohormone, siderophore, ACC

deaminase, and anti-phytopathogens, as well as solubilize Phosphate, which aid in plant growth and include Nitrogen.

In Türkiye, salinity issues in agricultural soils are exacerbated by factors such as saline irrigation water, low rainfall, poor soil management, high evaporation rates, and excessive chemical use. In this context, PGPR offer a promising strategy to enhance plant resistance and productivity in saline environments. This study investigates the impact of PGPR on the growth and yield of lettuce under varying salinity conditions, emphasizing its potential to mitigate salinity stress and promote sustainable agriculture.

## Materials and Methods

### *The Experiment's Variety and Characteristics of Lettuce*

The experiment used the Lital lettuce variety from Hazar Seed Company's. It is a type of lettuce with a core and is resistant to heat and gets up late. The average head weight is 800 – 1100 gr. Harvest period is 55 – 70 days. The leaf color is light green, reminiscent of light yellow. The leaves are erect, brittle and broad. Leaf margins are wavy and slightly toothed.

### *Plants, Cultivation, and Treatments*

This study was conducted in research greenhouses and as a pot experiment during the fall of 2022 at Mersin University Silifke Vocational School. Hybrid seeds of lettuce variety were used in the study. The trial planned as four replications according to the randomized blocks trial design; 7 applications including salt and nutrient solutions including control application and each application consisted of 40 plants. In the experiment, it was tried to determine the resistance of lettuce seedlings to drought stress, different salt concentrations and nutrient applications by using rhizobacteria, known to be effective in increasing stress tolerance in plants. Normal tap water was applied to the control plants. The seeds covered with bacteria in the laboratory were manually sown in seedling vials filled with the medium used in seedling production (peat + perlite + vermiculite) and then watered with appropriate methods. On the 21st day of the germination period, lettuce seedlings were transplanted into 750 ml pots in the same medium and were watered for one week for adaptation and necessary development. After this stage, salt and nutrient solutions were applied 5 times at intervals of 2 days and the experiment was terminated after 10 days. For measurements, 8 lettuce seedlings representing the average were selected from each plot and the average was taken. The greenhouse temperature during the experiment was conducted was 20-28 °C, with a humidity level of 65%. The fertilizers used in the experiment included a single dose of rhizobacteria, salt (NaCl) concentrations, 0 (control), 75 mM, 150 mM, Zinc (ZnSO<sub>4</sub>); 5mg L<sup>-1</sup> and phosphorus (P<sub>2</sub>O<sub>5</sub>); It was determined as 300 mg.kg<sup>-1</sup> P and added to Hoagland nutrient solution for general nutrition of plants (Hothem et al., 2003).

### *Disinfection of Seeds to Be Used in the Study*

Lettuce seeds were soaked in 70% ethanol for 5 minutes and then washed with distilled water (sdH<sub>2</sub>O). Then, they were then treated with 5% NaOCl for 3 minutes and at the end of the time, the seeds were washed with sdH<sub>2</sub>O. After the disinfection process was completed, the seeds were filtered and left to dry on blotting paper.

### Isolation and Purification of Microorganisms

The culturable bacterial population was isolated from the rhizospheric soil using a serial dilution approach. One gram of soil was combined with nine milliliters of deionized water in a test tube and vigorously stirred with a vortex mixer. Following the method of Johnson and Curl (1972), dilutions were created up to  $10^{-5}$ . On Nutrient Agar (HiMedia®) plates, 100 µl of each dilution was applied, and the plates were incubated for 24 hours at 30 °C. Then, using traits including color, texture, and shape, bacterial colonies were detected, enabling the computation of colony-forming units (CFU) and occurrence percentages. The CFU per gram of dry soil was determined using the formula:  $\text{CFU g}^{-1} \text{ dry soil} = (\text{Mean number of colonies} / \text{Dry weight of soil}) \times \text{Dilution factor}$ . For further purification, isolates were streaked on nutrient agar plates, and a single, well-isolated colony was selected and re-streaked to achieve a pure culture (Zhou, 1987).

### Bacteria Used in The Study and Their Properties

YÖ41: Bacillus cereus GC subgroup A

- MIS similarity index (%): 78
- Nitrogen-fixing properties: Strong
- Phosphorus and zinc dissolving properties: Strongly positive
- Isolated from plant roots of Thymus vulgaris from rhizospheric soil in Türkiye

### Phosphate Solubilization

The isolate's dissolution capacity was evaluated using the Tricalcium Phosphate (TCP) method as described by Wahyudi et al. (2011). In short, point inoculation of cultures was performed on Pikovskaya Agar (HiMedia®) plates, followed by incubation for 5 days at 30 °C. The appearance of a clear halo around the colonies indicated phosphate solubilization activity. Phosphate solubility was quantified as follows: fertile isolates were added to 100 ml of Pikovskaya solution in 250 ml Erlenmeyer flasks, along with 0.5 g of TCP as an insoluble phosphate source. The CFU per gram of dry soil was determined using the formula:  $\text{CFU g}^{-1} \text{ dry soil} = (\text{Mean number of colonies} / \text{Dry soil weight}) \times \text{Dilution factor}$ . The flasks were sterilized, and the medium was adjusted to an initial pH of 6.0. The isolates were then inoculated, and the flasks were incubated for 10 days at 27 °C. Each day, duplicate samples were taken, and the medium was filtered using Whatman 42 filter papers. The chlorostannous-reduced molybdophosphoric acid blue method was employed to measure the  $\text{P}_2\text{O}_5$  content of the filtrates, with absorbance recorded at 700 nm using an Eppendorf BioSpectrometer. For zinc solubilization, the isolates were incubated for 48 hours in a modified PVK medium supplemented with 0.1% insoluble zinc compounds ( $\text{ZnO}$ ,  $\text{ZnCO}_3$ ,  $\text{ZnS}$ ). Zinc dissolution was visually assessed by observing the clearance of the opaque medium, and the diameter of the clear zone was measured to calculate the Zinc Solubilization Index (ZSI) (Bapiri et al., 2012). Siderophore production was evaluated using Chrome Azurol S (CAS) agar (Schwyn et al., 1987). To assess the salinity tolerance (NaCl stress), selected isolates were grown in nutrient broth (NB) containing varying concentrations of NaCl (1.2% and 3% w/v) and their

growth was monitored by measuring absorbance at 600 nm (Mahajan et al., 1920). Nitrogen fixation ability was determined by growing the isolate in nitrogen-free solid malate (Nfb) medium at 33 °C for 24 hours (Okon et al., 1977). Bacterial growth indicated nitrogen-fixing capacity. Quantitative assessment of nitrogen fixation by the YO41 isolate was performed using the acetylene reduction assay (ARA) (Hardy et al., 1968), a reliable method to evaluate the activity of nitrogenase, the enzyme responsible for nitrogen fixation. Nitrogenase reduces acetylene gas to ethylene, which is measured using flame ionization gas chromatography to determine nitrogen fixation rates.

### Preparation of Bacterial Solutions and Vaccination Process

In the seed coating application, a bacterial suspension was first prepared. At this stage, bacteria preserved in 30% glycerol and liquid medium (Lauryl Broth) at -80 °C were seeded on Nutrient Agar solid medium. After the planted petri dishes were incubated for 48 hours in an incubator set at 27 °C, a loopful of each bacteria was taken and transferred to flasks containing 250 ml of Nutrient Broth. The broths contaminated with bacteria were incubated for 24 hours at 150 rpm in a shaker set at 27 °C for aerobic growth of bacteria. The prepared bacterial suspensions were diluted with sterile distilled water and the final concentration was adjusted to 107 cfu ml<sup>-1</sup> by spectrophotometric measurement (Turan et al. 2014).

### Covering Lettuce Seeds with Bacteria

Disinfected lettuce seeds were placed in bacterial suspensions with a concentration of 10<sup>7</sup> cfu/ml and left to incubate in a shaker at 140 rpm for 2 hours. At the end of the incubation period, the seeds were filtered and treated with sucrose to ensure the adhesion of the bacteria. The types of treatment to be applied in the trial is given in (Table 1)

Table 1. Trial subjects

1	Control (0 mM NaCl)
2	75 mM NaCl
3	150 mM NaCl
4	YÖ41
5	P +Zn+YÖ41
6	P+Zn+YO41+75 mM NaCl
7	P+Zn+YO41+150 mM NaCl

### Membrane Permeability (% EC)

Sample plant seedlings with 5-6 true leaves representing the mean were harvested and three samples of 1 cm diameter were taken from the leaves of each in the laboratory. These samples were washed with distilled water and placed in brown glass bottles, and the analyzes were repeated 3 times by adding 10 ml of distilled water. The prepared bottles were kept in the shaker for 24 hours and after that time, the solutions in the bottles were poured into the tubes and the EC1 value was measured in the EC meter. Then, the solutions were poured back into the bottles and kept in an autoclave at 120 °C for 20 minutes, and the EC value was calculated from the formula  $\text{EC1} / \text{EC2} \times 100$  by measuring the EC2 value at room temperature (Lutts et al., 1996).

### **Relative Water Content (% RWC)**

The turgor weights of the leaf samples were determined after they were immersed in distilled water for four hours to measure their relative water content (RWC). Three 1 cm<sup>2</sup> leaf discs were obtained to check for membrane damage. The discs were then shaken in closed vials at 25 °C for 24 hours after adding 10 mL of water. The EC values were calculated right away. EC measurements were taken again after the same samples were autoclaved for 20 minutes at 120°C and allowed to cool to 25 °C (Lutts et al., 1996).

### **Chlorophyll Amount (SPAD)**

The middle of the upper leaves of 4 randomly selected plants in each repetition for each application during the seedling period (5-6 leaves). With the SPAD device (SPAD-502 Chlorophyll) measuring devices; Konica Minolta, Tokyo, Japan) relative chlorophyll content was measured.

### **Antioxidant Enzyme Activity Assay**

Fresh lettuce leaves were processed following the protocol outlined by Angelini and Federico (1989). The resulting samples were analyzed at 560 nm, as described by Agarwal and Pandey (2004), to determine SOD activity by measuring the enzyme content responsible for inhibition. CAT activity was assessed using the method of Havir and McHale (1987), with absorbance changes recorded at 240 nm. POD activity was measured at 470 nm, based on the procedure established by Chance (1955). APX activity was determined at 290 nm, based on procedure established by Chaoui (1997).

### **Proline Analysis (µmol/g fresh weight)**

Proline levels were quantified using a spectrophotometric approach based on the acid-ninhydrin method, as described by Bates et al. (1973).

### **Dry Mass**

After harvesting, the leaves from the sample plants were placed in an oven at 65 °C for 48 hours. When the last two weight measurements were equal, indicating that the leaves were completely dried, they were removed from the oven, and their dry weights were calculated (Kacar, 1972).

### **Statistical Analysis**

The effects of varying irrigation levels and intervals on the yield and quality components were examined using variance analysis at two different probability levels (0.05 and 0.01), and the Duncan test was used to compare the averages. The statistical package IBM SPSS 23 (IBM Statistics for Windows, Version 23) was used to calculate all statistical values. Regression analysis was also used to determine the water-yield relationships.

## **Results**

In the control group, the plant root length is 6.2 cm. In plants treated with 75 mM NaCl, the root length decreased to 5.7 cm. In plants treated with 150 mM NaCl, the root length decreased to 4.6 cm, indicating that root length decreases as salt concentration increases. The YO41 treatment increased root length to 7 cm. The P+Zn+YO41 treatment increased root length to 10.2 cm, achieving the highest value. The P+Zn+YO41 treatment with salt resulted in a root length of 9.7 cm with 75 mM NaCl and

7.4 cm with 150 mM NaCl. The highest root length was obtained with the P+Zn+YO41 treatment, demonstrating that this combination significantly enhances plant root development (Tables 2).

In the control group, the plant diameter is 4.2 cm. The plant diameter decreased to 3.7 cm with 75 mM NaCl and to 3.1 cm with 150 mM NaCl. The YO41 treatment increased the plant diameter to 5.6 cm. The P+Zn+YO41 treatment achieved the highest plant diameter of 6.2 cm. The P+Zn+YO41 treatment with salt resulted in a plant diameter of 5.6 cm with 75 mM NaCl and 4.7 cm with 150 mM NaCl. Overall, the P+Zn+YO41 treatment increased plant diameter, and this increase continued despite salt stress (Tables 2).

In the control group, the number of leaves is 7.3. In plants treated with 75 mM NaCl, the number of leaves decreased to 7, and to 5.5 with 150 mM NaCl. The YO41 treatment increased the number of leaves to 8.5. The P+Zn+YO41 treatment achieved the highest number of leaves, with 10 leaves. The P+Zn+YO41 treatment with salt resulted in 9 leaves with 75 mM NaCl and 8.9 leaves with 150 mM NaCl. The P+Zn+YO41 combination maintained the highest number of leaves, promoting plant growth (Tables 2).

In the control group, the leaf width is 3.5 cm. The leaf width decreased to 3.1 cm with 75 mM NaCl and to 2.9 cm with 150 mM NaCl. The YO41 treatment increased leaf width to 4.9 cm. The P+Zn+YO41 treatment increased leaf width to 4.7 cm. The P+Zn+YO41 treatment with salt resulted in a leaf width of 4.4 cm with 75 mM NaCl and 3.6 cm with 150 mM NaCl. The P+Zn+YO41 combination significantly increased leaf width (Tables 2).

In the control group, the root length is 7.7 cm. The root length decreased to 6.9 cm with 75 mM NaCl and to 4.9 cm with 150 mM NaCl. The YO41 treatment increased root length to 7.8 cm. The P+Zn+YO41 treatment increased root length to 8.4 cm, achieving the highest value. The P+Zn+YO41 treatment with salt resulted in a root length of 7.6 cm with 75 mM NaCl and 6.3 cm with 150 mM NaCl. The P+Zn+YO41 combination significantly increased root length and supported root development despite salt stress (Tables 2).

The P+Zn+YO41 combination significantly enhanced plant growth and protected the plant against salt stress. Notable increases were observed in root length, plant diameter, and number of leaves. As salt concentration increased, a general decrease in plant growth was observed, but the P+Zn+YO41 treatment largely compensated for this decrease. These data suggest that the P+Zn+YO41 treatment could be an effective method for improving plant growth and managing salt stress (Tables 2).

In the control group, the fresh and dry weights of the plants were 3.8 g and 0.7 g, respectively, and the fresh and dry weights of the roots were 1 g and 0.27 g, respectively. In plants treated with 75 mM NaCl, the fresh and dry weights of the plants decreased to 3.2 g and 0.6 g, respectively, and the fresh and dry weights of the roots decreased to 0.71 g and 0.21 g, respectively. This indicates that salt stress reduces both plant and root weights. In plants treated with 150 mM NaCl, the fresh and dry weights of the plants decreased to 2.7 g and 0.5 g, respectively, and the fresh and dry weights of the roots decreased to 0.62 g and 0.16 g, respectively. This shows that as salt concentration increases, plant and root weights decrease further (Table 3).

Table 2. The effects of P, Zn and PGPR (YO41) applications on lettuce seedlings exposed to salt stress on seedling growth parameters

Treatments	Plant Rootless Length (cm)	Plant Diameter (cm)	Number Of Leaves (Plant/Piece)	Leaf Width (cm)	Root Length (cm)
Control	6.2 ± 0.2cd	4.2 ± 0.1bc	7.3 ± 0.5c	3.5 ± 0.1b	7.7 ± 0.4ab
75 mM NaCl	5.7 ± 0.2de	3.7 ± 0.1c	7 ± 0.4c	3.1 ± 0.1b	6.9 ± 0.2bc
150 mM NaCl	4.6 ± 0.2e	3.1 ± 0.2d	5.5 ± 0.3d	2.9 ± 0.2b	4.9 ± 0.2d
YO41	7 ± 0.3bc	5.6 ± 0.2a	8.5 ± 0.3b	4.9 ± 0.1a	7.8 ± 0.3ab
P+Zn+YO41	10.2 ± 0.7a	6.2 ± 0.2a	10 ± 0.4a	4.7 ± 0.3a	8.4 ± 0.4a
P+Zn+YO41+75 mM NaCl	9.7 ± 0.5a	5.6 ± 0.2a	9 ± 0.4ab	4.4 ± 0.5a	7.6 ± 0.3ab
P+Zn+YO41+150 mM NaCl	7.4 ± 0.5b	4.7 ± 0.3b	8.9 ± 0.5b	3.6 ± 0.2b	6.3 ± 0.3c
Average	7.25	4.73	6.76	3.87	7.08

\* When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

Table 3. The effects of P, Zn and PGPR (YO41) applications on lettuce seedlings exposed to salt stress on seedling growth parameters

Treatments	Plant Fresh Weight (g)	Plant Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Control	3.8 ± 0.2bcd	0.7 ± 0b	1 ± 0.1ab	0.27 ± 0d
75 mM NaCl	3.2 ± 0.2de	0.6 ± 0b	0.71 ± 0.1bc	0.21 ± 0de
150 mM NaCl	2.7 ± 0.2e	0.5 ± 0c	0.62 ± 0c	0.16 ± 0e
YO41	4.2 ± 0.2bc	0.9 ± 0.1a	0.84 ± 0.2bc	0.43 ± b
P+Zn+YO41	4.8 ± 0.3a	1 ± 0.1a	1.2 ± 0a	0.46 ± 0a
P+Zn+YO41+75 mM NaCl	4.4 ± 0.1ab	1 ± 0.1a	1.1 ± 0.1ab	0.44 ± 0b
P+Zn+YO41+150 mM NaCl	3.7 ± 0.2dc	0.7 ± 0.1b	0.8 ± 0.1bc	0.23 ± 0c
Average	3.82	0.77	0.79	0.24

When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

The YO41 treatment increased the fresh and dry weights of the plants to 4.2 g and 0.9 g, respectively, and the fresh and dry weights of the roots to 0.84 g and 0.43 g, respectively. This demonstrates that YO41 supports plant growth. The P+Zn+YO41 treatment increased the fresh and dry weights of the plants to 4.8 g and 1 g, respectively, and the fresh and dry weights of the roots to 1.2 g and 0.46 g, respectively, achieving the highest values. This combination significantly enhances plant growth (Table 3).

In plants treated with P+Zn+YO41+75 mM NaCl, the fresh and dry weights of the plants were 4.4 g and 1 g, respectively, and the fresh and dry weights of the roots were 1.1 g and 0.44 g, respectively. This indicates that despite salt stress, this combination supports plant growth. In plants treated with P+Zn+YO41+150 mM NaCl, the fresh and dry weights of the plants were 3.7 g and 0.7 g, respectively, and the fresh and dry weights of the roots were 0.8 g and 0.23 g, respectively. This shows that even at high salt concentration, the combination helps maintain plant growth to some extent (Table 3).

Overall, the highest plant and root weights were obtained with the P+Zn+YO41 combination. Salt stress was observed to reduce plant and root weights, while YO41 and P+Zn+YO41 treatments mitigated these adverse effects (Table 3).

The table 4 presents the impact of P, Zn, and PGPR (YO41) applications on the antioxidant levels of lettuce seedlings under salt stress. SOD, CAT, POD, and APX activities were measured in enzyme units (EU) per gram of sample.

In the control group, SOD, CAT, POD, and APX activities were 116.4 EU g<sup>-1</sup>, 324 EU g<sup>-1</sup>, 57.5 EU g<sup>-1</sup>, and 8.2 EU g<sup>-1</sup>, respectively. Treatment with 75 mM NaCl

decreased these activities to 100.2 EU g<sup>-1</sup>, 293.9 EU g<sup>-1</sup>, 58.9 EU g<sup>-1</sup>, and 6 EU g<sup>-1</sup>, respectively. Under 150 mM NaCl, the activities further decreased to 97.8 EU g<sup>-1</sup>, 237.6 EU g<sup>-1</sup>, 55.7 EU g<sup>-1</sup>, and 4.8 EU g<sup>-1</sup>, respectively (Table 4).

YO41 treatment significantly increased all enzyme activities, with SOD at 231.8 EU g<sup>-1</sup>, CAT at 517.8 EU g<sup>-1</sup>, POD at 62.1 EU g<sup>-1</sup>, and APX at 13 EU g<sup>-1</sup>. P+Zn+YO41 treatment further increased these activities to 249.9 EU g<sup>-1</sup>, 571.2 EU g<sup>-1</sup>, 118.3 EU g<sup>-1</sup>, and 15.3 EU g<sup>-1</sup>, respectively (Table 4).

Combining YO41, P, and Zn with 75 mM NaCl resulted in intermediate activities, with SOD at 194.1 EU g<sup>-1</sup>, CAT at 497 EU g<sup>-1</sup>, POD at 102.9 EU g<sup>-1</sup>, and APX at 10.4 EU g<sup>-1</sup>. Similarly, with 150 mM NaCl, the activities were SOD 130.1 EU g<sup>-1</sup>, CAT 315.7 EU g<sup>-1</sup>, POD 101.7 EU g<sup>-1</sup>, and APX 7.2 EU g<sup>-1</sup>. Overall, application of YO41, P, and Zn, alone or combined, increased antioxidant enzyme activities in lettuce seedlings under salt stress, indicating a protective effect against oxidative stress (Table 4).

The table 5 presents the effect of P, Zn, and PGPR (YO41) applications on some physiological parameters of lettuce seedlings under salt stress. The parameters measured are membrane damage (%), chlorophyll ratio (%), relative humidity (%), and proline content (µg g<sup>-1</sup>).

In the control group, the values for membrane damage, chlorophyll ratio, relative humidity, and proline content were 33.7%, 41.5%, 73%, and 170 µg g<sup>-1</sup>, respectively. Treatment with 75 mM NaCl increased membrane damage to 64.1%, decreased chlorophyll ratio to 34.9%, decreased relative humidity to 58.7%, and decreased proline content to 110.6 µg g<sup>-1</sup>. Under 150 mM NaCl, the values further increased to 71.4%, 33.1%, 54.5%, and 106 µg g<sup>-1</sup>, respectively (Table 5).



Table 4. Effects of P, Zn and PGPR (YO41) applications on antioxidant levels of lettuce seedlings exposed to salt stress

Parameters	SOD (EU g <sup>-1</sup> )	CAT (EU g <sup>-1</sup> )	POD (EU g <sup>-1</sup> )	APX (EU g <sup>-1</sup> )
Control	116.4 ± 6.8cd	324 ± 15.4c	57.5 ± 1.5c	8.2 ± 0.5d
75 mM NaCl	100.2 ± 7.3d	293.9 ± 5.2c	58.9 ± 1.4c	6 ± 0.3e
150 mM NaCl	97.8 ± 2.3d	237.6 ± 9.7d	55.7 ± 1.6c	4.8 ± 0.2f
YO41	231.8 ± 6.6a	517.8 ± 14.1b	62.1 ± 2c	13 ± 0.6b
P+Zn+YO41	249.9 ± 6.2a	571.2 ± 4.1b	118.3 ± 5.4a	15.3 ± 0.2a
YO41+P+Zn+75 mM NaCl	194.1 ± 9b	497 ± 14.6b	102.9 ± 4.1b	10.4 ± 0.3c
YO41+P+Zn+150 mM NaCl	130.1 ± 10.3c	315.7 ± 13.1c	101.7 ± 5.6b	7.2 ± 0.3d
Average	160.16	393.8	79.59	9.34

When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

Table 5. The effect of P, Zn and PGPR (YO41) applications on some physiological parameters of lettuce seedlings exposed to salt stress.

Parameter	Membrane Damage (%)	Chlorophyll Ratio (%)	Relative Humidity (%)	Prolin (µg g <sup>-1</sup> )
Control	33.7 ± 1.1d	41.5 ± 2.1bc	73 ± 0.1b	170 ± 6.6c
75 mM NaCl	64.1 ± 3.1b	34.9 ± 0.1d	58.7 ± 1.5d	110.6 ± 5.9d
150 mM NaCl	71.4 ± 2.2a	33.1 ± 0.1d	54.5 ± 0.1e	106 ± 3.4d
YO41	34.9 ± 0.1d	43.6 ± 1.3bc	71.8 ± 2.2ab	315.5 ± 9.8a
P+Zn+YO41	32.5 ± 0.1d	53.2 ± 1.9a	77.3 ± 0.1a	344.7 ± 9.3a
YO41+P+Zn+75 mM NaCl	56.6 ± 1.5	44.9 ± 0.1b	69.6 ± 0.6b	330.2 ± 13.4b
YO41+P+Zn+150 mM NaCl	61.7 ± 1.9	40.1 ± 0.5d	64.6 ± 0.1c	160 ± 6.2c
Average	50.7	41.61	67.07	219.57

When the columns are examined from top to bottom, the averages containing the same letter are not statistically different according to the Duncan (p = 0.05) test.

YO41 treatment reduced membrane damage to 34.9%, increased chlorophyll ratio to 43.6%, slightly decreased relative humidity to 71.8%, and significantly increased proline content to 315.5 µg g<sup>-1</sup>. P+Zn+YO41 treatment further reduced membrane damage to 32.5%, significantly increased chlorophyll ratio to 53.2%, slightly increased relative humidity to 77.3%, and significantly increased proline content to 344.7 µg g<sup>-1</sup> (Table 5).

Combining YO41, P, and Zn with 75 mM NaCl resulted in moderate changes in the parameters, with membrane damage at 56.6%, chlorophyll ratio at 44.9%, relative humidity at 69.6%, and proline content at 330.2 µg g<sup>-1</sup>. Similarly, with 150 mM NaCl, the changes were membrane damage at 61.7%, chlorophyll ratio at 40.1%, relative humidity at 64.6%, and proline content at 160 µg g<sup>-1</sup> (Table 5).

Overall, the application of P, Zn, and YO41, either alone or in combination, had varying effects on the physiological parameters of lettuce seedlings under salt stress, with significant improvements in most parameters observed with the combined treatment (Table 5).

## Discussion

Salinity is a critical abiotic stress factor that significantly affects plant productivity and quality. Plants under salinity stress exhibit notable symptoms, including reduced turgor pressure, stunted leaf growth, and decreased photosynthesis rates. Salinity impairs growth, yield, and quality by disrupting essential metabolic processes in plants (Alp and Kabay, 2019; Kabay, 2019; Yılmaz et al., 2011). The increased severity of salinity also leads to mechanical, metabolic, and oxidative damage. In our study, we observed a marked decrease in lettuce growth as salinity stress intensified. Specifically, growth parameters such as root length, plant diameter, number of leaves, leaf width, plant fresh and dry weight, and root fresh and dry

weight were significantly reduced under higher salt concentrations (Tables 2, 3). These findings are consistent with those of Ouhammadou et al. (2022), who reported that salinity adversely affected growth and physiological traits. However, their study also noted that compost application and mycorrhizal colonization under 100 mM NaCl positively influenced growth parameters.

Salinity negatively impacts physiological parameters, potentially reducing crop yields through three primary mechanisms: degradation of the thylakoid membrane, a decrease in photosynthetic efficiency, and a subsequent decline in plant growth (Baslam et al., 2020). Growth inhibition under salinity stress is commonly linked to increased osmotic pressure, nutrient deficiencies, and damage to physiological mechanisms (Alqarawi et al., 2014). Our results showed that PGPR inoculation improved root length in lettuce (Table 2), with plant growth enhancing upon application of PGPR isolates combined with P+Zn. However, bacterial activity decreased with higher NaCl concentrations, indicating a negative effect of salt on bacterial cell numbers. The observed growth promotion can be attributed to improved P-solubility and Zn uptake by the bacterial isolates, similar to findings by Qin et al. (2017).

During the seedling stage, plants are particularly vulnerable to salt stress, with parameters such as plant height, diameter, and fresh and dry weights responding to salt levels. Stress conditions lead to reduced root growth and stem elongation due to insufficient water uptake. Advanced salt applications further reduce stem dimensions and can lead to seedling mortality depending on severity. Significant reductions in dry matter and fresh weight under stress have been reported for various plants (Irshad et al., 2002; Ghoulam et al., 2002; Dasgan et al., 2002). Nevertheless, our study found that YO41, P, and Zn applications mitigated salt stress effects. Salt stress induces oxidative stress by altering water potential, causing

physiological drought, and imbalancing mineral elements, which triggers the release of reactive oxygen species (ROS) that inhibit growth and metabolism (Evelin et al., 2012). ROS accumulation also damages cell membranes. Plants counteract ROS with various defense systems involving both enzymatic (e.g., SOD and CAT) and non-enzymatic antioxidants (Navarro-León et al., 2020).

Using plant growth-promoting rhizobacteria (PGPR) offers an eco-friendly solution to combat abiotic stresses, including salinity (Glick and Bashan, 1997). The combined treatment with YÖ41, P, and Zn significantly enhanced enzyme activities even under NaCl stress, suggesting protective effects of these treatments. Similar findings were reported by Han and Lee (2005), where PGPRs like *Serratia* sp. and *Rhizobium* sp. reduced APX and GR activity under increasing salinity stress. Upadhyay et al. (2012) observed reduced antioxidant enzyme activities (APX, CAT, GR) in wheat leaves treated with PGPR strains under salinity stress. Kadmiri et al. (2018) noted increased antioxidant enzyme levels with phosphate-solubilizing and IAA-producing *Pseudomonas fluorescens* and *Azospirillum brasilense* in saline soil. Kallala et al. (2018) found that rhizobial inoculants alleviated stress in legumes by inducing antioxidant enzymes. Increased carbohydrate content in plants treated with *R. radiobacter* LB2 was also observed, attributed to enhanced photosynthesis (Kang et al., 2014). Our study supports these findings, demonstrating that PGPR positively impacts enzyme activities and stress resistance, aligning with existing literature.

## Conclusion

Salinity poses a significant challenge to Turkish agricultural lands. The ability of plant growth-promoting rhizobacteria (PGPR) to thrive in saline soils offers a distinct advantage. The bacterial strain YO41, known for its role in enhancing plant defense mechanisms under stress, mitigates stress effects by improving the solubility of nutrients such as phosphorus (P) and zinc (Zn). During the development of salt tolerance, YO41 was found to increase chlorophyll content, repair membrane damage, enhance leaf relative humidity, proline content, and antioxidant capacity in response to salt stress. PGPR is believed to support plant growth through mechanisms such as auxin and siderophore production, with phosphorus solubilization balancing the antagonistic effects between soluble Zn and P.

As salinity levels increased, there was a noticeable decline in the fresh and dry weights of lettuce, as well as the fresh and dry weights of roots. Additionally, root height, diameter, leaf width, and root length were adversely affected. Plants subjected to 150 mM NaCl treatment ceased development after a certain period, whereas seedling growth was slower in treatments with YO41+P+Zn+150 mM NaCl. In contrast, control plants exhibited better growth compared to those treated with 150 mM NaCl. The combination of YO41+75 mM NaCl+P+Zn was found to alleviate the impact of salt stress. Notably, treatments with YO41+P+Zn+75 mM NaCl led to increased levels of SOD, CAT, APX, POD, and proline, whereas these components remained low in treatments with YO41+P+Zn+150 mM NaCl. Further research is needed to explore the effects of beneficial mineral applications in mitigating salt stress.

## Declarations

### Author Contribution

**Y.Ç.:** Investigation; conceptualization; funding acquisition; writing – original draft; methodology; validation; writing– review and editing.

**A.Ö.:** Investigation; writing – review and editing; methodology

**N.K.:** Conceptualization; methodology; writing – review and editing.

### Conflict of Interest Statement

The authors declare no conflict of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Comparison of Different Twin Row and Narrow Row Sowing Methods in Corn A Clay-Textured Soil

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### ABSTRACT

Narrow and twin row sowing methods are agronomic applications that aim to increase plant growth and yield by expanding the plant's growing area but, they are highly affected by environmental conditions. The aim of this study is to determine the applicability of different narrow row and twin row sowing methods in clay textured soil in main crop cultivation of corn, which an important grain. The study was carried out Diyarbakır province in the Southeastern Anatolia Region of Türkiye in 2016 and 2017. In the study carried out with two different corn varieties, line abreast narrow row, diagonal narrow row, line abreast twin row, diagonal twin row, single row 1 (70 cm row spacing, 20 cm intra-row spacing) and single row 2 (70 cm row spacing, 12.5 cm intra-row spacing) applications were tried. ADA 351 and Sakarya corn varieties were used in the study. As a result it was determined that line abreast narrow row, diagonal narrow row, line abreast twin row and diagonal twin row applications are not suitable for corn cultivation. The highest grain yield was obtained from single row and 12.5 cm intra-row spacing application.

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## Introduction

Corn is a plant that is important for both human and animal nutrition and has a wide range of uses (Öztürk et al. 2019; Şahin, 2001; Özcan 2009; Prasanna 2012; Akkurt & Demirbaş 2021). Also it is an important industrial raw material due to its wide range of uses. Corn is the most produced grain in the world (Murdia et al. 2016; Özcan 2009; Turhal 2021). The yield of corn is about double that of wheat and barley (Özcan 2009). According to the data of the Turkish Statistical Institute for 2023, considering the total production amount in Türkiye, corn ranks third among cereals. In 2023, an average of 9 000 000 tons of corn was produced. The highest production among the regions is made in the Southeastern Anatolia Region. 24.90% of the corn cultivation area and 21.16% of the production amount are in this region in 2022. The corn plant is grown in many

regions of Türkiye due to its adaptability to climatic conditions. Due to the suitable climatic conditions, corn can be grown as both the main crop and the second crop in the Southeastern Anatolia Region. Corn production has increased considerably with increasing irrigated areas within the scope of the GAP Project (Akkurt & Demirbaş 2021). It is thought that this rate will increase with more irrigated areas.

As in all cultivated plants, the success of cultivation in corn is directly related to the appropriate cultivation techniques for the region where the cultivation will be carried out. In the production of corn, it is necessary to fully implement the cultivation technique, use the suitable varieties and suitable seeds (Şahin 2001).

Significant advantages of twin row and narrow row sowing technique are known in corn. Some of these include increasing irrigation efficiency by reducing evaporation loss, increasing the chance of controlling weeds by increasing the plant population, and increasing the yield by creating a life triangle for the plant. In sowing made in narrow row spacing, the amount of seed deviation from row as well as the distance between plants in the row center affects the living area and therefore the seed distribution in the horizontal plane (Karayel 2010). However, it is known that narrow and twin row planting methods are highly affected by environmental conditions. Greveniotis et al. (2019) reported that environmental conditions can distort all other effects for 11 of the 12 traits studied, as a result of a 4-year study conducted with different plant populations in single and twin rows in corn. Therefore, positive results may not be obtained from these methods in every region.

The application of narrow row and twin row in corn has been tried in some regions in the world and in Türkiye. However, different shapes of narrow row and twin row spacing such as diagonal and line abreast have not been compared with single row spacing. A twin row corn study was conducted in Hatay province in Türkiye (Gözübenli et al. 2004). There are studies conducted under the conditions of the main crop in Konya province (Kırılmaz 2018) and the second crop in Sanliurfa province (Koşar 2015). However, there is no study conducted under main crop conditions in the Southeastern Anatolia Region, where there is a large corn production. For this reason, this study was planned to determine the performance of different twin and narrow row sowing methods in corn under the main crop cultivation conditions of the Southeastern Anatolia Region for two years.

## Materials and Methods

### Soil Properties of the Trial Area

The soil properties of the area where the trial was conducted were determined. As can be seen from Table 1,

Table 1. Soil properties of the trial area.

Soil Properties	Units	Contents
Texture	-	Clay
Total salt	%	0.034
pH	-	8.10
Lime content (CaCO <sub>3</sub> )	%	8.64
Available P <sub>2</sub> O <sub>5</sub>	kg ha <sup>-1</sup>	28.60
Available K <sub>2</sub> O	kg ha <sup>-1</sup>	1421.80
Organic matter	%	0.98
Field capacity	%	49.06
Wilting point	%	21.66
Bulk density	g cm <sup>-3</sup>	1.47

Table 2. Meteorological data of long-term (1929-2023) averages of Diyarbakir province

	Months							
	April	May	June	July	August	September	October	December
Average Temperature (°C)	13.8	19.3	26.1	31.0	30.5	25.1	17.6	9.8
Average Highest Temperature (°C)	20.5	26.6	33.6	38.4	38.3	33.4	25.4	16.4
Average Sunbathing Time (hours)	7.2	9.6	12.1	12.4	11.6	10.0	7.5	5.5
Monthly Total Rainfall Average (kg m <sup>-2</sup> )	68.3	44.4	8.6	1.3	1.0	5.3	32.5	55.9

the area where the study was conducted has a low content of organic matter, low P<sub>2</sub>O<sub>5</sub>, high K<sub>2</sub>O, and a clayey structure. The salt content of the trial area is low and contains 8.64% lime.

In the trial area, triple super phosphate and ammonium sulfate were used before sowing and urea fertilizer was used for topdressing. Triple super phosphate and ammonium sulfate were mixed into the soil before sowing, and urea was applied by fertigation method. With sowing, 8 kg of P<sub>2</sub>O<sub>5</sub> and 5 kg of N were applied, and 20 kg of N was applied as topdressing.

### Climate Data

Diyarbakır province, where the experiment was conducted, the climate is hot and dry in summers and warm and rainy in winters. Most of the precipitation generally occurs in winter and early spring. Some meteorological data for the long-term average (1929-2023) in the months when corn is grown in Diyarbakır province are given in Table 2.

### The Characteristics of the Varieties

Both ADA 351 and Sakarya varieties have vertical and wide leaves, yellow dent corn grain structure and number of days to ripening are medium-late (FAO 650). The plant height of ADA 351 variety is around 260-320 cm and the yield potential is 15000 kg ha<sup>-1</sup> on average. The plant height of Sakarya variety is 245-275 cm and the yield potential is 12500-15500 kg ha<sup>-1</sup>. The leaf angle of ADA 351 variety is narrower than Sakarya variety.

### Irrigation

The trial was irrigated by drip irrigation. The amount of irrigation water applied during the irrigation season was determined by the Class A pan evaporation method every 4 days (Vural, 2007). The spacing of the laterals was 70 cm and equal irrigation amount applied to all applications. While 634 mm irrigation amount applied in 2016, 700 mm applied in 2017.

### Methods

Separate trials have been conducted for both varieties of corn. The study was carried out for 2 years in 2016 and 2017. Both trials were conducted with 4 replications according to the randomized block trial design. In the trial, the width of the parcel is 2.8 m and the length of the parcel is 6 m. There is a 2 m gap between parcels and between replications.

#### Sowing methods:

- (1) Line abreast narrow row application (35 cm row spacing, 25 cm intra-row spacing)
- (2) Diagonal narrow row application (35 cm row spacing, 25 cm intra-row spacing)
- (3) Line abreast twin row application (20-50 cm row spacing, 25 cm intra-row spacing)
- (4) Diagonal twin row application (20-50 cm row spacing, 25 cm intra-row spacing)
- (5) Single row 1 (70 cm row spacing, 20 cm intra-row spacing)
- (6) Single row 2 (70 cm row spacing, 12.5 cm intra-row spacing)

Single row 1 is the method used in conventional corn cultivation in this region. Single row 2 was tested in order to determine the result that will be obtained if the plant population used in narrow and twin row applications is applied in single row. In order to determine whether it is important for plants to be diagonal and line abreast in narrow row and twin row applications, both diagonal and line abreast applications were carried out.

Sowing date of corn was in the first week of May according to the weather conditions. All of the phosphorus ( $P_2O_5$ ) and one-fifth of the nitrogen were applied into the soil before sowing. The remaining part of the nitrogen was given by fertigation method in equal amounts once in two irrigations until the milk stage. Sowing was done by hand. Harvest was done in the second week of October in both years.

### Examined Features

Plant height, ear height, number of ears/plant and stem diameter parameters were measured and recorded in 10 randomly selected plants in each plot. Ear length and ear diameter were measured in 10 randomly selected ears from each plot, and grain/ear rate was determined by separating

the grains from the ears. Grain yield was determined by converting plot yields to unit area yield based on 15% grain moisture.

### Statistical Analysis

Analysis of variance was applied to the data obtained from the study and the difference between applications is grouped by the LSD test. JMP 5.01 statistical program was used for statistical analysis.

### Results

The results obtained from different twin row and narrow row applications in the main crop corn cultivation for two years in a soil with a clay texture and climatic conditions that hot and dry summer months in the Southeastern Anatolia Region of Türkiye are as follows; as seen in Table 3, in the trial conducted with ADA 351 corn variety, the highest plant height was obtained from single row 1 application. The closest application to this is the single row 2 application. While there was no difference between applications in terms of ear height, the highest number of ears/plant was obtained from single row 2 and single row 1 applications, respectively (Table 3). In the experiment with Sakarya corn variety, the highest number of ears/plant was obtained from single row 1 and single row 2 applications respectively (Table 4).

It was observed that the highest ear length and ear diameter were obtained from single row 1 and single row 2 applications, respectively in the trial with ADA 351 variety (Table 5). In the experiment with Sakarya variety, the highest ear diameter was obtained from the single row 1 application (Table 6).

In terms of stem diameter parameter, the highest values were obtained in single row 1 application in the trial conducted with both varieties (Table 7, Table 8). Stem diameter is very important for plant growth and lodging resistance. The thin stem of the plants causes the plants to be easily overturned in windy weather. The highest grain yield was obtained from the single row 2 application in the trial carried out with ADA 351 corn variety.

In the trial carried out with Sakarya corn variety, there was no statistical difference between the applications, but it is seen that the highest yield value was in the single row 2 application.

Table 3. The averages and multiple comparison results of the parameters of plant height, ear height and number of ears/plant in the experiment carried out with ADA 351 variety.

Sowing Methods	Plant height (cm)			Ear height (cm)			Number of ears/plant		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
L.A.N.R.	237.7	218.2	228.0 C	137.0	107.3	122.1	0.75	0.61	0.68 C
D.N.R.	241.8	228.8	235.3 A-C	138.2	108.8	123.5	1.02	0.59	0.81 A-C
L.A.T.R.	236.8	224.1	230.5 BC	137.5	106.2	121.8	0.99	0.55	0.77 BC
D.T.R.	238.8	226.3	232.6 BC	137.7	109.8	123.8	0.86	0.57	0.71 C
S.R. 1	246.5	236.0	241.2 A	139.0	112.6	125.8	0.90	0.85	0.87 AB
S.R. 2	246.5	229.0	237.7 AB	144.0	111.3	127.6	1.08	0.83	0.95 A
Mean	241.3	227.1		138.9A	109.3B		0.93A	0.67B	
CV		3.17			4.19			17.50	
LSD sowing methods		7.58**			n.s.			0.14**	
LSD year		n.s.			7.71**			0.14**	
LSD year*sowing methods		n.s.			n.s.			n.s.	

n.s. non-significant, \*: significant at  $P < 0.05$ , \*\*: significant at  $P < 0.01$ . L.A.N.R.: Line abreast narrow row, D.N.R.: Diagonal narrow row, L.A.T.R.: Line abreast twin row, D.T.R.: Diagonal twin row, S.R. 1: Single row 1, S.R. 2: Single row 2, CV: coefficient of variation; LSD: least significant difference



Table 4. The averages and multiple comparison results of the parameters of plant height, ear height and number of ears/plant in the experiment carried out with Sakarya variety.

Sowing Methods	Plant height (cm)			Ear height (cm)			Number of ears/plant		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
L.A.N.R.	226.2	208.7	217.5	116.1	100.8	108.4	0.94 ab	0.63 c	0.78 B
D.N.R.	227.1	207.3	217.2	112.8	98.4	105.6	0.99 a	0.54 c	0.76 B
L.A.T.R.	222.5	205.5	214.0	114.1	98.8	106.4	0.99 a	0.54 c	0.76 B
D.T.R.	227.0	208.1	217.5	114.6	99.8	107.2	0.96 ab	0.53 c	0.75 B
S.R. 1	229.5	222.8	226.1	116.2	104.3	110.2	0.97 ab	0.90 b	0.93 A
S.R. 2	226.0	212.0	219.0	115.7	100.8	108.2	0.86 ab	0.82 b	0.84 AB
Mean	226.3 A	210.7 B		114.9 A	100.4 B		0.95 A	0.66 B	
CV		3.93			3.48			12.50	
LSD sowing methods		n.s.			n.s.			0.10**	
LSD year		14.68*			4.88**			0.10**	
LSD year*sowing methods		n.s.			n.s.			0.14**	

n.s. non-significant, \*: significant at  $P < 0.05$ , \*\*: significant at  $P < 0.01$ . L.A.N.R.: Line abreast narrow row, D.N.R.: Diagonal narrow row, L.A.T.R.: Line abreast twin row, D.T.R.: Diagonal twin row, S.R. 1: Single row 1, S.R. 2: Single row 2, CV: coefficient of variation; LSD: least significant difference

Table 5. The averages and multiple comparison results of the parameters of ear length, ear diameter and grain/ear rate in the experiment carried out with ADA 351 variety.

Sowing Methods	Ear length (cm)			Ear diameter (cm)			Grain/ear rate (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
L.A.N.R.	19.35	19.12	19.24 C	3.38	3.58	3.48 BC	88.98	85.76	87.37
D.N.R.	20.46	19.45	19.95 BC	3.45	3.54	3.49 BC	87.88	85.59	86.74
L.A.T.R.	20.01	18.90	19.45 C	3.37	3.54	3.45 BC	87.82	85.51	86.66
D.T.R.	20.37	18.55	19.46 C	3.25	3.51	3.38 C	87.56	85.57	86.57
S.R. 1	22.77	23.43	23.10 A	3.68	3.90	3.79 A	88.18	86.21	87.20
S.R. 2	20.28	21.56	20.92 B	3.51	3.62	3.56 B	88.62	86.47	87.54
Mean	20.54	20.16		3.44 B	3.61 A		88.17 A	85.85 B	
CV		6.53			3.11			1.52	
LSD sowing methods		1.34**			0.12**			n.s.	
LSD year		n.s.			0.17*			1.46**	
LSD year*sowing methods		n.s.			n.s.			n.s.	

n.s. non-significant, \*: significant at  $P < 0.05$ , \*\*: significant at  $P < 0.01$ . L.A.N.R.: Line abreast narrow row, D.N.R.: Diagonal narrow row, L.A.T.R.: Line abreast twin row, D.T.R.: Diagonal twin row, S.R. 1: Single row 1, S.R. 2: Single row 2, CV: coefficient of variation; LSD: least significant difference

Table 6. The averages and multiple comparison results of the parameters of ear length, ear diameter and grain/ear rate in the experiment carried out with Sakarya variety.

Sowing Methods	Ear length (cm)			Ear diameter (cm)			Grain/ear rate (%)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
L.A.N.R.	17.14	15.77	16.45	3.99	4.21	4.10 A-C	81.18	79.15	80.16
D.N.R.	16.16	15.72	15.94	3.82	4.21	4.02 BC	80.24	79.56	79.90
L.A.T.R.	16.53	16.22	16.37	3.91	4.15	4.03 BC	80.98	81.54	81.26
D.T.R.	16.76	16.32	16.54	3.95	4.06	4.00 C	82.23	79.46	80.84
S.R. 1	17.63	17.87	17.75	4.09	4.42	4.26 A	81.87	82.45	82.16
S.R. 2	16.49	16.12	16.30	4.08	4.29	4.18 AB	82.66	80.64	81.65
Mean	16.78	16.34		3.97 B	4.22 A		81.53	80.47	
CV		6.88			3.90			2.51	
LSD sowing methods		n.s.			0.16*			n.s.	
LSD year		n.s.			0.19*			n.s.	
LSD year*sowing methods		n.s.			n.s.			n.s.	

n.s. non-significant, \*: significant at  $P < 0.05$ , \*\*: significant at  $P < 0.01$ . L.A.N.R.: Line abreast narrow row, D.N.R.: Diagonal narrow row, L.A.T.R.: Line abreast twin row, D.T.R.: Diagonal twin row, S.R. 1: Single row 1, S.R. 2: Single row 2, CV: coefficient of variation; LSD: least significant difference

## Discussion

Gözübenli et al. (2004) and (Balem et al. (2014) found no difference in plant height between single row and twin row. Similar results were obtained in the experiment carried out with Sakarya variety and in these studies. As a result of the study comparing 50 cm row spacing, 75 cm row spacing and twin row planting in corn, the highest

plant height was obtained in 50 cm row spacing and twin row (Greveniotis et al. 2019). Ahmad et al. (2010) reported that plant height increased by narrowing the row spacing from 75 to 45 cm. In this study, different results were obtained with these literatures.

Gözübenli et al. (2004) found no difference between single row and twin row in terms of ear length and ear diameter. Contrasting results with this literature were found in the trial conducted with the ADA 35 variety. In the experiment carried out with Sakarya variety, similar results were obtained in terms of ear length and opposite results in terms of ear diameter. Greveniotis et al. (2019) reported that among corn planted with twin row, 75 and 50 cm row spacing, the highest ear length and ear diameter were obtained from 50 cm row spacing. Gözübenli et al. (2004) and Balem et al. (2014) informed that sowing twin row corn increased the stem diameter parameter in corn plant compared to single row. Contrasting results with these studies were found in trials with both variety. Stem diameter was found to be higher in single row 1 corn sowing method.

Robles et al. (2012) reported that twin row corn sowing did not increase yield compared to single row. Haegele et al. (2014) informed that twin row planting did not increase yield compared to single row in their study in Lewisville. Balkcom et al. (2011) reported that irrigated twin row corn gives the same yield as single row corn. It was also stated that the effect of hybrid and plant population was greater. Balem et al. (2014) determined that twin row corn sowing increased yield compared to conventional spacing (0.7 m).

Novacek (2011) determined that twin row corn cultivation had little effect on yield and plant growth. According to Gözübenli et al. (2004) determined that the twin row corn sowing increased the grain yield parameter in the corn plant compared to the single row. Acciares & Zuluaga (2006) reported that narrow row corn planting increased yield compared to wide row spacing sowing. The study conducted by Kratochvil & Taylor (2005) twin rows (two rows 19.05 cm inches apart on 76.2 cm centers) to corn produced in rows spaced 76.2 cm apart over a range of plant populations 4 different locations was compared. As a result of the study difference was found in only one location and the yield was higher in the 76.2 cm rows. Barbieri et al. (2008) determined that narrow rows increased corn grain yield. Stone et al. (2000) in a study conducted in three different environments (Waikato, Hawke's Bay & Manawatu), it was found that the effect of row spacing on yield and quality of corn was minimal and inconsistent. Maddonni et al. (2006) emphasized that the benefit of narrowing the row spacing (narrow row) in increasing the grain yield of the corn plant is not expected. Lancaster & Adey (2023) reported no difference in corn yield between 38.1 and 76.2 cm row spacing. Fuksa et al. (2023) stated that only limited success was achieved in increasing yield in narrow row spacing.

Table 7. The averages and multiple comparison results of the parameters of stem diameter and grain yield in the experiment carried out with ADA 351 variety.

Sowing Methods	Stem diameter (mm)			Grain yield (kg ha <sup>-1</sup> )		
	2016	2017	Mean	2016	2017	Mean
L.A.N.R.	17.15 cd	19.47 b	18.31 B	8187.30	6636.87	7412.08 C
D.N.R.	18.92 b	19.87 b	19.40 B	11307.52	7404.70	9356.11 B
L.A.T.R.	16.90 d	19.82 b	18.36 B	9953.75	7541.65	8747.70 BC
D.T.R.	18.72 bc	20.00 b	19.36 B	8791.42	7285.65	8038.53 BC
S.R. 1	22.42 a	21.85 a	22.13 A	9451.57	9110.05	9280.81 B
S.R. 2	18.91 b	19.65 b	19.28 B	11928.20	11383.90	11656.05 A
Mean	18.84 B	20.11 A		9936.63	8227.14	
CV		5.75			17.94	
LSD sowing methods		1.14**			1662.43**	
LSD year		1.14*			n.s.	
LSD year* sowing methods		1.61*			n.s.	

n.s. non-significant, \*: significant at P < 0.05, \*\*: significant at P < 0.01. L.A.N.R.: Line abreast narrow row, D.N.R.: Diaogonal narrow row, L.A.T.R.: Line abreast twin row, D.T.R.: Diaogonal twin row, S.R. 1: Single row 1, S.R. 2: Single row 2, CV: coefficient of variation; LSD: least significant difference

Table 8. The averages and multiple comparison results of the parameters of stem diameter and grain yield in the experiment carried out with Sakarya variety.

Sowing Methods	Stem diameter (mm)			Grain yield (kg ha <sup>-1</sup> )		
	2016	2017	Mean	2016	2017	Mean
L.A.N.R.	18.12	20.44	19.28 B	9466.04	7574.37	8520.21
D.N.R.	17.97	19.85	18.91 B	9266.06	6205.32	7735.69
L.A.T.R.	18.32	19.47	18.90 B	8902.52	6755.90	7829.21
D.T.R.	17.70	20.05	18.87 B	9416.32	6092.22	7754.28
S.R. 1	21.45	22.12	21.78 A	8334.34	6696.37	7515.36
S.R. 2	18.15	20.72	19.43 B	11077.14	7651.75	9364.45
Mean	18.62 B	20.44 A		9410.41	6829.33	
CV		4.96			18.14	
LSD sowing methods		0.97**			n.s.	
LSD year		1.02**			n.s.	
LSD year* sowing methods		n.s.			n.s.	

n.s. non-significant, \*: significant at P < 0.05, \*\*: significant at P < 0.01. L.A.N.R.: Line abreast narrow row, D.N.R.: Diaogonal narrow row, L.A.T.R.: Line abreast twin row, D.T.R.: Diaogonal twin row, S.R. 1: Single row 1, S.R. 2: Single row 2, CV: coefficient of variation; LSD: least significant difference

It is seen that different results were obtained from different studies conducted. Different narrow row and twin row corn cultivation did not provide an increase in yield compared to single row corn cultivation in this study. Haegele et al. (2014), Maddonni et al. (2006), Robles et al. (2012), and Balkcom et al. (2011) found similar results with this study in terms of grain yield.

## Conclusion

As a result of the data obtained, it was concluded that line abreast narrow row, diagonal narrow row, line abreast twin row and diagonal twin row applications are not suitable in terms of both yield and yield parameters for corn cultivation under main crop conditions in the Southeastern Anatolia Region of Türkiye. Because better results were obtained from single row applications in all parameters examined. The highest grain yield was obtained from single row 2 application, which has the same plant density with different twin row and narrow row applications. Therefore, narrow row and twin row applications were not found to be advisable in the main crop grain corn cultivation in this region.

## Declarations

### Author Contribution Statement

Betül Kolay: Project administration, supervision, data collection, investigation, formal analysis, conceptualization, methodology, review and editing and writing the original draft  
 Özlem Avşar: Data collection and investigation  
 Uğur Bilge: Data collection and investigation  
 Kudret Berekatoğlu: Data collection and investigation  
 Sevda Kılınc: Data collection and investigation  
 Ferhat Oğurlu: Laboratory analysis  
 Şehmus Atakul: Data collection and investigation  
 Yener Çelik: Disease and pest monitoring in plants  
 Abdullah Eren: Data collection and investigation  
 Ali Rıza Öztürkmen: Supervision, conceptualization, methodology, review and editing

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### Conflict of Interest

The authors declare no conflict of interest.

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## In vitro Antifungal Effects of Various Essential Oils against Aspergillus Crown Rot of Peanut

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ARTICLE INFO	ABSTRACT
<p>Research Article</p> <p>Received : 29.09.2024 Accepted : 07.11.2024</p> <p>Keywords: Antifungal activity Essential oil <i>Arachis hypogea</i> L. <i>Aspergillus niger</i> Crown rot</p>	<p>Synthetic fungicides that combat plant pathogenic fungi can enhance crop yields, ensuring stable crop production and market quality. However, the increase in the use of fungicides may cause to development of fungicide-resistant pathogen strains and the accumulation of fungicide residues in the food chain above safe limits. This situation underscores the need for improved fungal disease management through alternatives to synthetic fungicides. These alternatives include plant-derived compounds such as essential oils and extracts. Essential oils are known to be potent antifungal compounds against both human and plant pathogens. <i>Aspergillus niger</i> is a toxin-producing fungal disease agent that causes Aspergillus crown rot in peanuts. In this study, the antifungal activities of nine different essential oils from <i>Foeniculum vulgare</i>, <i>Lippia citriodora</i>, <i>Origanum majorana</i>, <i>Origanum minutiflorum</i>, <i>Origanum onites</i>, <i>Origanum syriacum</i>, <i>Origanum vulgare</i>, <i>Salvia aramiensis</i> and <i>Thymus syriacus</i> plants were evaluated against <i>A. niger</i> under <i>in vitro</i> conditions by using disc diffusion test. Among the nine essential oils tested, the highest antifungal activities were displayed by <i>O. vulgare</i> essential oil (with an inhibition zone diameter of 49.33 mm) which was followed by <i>T. syriacus</i>, <i>O. onites</i>, <i>O. syriacum</i> and <i>O. minutiflorum</i> essential oils (48.67, 47.00, 46.33 and 43.33 mm, respectively). The essential oils of <i>F. vulgare</i>, <i>L. citriodora</i>, and <i>O. majorana</i> showed relatively lower antifungal effects. The essential oil of <i>S. aramiensis</i> did not show antifungal effect against pathogen. The results indicated that plant essential oils could be valuable in promoting research aimed at developing new antifungal agent(s) for fungal disease management. However, further studies are needed to optimize the <i>in vivo</i> application conditions of essential oils against <i>A. niger</i>.</p>

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## Yer Fıstığında Aspergillus Kök Boğazı Çürüklüğüne Karşı Çeşitli Uçucu Yağların in vitro Antifungal Etkileri

MAKALE BİLGİSİ	ÖZ
<p>Araştırma Makalesi</p> <p>Geliş : 29.09.2024 Kabul : 07.11.2024</p> <p>Anahtar Kelimeler: Antifungal etki Uçucu yağ <i>Arachis hypogea</i> L. <i>Aspergillus niger</i> Kök boğazı çürüklüğü</p>	<p>Bitki patojeni funguslarla mücadelede sentetik fungusitler, ürün verimini artırarak istikrarlı üretim ve pazar kalitesi sağlayabilir. Ancak fungusit kullanımındaki artış, fungusite toleranslı patojen türlerinin gelişmesine ve gıda zincirinde güvenli sınırların üzerinde fungusit kalıntılarının birikmesine yol açmıştır. Bu durum, sentetik fungusitlere alternatif olarak geliştirilen fungal hastalıklarla mücadele yöntemlerine olan ihtiyacı doğurmuştur. Bu alternatifler arasında, uçucu yağlar ve ekstraktlar gibi bitkisel kaynaklı bileşikler yer almaktadır. Uçucu yağların, hem insan hem de bitki patojenlerine karşı güçlü antifungal etkiye sahip olduğu bilinmektedir. <i>Aspergillus niger</i>, yer fıstığında Aspergillus kök boğazı çürüklüğüne neden olan, toksin üreten bir fungal hastalık etmenidir. Bu çalışmada, <i>Foeniculum vulgare</i>, <i>Lippia citriodora</i>, <i>Origanum majorana</i>, <i>Origanum minutiflorum</i>, <i>Origanum onites</i>, <i>Origanum syriacum</i>, <i>Origanum vulgare</i>, <i>Salvia aramiensis</i> ve <i>Thymus syriacus</i> bitkilerinden elde edilen dokuz farklı uçucu yağın antifungal etkileri disk difüzyon testi kullanılarak <i>in vitro</i> koşullar altında <i>A. niger</i>'e karşı değerlendirilmiştir. Test edilen dokuz uçucu yağ arasından en yüksek antifungal etkiyi <i>O. vulgare</i> uçucu yağı (49.33 mm engelleme zon çapı ile) göstermiş, bunu <i>T. syriacus</i>, <i>O. onites</i>, <i>O. syriacum</i> ve <i>O. minutiflorum</i> uçucu yağları (sırasıyla 48.67, 47.00, 46.33 ve 43.33 mm) izlemiştir. <i>F. vulgare</i>, <i>L. citriodora</i> ve <i>O. majorana</i> uçucu yağları nispeten daha düşük antifungal etkiler göstermiştir. <i>S. aramiensis</i> uçucu yağı patojene karşı herhangi bir antifungal etki göstermemiştir. Bu çalışmanın sonuçları, bitki uçucu yağlarının fungal hastalık yönetimi için yeni antifungal etmen(ler) geliştirmeyi amaçlayan araştırmaları teşvik etmede değerli olabileceğini göstermektedir. Bununla birlikte, <i>A. niger</i>'e karşı uçucu yağların <i>in vivo</i> uygulama koşullarını optimize etmek için daha fazla çalışmaya ihtiyaç vardır.</p>

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## Introduction

Groundnut (*Arachis hypogaea* L.) is an agricultural crop of great importance in both human nutrition and economic terms worldwide. Thanks to its high oil and protein content, it is both consumed directly as food and used in various fields such as animal husbandry and industrial oil production (FAO, 2020). Fungal disease agents such as *Aspergillus niger*, *A. oryzae*, *Penicillium georgiense*, *P. polonicum*, *P. glabrum* and *P. expansum* cause rot diseases in agriculturally important crops before and after harvest (Kara & Soyly, 2023). Peanuts are attacked by fungal pathogens in post-harvest processes, causing significant quality and yield losses. *A. niger*, one of these pathogens, multiplies rapidly on peanuts, causing both physical deterioration and food safety hazards with mycotoxin production (Pitt & Hocking, 2009).

*A. niger* grows under favorable moisture and temperature conditions, especially during storage, and produces toxic compounds such as aflatoxins. Aflatoxins are extremely dangerous for both humans and animals and can cause serious health problems such as liver cancer (Williams et al., 2004). The World Health Organization (WHO) and the Food and Agriculture Organisation (FAO) emphasize that aflatoxins pose a serious public health risk in food products and should therefore be checked regularly (WHO, 2018). Therefore, control of *A. niger* and prevention of damage caused by this fungus in groundnut products is of critical importance.

Traditionally, chemical fungicides have been widely used to control fungal pathogens. However, overuse of chemical fungicides leads to problems such as environmental pollution, loss of biodiversity, and fungicide resistance in pathogens (Fernández-Baldo et al., 2013). In addition, chemical residues can accumulate in foods and this situation creates risks for consumer health (Pimentel, 2009). In this context, environmentally friendly, non-toxic, and safe alternative solutions are of great interest.

Plant essential oils (EOs) have been increasingly investigated as natural antifungal agents in recent years. Essential oils are rich in components such as terpenes, phenols, and aldehydes and show broad-spectrum biological activity against pests and pathogenic microorganisms (Bakkali et al., 2008). For example, essential oils of plants such as thyme (*Origanum vulgare*), peppermint (*Mentha piperita*) and tea tree (*Melaleuca alternifolia*) have shown significant antifungal activity on various microorganisms (Soković et al., 2010). The natural and multicomponent nature of essential oils attracts attention as an alternative control method against not only fungal pathogens but also plant pests (Sertkaya et al., 2010).

Several studies have been conducted to investigate the antifungal effects of essential oils against *A. niger*. Especially essential oils obtained from *Origanum* species attract attention due to their broad-spectrum antimicrobial effects. For example, essential oils of species such as *O. vulgare* and *O. syriacum* have been reported to exhibit strong antifungal properties against different microorganisms (Fikry et al., 2019; Žitek et al., 2021). Active components such as carvacrol and thymol in these

oils were found to inhibit the growth of pathogens by damaging the cell membrane (Sakkas & Papadopoulou, 2017). Similarly, essential oils of plants such as *Foeniculum vulgare* (fennel) and *Salvia aramiensis* (sage) have also been shown to be effective against *Aspergillus* species. While fennel oil shows antifungal properties thanks to its high amount of anethole, components such as salvin and rosmarinic acid in sage oil have been reported to inhibit the growth of pathogens (Sharma et al., 2019; Ghasemian et al., 2020; Zulu et al., 2023). Recently, *in vitro* antifungal effects of different concentrations of essential oils of different thyme species such as *T. vulgare*, *T. spicata* and *O. syriacum* L., fennel (*F. vulgare*), laurel (*Laurus nobilis*) and eucalyptus (*Eucalyptus camaldulensis*) were investigated against pepper fungal disease agents *Alternaria alternata* and *A. niger* (Atay & Soyly, 2023). The highest antifungal activities (100% inhibition) against fungal isolates were shown by essential oils of *T. spicata*, *O. syriacum* and *T. vulgare*. *E. camaldulensis* displayed the lowest antifungal activity against tested fungal isolates. Previous studies showed that essential oils not only inhibit the growth of pathogens but also contribute to making plants more resistant by enhancing the overall immune response (Valdivieso-Ugarte et al., 2019; Sandner et al., 2020). These findings emphasize that essential oils can be used as an environmentally friendly alternative to chemical fungicides.

In this study, the antifungal activities of nine different essential oils (EOs) obtained from *Foeniculum vulgare*, *Lippia citriodora*, *Origanum majorana*, *Origanum minutiflorum*, *Origanum onites*, *Origanum syriacum*, *Origanum vulgare*, *Salvia aramiensis* and *Thymus syriacus* plants were investigated *in vitro* conditions against *A. niger*. This study aimed to investigate the potential of using plant essential oils as a natural solution for the protection of agricultural products and to determine whether these oils can be effective in the control of *A. niger*. Thus, environmentally friendly alternatives that can replace chemical fungicides were evaluated.

## Materials and Methods

### Isolation and Identification of Fungal Disease Agent

Fungal disease agent *Aspergillus niger* was isolated from the rind and inner tissues of infected peanut plants growing in Hatay province. Small pieces were taken from infected peanut samples with a sterile scalpel and surface sterilised with 70% alcohol. In a sterile environment, the samples were placed on potato sucrose agar (PSA) medium and incubated at 25°C for 7 days. Fungal disease agent was tested for pathogenicity and identified based on morphological characteristics (Ahmed & Ravinder Reddy, 1993) and MALDI-TOF (Bruker Daltonics GmbH, Bremen, Germany) analysis (Kara & Soyly, 2023). Spore suspension ( $10^3$  spores/ml) of *A. niger* was prepared from the actively growing culture (5 days old) in distilled sterile water. These suspensions were used to inoculate PSA in antifungal activity studies.

### Determination of Antifungal Effects of Essential Oils

The essential oils used in the study (*Foeniculum vulgare*, *Lippia citriodora*, *Origanum majorana*, *Origanum minutiflorum*, *Origanum onites*, *Origanum syriacum*, *Origanum vulgare*, *Salvia aramiensis* and *Thymus syriacus*) were obtained from the stocks of Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, Phytopathology Laboratory. The hydrodistillation method with a Neo-Clevenger device (Ildam, Ankara, Turkey) was used to extract the EOs. The main compounds and % ratios of essential oils were analysed using a GC-MS device (Thermo Scientific ISQ Single Quadrupole, Milan, Italy) and the results have been reported in previous studies (Kara et al., 2020; Soylu et al., 2020; Kara et al., 2021; Kara et al., 2022a). The antifungal effect of each essential oil under *in vitro* conditions was determined by using disc diffusion technique in petri dishes. On the surface of the petri dishes containing PSA medium were inoculated 200 µl of fungal suspension prepared as previously described was spread. Sterile filter paper discs (6 mm in diameter) were placed on the surface of the petri dishes, 5 µl of essential oils were added, and the petri dishes were sealed with sterile parafilm and incubated at 25°C for 48 hours. In negative control petri dishes, sterile filter paper discs amended with distilled water were used. The antifungal effect of essential oils was evaluated by measuring the diameter of the inhibition zone around the filter paper disc 48 hours after treatment.

### Statistical Analysis

*In vitro* antifungal activity trials were carried out in at least three replicates for each essential oil/fungal isolate and the experiment was repeated at two different times. One-way analysis of variance was performed on the values obtained in the trials using SPSS statistics software (SPSS Statistics 17.0) and the difference between the treatments was analysed by Duncan Multiple Comparison Test ( $P \leq 0.05$ ).

### Results and Discussion

The fungal disease agent was isolated from infected groundnut shells. Preliminary identification of the fungal isolate was based on the morphological characteristics of the isolate grown on PDA medium and confirmed by MALDI-TOF MS.

The antifungal effects of the essential oils obtained from each plant species were determined using the disc diffusion technique and the diameters of the inhibition zones of these essential oils against the fungal disease agent were measured and given in Table 1. The findings of this study showed that plant essential oils have a significant antifungal potential against *A. niger*. The essential oils of different plant species showed significantly varying levels of antifungal activity. The essential oils of *F. vulgare*, *L. citriodora*, *O. majorana*, *O. minutiflorum*, *O. onites*, *O. syriacum*, *O. vulgare*, and *T. syriacus* significantly inhibited the growth of *A. niger* (Figure 1). According to the inhibition zone diameter values, the highest antifungal activities were displayed by *O. vulgare* EO (with an inhibition zone diameter of 49.33 mm) which was followed by *T. syriacus*, *O. onites*, *O. syriacum* and *O. minutiflorum*, EOs (48.67, 47.00, 46.33 and 43.33 mm, respectively). The EOs of *F. vulgare*, *L. citriodora*, and *O. majorana* showed relatively lower antifungal effects. The EO of *S. aramiensis* did not show antifungal effect against the pathogen.

EOs of different thyme and oregano plant species are known to contain high levels of phenolic compounds, such as carvacrol and thymol (Burt, 2004; Soylu et al., 2006). These compounds have been also reported to disrupt the fungal cell membrane, increasing cell wall permeability and weakening the cellular structure (Burt, 2004; Soković et al., 2010; Soylu et al., 2006). Thyme and oregano EOs have been reported to show high antifungal activity against other common fungal species such as *Candida albicans*, *P. infestans*, *Botrytis cinerea*, *Aspergillus flavus* and *Penicillium* spp. (Rasooli & Abyaneh, 2004; Soylu et al., 2005; Soylu et al., 2006; Soylu et al., 2010; Kara et al., 2022a). This study shows that oregano oil can be used against fungi, especially *A. niger*, which damage agricultural products during storage. However, there are some practical difficulties for EOs to be used for the large-scale protection of agricultural products. Large-scale production and application of essential oils may increase production costs. Furthermore, factors such as the stability of essential oils, application methods, and possible flavour or aroma on products should be taken into account (Isman, 2000). Therefore, future research could be focussed on the development of formulations of oregano oil and improving its efficacy. *T. syriacus*, one of the thyme species, is known for its high content of thymol and carvacrol.

Table 1. Antifungal activities of essential oils on the mycelial growth of *Aspergillus niger*

Essential oils	Inhibition Zone <sup>a</sup>
<i>Origanum vulgare</i>	49.33 <sup>d</sup>
<i>Thymus syriacus</i>	48.67 <sup>d</sup>
<i>Origanum onites</i>	47.00 <sup>cd</sup>
<i>Origanum syriacum</i>	46.33 <sup>cd</sup>
<i>Origanum minutiflorum</i>	43.33 <sup>c</sup>
<i>Foeniculum vulgare</i>	18.67 <sup>b</sup>
<i>Lippia citriodora</i>	16.67 <sup>b</sup>
<i>Origanum majorana</i>	16.67 <sup>b</sup>
<i>Salvia aramiensis</i>	0.00 <sup>a</sup>
Control	0.00 <sup>a</sup>

<sup>a</sup>The values obtained are the mean values of fungal growth zone diameter (mm) developed in 3 different petri dishes. Similar lower-case letters next to the mean values in the same column or similar upper-case letters next to the mean values in the same row indicate that the difference between treatments is not statistically significant (Duncan's Multiple Range Test,  $P \leq 0.05$ ).



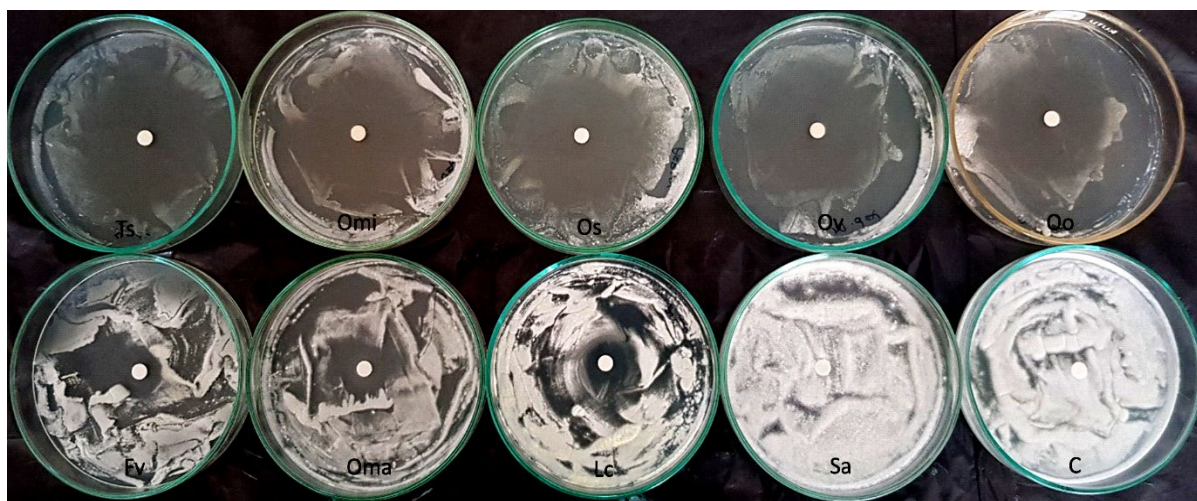


Figure 1. Antifungal activity of essential oils against *A. niger*, characterised by the formation of inhibition zones around filter discs (arrow). (Ts: *Thymus syriacus*, Omi: *Origanum minutiflorum*, Os: *Origanum syriacum*, Ov: *Origanum vulgare*, Oo: *Origanum onites*, Fv: *Foeniculum vulgare*, Oma: *Origanum majorana*, Lc: *Lippia citriodora*, Sa: *Salvia aramiensis*, C: Control)

These two components show antifungal effect by destroying the fungal cell membrane. Studies show that *Thymus* species have broad-spectrum antifungal effects (Soković et al., 2010; Atay & Soylu, 2023). This species, which is rarer than other thyme species, attracts attention especially with its high carvacrol content. Its antifungal effect is largely dependent on the concentration of active compounds it contains (Burt, 2004).

Fennel oil contains compounds such as anethole and fenchon and showed weaker milder antifungal effects. In the literature, it has been reported that this essential oil is more prominent with its mild antibacterial and antioxidant properties (Soylu et al., 2009; Bozkurt et al., 2020; Soylu et al., 2024), but its antifungal effect is lower compared to thyme species (Isman, 2000; Soylu et al., 2007; Soylu et al., 2010; Kara et al., 2020). Lemongrass oil contains components such as citral and limonene. Its antifungal effect is particularly associated with the weakening of the fungal cell wall by these components. However, the effect of this oil against fungal species is reported to be moderate (Kalemba and Kunicka, 2003). Marjoram oil shows mild antibacterial and antifungal properties. However, its antifungal effect is generally more limited compared to thyme species (Burt, 2004). Sage oils contain high levels of cineole and camphor. Their antifungal effects may be milder than other oils; however, they have shown suppressive effects on fungal growth (Bakkali et al., 2008). The lowest antifungal effect of *S. aramiensis* oil may be related to the fact that the active components it contains are not effective on *A. niger* at the expected level. It is thought that this plant oil contains a lower proportion of antifungal components compared to the others or that it causes less damage to the fungal cell wall. Some studies have reported that this species shows particular antibacterial activity, but antifungal activity may be limited (Pitarokili et al., 2003). This may explain why *S. aramiensis* is less effective compared to other more potent essential oils from *Origanum* or *Thymus* species. The low antifungal activity of *S. aramiensis* suggests that the use of this essential oil as a stand-alone antifungal agent in agriculture is limited. However, its efficacy may increase when used in

synergistic combinations or combination with other essential oils (Kara et al., 2022b; Kara, 2024).

## Conclusion

This study shows that oregano and thyme oils can be used as a potent antifungal agents against *A. niger* in groundnut. This essential oil, which offers a more environmentally friendly and natural alternative to chemical fungicides, is promising in reducing fungal losses, especially in organic farming practices.

## Declarations

### Author Contribution Statement

All the authors contributed equally for analyses, writing and interpretation of the article. The authors read and approved the final version of the manuscript.

### Conflict of Interest

The authors declare that there is no conflict of interest between them.

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## Physiological Features of Yield Formation of Sunflower Breeding Samples in Arid Conditions of the Ukrainian Steppe

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### ABSTRACT

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The research methodology was based on determining the soil moisture consumption per unit of photosynthesis productivity, establishing the influence of the moisture consumption coefficient on the productivity of photosynthesis on the yield of sunflower genotypes. The aim of the research was to determine the physiological characteristics of the formation of plant productivity of different sunflower samples and use them in assessing and creating genotypes for cultivation in arid conditions. The conducted investigations allowed to determine physiological factors and properties of plants that determine the level of formation of the weight of seeds of the head and the yield of sunflower. The weight of seeds of the head is determined by the amount of soil moisture consumption per unit of net productivity. Between the indicators of soil moisture consumption per unit of net productivity at the stage of formation of the head - the beginning of flowering, a direct negative correlation interdependence was established. Sunflower varieties with a minimum consumption of soil moisture per unit of net productivity of photosynthesis of 1.01-1.05 m<sup>3</sup> g m<sup>-2</sup> per day form in arid conditions of the Steppe of Ukraine the maximum level of weight of seeds of the head of 58.7-78.7 g, which ensures obtaining a high yield within 2.68-3.49 t ha<sup>-1</sup>. The conducted assessment of genotypes by indicators of soil moisture consumption per unit of net productivity made it possible to create highly productive varieties of sunflower Emelard, Igolya, Orlik.

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## Introduction

The main regions of sunflower cultivation in Ukraine are located in the steppe zone and are characterized by dry weather conditions during the growing season.

The varieties and hybrids of sunflower have a high level of adaptation to growing in arid conditions. The root system of sunflower plants is able to absorb moisture from deep soil layers (El-Bially et al., 2022). However, over the past decades in the Steppe of Ukraine, the amount of precipitation during the growing season of sunflower has decreased and falls within the range of 80 to 110 mm. The deficit of moisture content in the soil limits and restricts the level of formation of the productivity of sunflower varieties and hybrids in such weather conditions of the growing season (Aksyonov, 2010; Aksyonov and Gavrilyuk, 2013). Guaranteed and stable yield of sunflower is possible with the creation of highly productive varieties and hybrids that are adapted to growing in drought conditions and understanding the physiological properties of productivity formation.

Studying and understanding the processes of formation of high productivity of plants under conditions of moisture deficiency is the key to creating drought-resistant sunflower source material (Salem et al., 2021; Ramadan et al., 2023). The studies show that in dry conditions, the activity of plant growth and development is largely determined by photosynthesis processes. The photosynthesis processes ensure the accumulation of dry matter in the vegetative and generative organs of plants, ensure the level of formation of plant productivity. The influence of photosynthesis processes on the formation of generative organs in the conditions of abiotic stress of plants during drought is one of the main points in the formation of the productivity of varieties and hybrids (Gao et al., 2018; Oikawa and Ainsworth, 2016).

At the same time, drought, moisture deficiency in the soil during the vegetation period of plants can suppress the processes of vegetative development of sunflower, reduce the area of the leaf surface of plants, and reduce the rate of

photosynthesis. A decrease in the level of aboveground biomass of sunflower plants contributes the formation of photosynthetic products in the plant to a lesser extent. A decrease of the photosynthetic capacity of leaves and the amount of dry matter accumulation by vegetative organs of the plant limits reproductive growth and can lead to a significant decrease of the productivity of sunflower plants (Cao et al., 2009; Saady et al., 2023).

The conducted studies did not establish a direct correlation between the elements of productivity and net productivity of photosynthesis and the amount of moisture in the soil. Plant productivity of sunflower varieties and hybrids depended on the greater extent on individual traits of photosynthesis and growing conditions that contributed to a change in the intensity of photosynthesis processes (Aksyonov, 2007).

Regarding the moisture deficit in the soil, studies show that sunflower genotypes have different reactions to the moisture content in the soil depending on the stage of plant development. Deficiency of moisture content in the soil during the drought period in the stage of sunflower flowering can reduce the level of formed yield in the agrocenosis. At the same time, the moisture deficit in the soil in the stage of initial seed formation does not contribute the decrease of yield. In this case, the decrease of the the number of seeds in the head is compensated by an increase of the weight of the seeds in the head and the yield level is stabilized (Jensen et al., 2010; Karam et al., 2007).

Establishing and evaluating the physiological characteristics of sunflower genotypes allows in the breeding process to create varieties and hybrids with a high level of productivity for growing in the conditions of moisture deficit. In the breeding of varieties and hybrids, breeders use several strategies for assessing physiological characteristics in order to create drought-resistant genotypes. One of the directions in creating such genotypes is the assessment of only the lower leaf canopy of plants, reducing transpiration, reducing water consumption on forming a unit of yield. This approach in breeding allows to create genotypes with a high level of drought resistance, but with a low level of productivity (Birck et al., 2017; Rauf and Sadaqat, 2008). Therefore, for creation varieties and hybrids in drought conditions, a breeder must understand the physiological characteristics that ensure the creation of highly productive genotypes and consider the formation of plant productivity in a complex relationship with the intensity of photosynthesis and the moisture content in the soil.

Therefore, the study and establishment of physiological characteristics for the comprehensive assessment of the criteria of photosynthetic activity and moisture reserves in the soil in the specific phases of sunflower plant development in relation to droughty periods of vegetation in the steppe zone, allowing the creation of highly productive genotypes, determine the prospects and practical significance of the studies.

The purpose of this research work was to establish the physiological properties of the formation of high plant productivity, identify genotypes with high productivity potential for cultivation in the steppe zone of Ukraine.

## Materials and method

### Materials

The studies were carried out at the Luhansk National University and the Dnipro State Agrarian and Economic University during 2016-2023.

Field experiments were laid in a 7-field crop rotation. The soil of the experimental plot is ordinary chernozem, medium-deep, low-humus, heavy loamy soil with a humus content of 3.2-3.6%.

The material for research is self-pollinated samples and new sunflower varieties. Sunflower in the experiment was sown after winter wheat.

The method of basic tillage - plowing to a depth of 27-30 cm.

Two soil cultivations were carried out in spring:

- early spring soil cultivation to a depth of 8.0-10.0 cm with simultaneous harrowing;
- pre-sowing cultivation to a depth of 6.0-8.0 cm.

Cultivations were carried out with KPS-4 cultivator.

Sowing of sunflower was carried out at the end of the third decade of April: April 25-30. The width of row spacing was 70 cm. Plant stand density before harvesting was equal to 40 thousand hectares. The plots in the field experiment had 4 rows. The counted area of each plot was 12.2 m<sup>2</sup>. Each variant (sample, variety) of the experiment had three replications.

The assessment of plants by morphological characteristics was carried out in the field on experimental plots. During the growing season of sunflower, we carried out phenological observations of plants, a description of plants by morphological characteristics, determination of soil moisture content, and calculations based on the net productivity of photosynthesis. Determination of the content of soil moisture reserves, the area of the leaf surface of plants, and the content of dry matter in a sunflower plant were performed at stages 6-8 of ontogenesis at the stage of head formation - the beginning of flowering of plants.

Sunflower samples that had different qualitative morphological characteristics of plants and a vegetation period of 100-110 days were included in the research.

### Methods

The studies were conducted in accordance with generally accepted methods in plant growing.

Crop care consisted of formation of plant stand density, two row spacing cultivations. During the period of crop care, two inter-row cultivations were performed. Sunflower harvesting from experimental plots was carried out hand with the subsequent threshing of heads in laboratory. Phenological observations, biometrics were carried out, the water regime of the soil, dry matter content, leaf surface area, the net productivity of photosynthesis of plants were determined in the period of vegetation.

After harvest and threshing the heads of sunflower samples, the weight and quantity of seeds of one head were calculated, the mass of 1000 seeds, the yield of sunflower were determined.

Determining the reserves of soil moisture, the leaf surface area of plants, the content of dry matter in plants were performed at the of the phase head formation - the beginning of flowering of plants.

Taking soil samples for moisture determination was carried out in the soil horizon of 0-100 cm every 10 cm in three repetitions along the diagonal of the plot.

To determining the moisture reserve of the soil horizon of 0-100 cm, calculations were made for each individual layer of soil: 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50-60 cm, 60-70 cm, 70-80 cm, 80-90 cm, 90-100 cm. The reserves of moisture of each layer of soil were calculated by the next formula:

$$W_{ozv} = \frac{V \times d \times h}{10} \quad (1)$$

Where:

$W_{ozv}$ : Total moisture reserve in a separate soil layer, mm;

$W$  : Total moisture of the soil layer, %;

$d$  : Density of the studied soil layer, g cm<sup>-3</sup>;

$h$  : Thickness of the studied soil layer, cm.

The determining of moisture reserve was established by calculating for each separately soil layers, which have different soil density -  $d$ .

Next, the content of productive moisture was determined on each layer of soil:

$$W_{pr} = \frac{(W_{ozv} - W_{wilt}) \times d \times h}{10} \quad (2)$$

Where:

$W_{pr}$  : Productive moisture in soil layer, mm;

$W_{ozv}$  : Total moisture of soil layer, %;

$W_{wilt}$  : Wilting moisture, %;

$d$  : Density of soil layer, g/cm<sup>3</sup>;

$h$  : Thickness of the studied soil layer, cm.

The total content of moisture in the studied meter profile of the soil was obtained by summing the indicators calculated separately for each layer: ( $W_{pr} = W_{pr1} + W_{pr2} + W_{pr3} + \dots + W_{pr10}$ ).

The calculation of total water consumption for the studied period of development of sunflower (phase of head formation - the beginning of flowering of plants) was determined by the formula:

$$\Sigma W = (W_{pr0} + Pr_{0-1} - W_{pr1}) \times KO \quad (3)$$

Where:

$\Sigma W$  – total water consumption during the study period: the phase of head formation - the beginning of flowering of plants, mm ha<sup>-1</sup>.

$W_{pr0}$  – total content of productive moisture at head formation phase, mm;

$Pr_{0-1}$  – precipitation for the period: phase of head formation - the beginning of flowering of plants, mm;

$W_{pr1}$  – total moisture supply at the end of the studied period of development of sunflower - phase of beginning of flowering of plants, mm;

$KO$  – coefficient of precipitation using (adopted of 0.7).

To transfer the soil moisture supply in m<sup>-3</sup>, the amount of moisture in millimeters (mm) was multiplied by 10, since a layer of water with a thickness of 1.0 mm on an area of 1.0 ha corresponds to 10.0 tons (1.0 m<sup>-3</sup>) of water.

The area of leaf surface of the sunflower plant was determined by the method of die-cutting:

$$S = \frac{M_l \times a \times \pi D^2}{M_{dc} \times 10000} \quad (4)$$

Where:

$S$  : Area of leaf surface of one plant, m<sup>-2</sup>;

$M_l$  : Total weight of leaves of one plant, g;

$a$  : The number of die-cutting from the leaves of one plant, pieces;

$\pi D^2$  : The area of one die-cutting from the leaves of a one plant, cm<sup>-2</sup>;

$\pi$  : 3,14;

$D$  : The diameter of the drill, which is used to make die-cutting in the leaves of the plant, cm;

$M_{dc}$  : Weight of die-cutting per plant, g;

10000: Square centimeter to square meter conversion coefficient (conversion base:

1 m<sup>-2</sup> = 10000 cm<sup>-2</sup>).

The content of dry matter in sunflower plants was determined by the gravimetric method by drying in an oven at a temperature of 105°C.

The net productivity of photosynthesis (NPP), showing the accumulation intensity of biomass plants by unit area of leaves per unit time (g m<sup>-2</sup> per day) and characterizing the difference between photosynthesis and respiration of plants, was calculated by the formula:

$$NPP = \frac{(B_2 - B_1)}{0.5 (S_1 + S_2) \times t} \quad (5)$$

Where:

$NPP$ : Net productivity of photosynthesis of sunflower plants, g m<sup>-2</sup> per day;

$B_2$ : Dry weight of sunflower plants per square meter of sowing at the end of the accounting (investigated) period (beginning of plants flowering), g;

$B_1$ : Dry weight of sunflower plants per square meter of sowing at the beginning of the accounting (investigated) period (phase of head formation), g;

$S_1$ : Leaf area of sunflower plants at the beginning of the accounting (investigated) period (phase of head formation), m<sup>-2</sup>;

$S_2$ : Leaf area of sunflower plants at the end of the accounting (investigated) period (beginning of plants flowering), m<sup>-2</sup>;

$t$ : Period (in days) between selection samples in the experiment at the beginning and end of the accounting (investigated) period, days.

### Statistical Analysis

In investigation was used to test of the experiment data reliability using analysis of variance the software packages: ANOVA, Microsoft Office Excel. Reliability of experimental data, verification of significance between the studied indicators were carried out on the basis of the least significant difference (LSD) test. The indicators values of experiment in article are expressed as mean at years investigations. Statistical processing of the research results was performed in accordance also with methods Dospekhov (1985), Aksyonov et al. (2023).



**Results**

The results of the conducted studies showed that during the period of plant development from the stage of head formation to flowering, sunflower samples 52 and 129 had the highest consumption of the moisture from the soil: 38.1 and 43.5 m<sup>-3</sup> ha, respectively (Table 1).

The minimum consumption of soil moisture during the period of head formation and the beginning of flowering of 10.2-13.2 m<sup>-3</sup> ha was observed at the sunflower varieties Emerald, Igolya, Orlik. Sunflower sample 52, with a high level of the consumption of soil moisture, was characterized by the lowest net photosynthetic productivity – 9.6 g m<sup>-2</sup> per day.

The maximum weight of the head seeds 78.7 and 60.2 g was formed at new varieties Igolya and Emerald. The maximum weight of the head seeds 78.7 and 60.2 g was formed at new varieties Igolya and Emerald. The seeds weight of head these varieties was more on 7.3 – 15.6 g, than at variety-control Sur.

Research shown, that in the arid conditions of the Ukrainian Steppe, the duration of the period and the duration of the period of plant development "seedlings - flowering" did not determine to a significant extent the level of yield of these samples and varieties of sunflower with a vegetation period of 102 - 110 days (Table 2).

For example, with the same growing season 110 days sample of 129 and variety Igolya had the different level yield. The yield of sample 129 was formed on the level 1.7 t ha<sup>-1</sup>, the variety yield of Igolya was formed on the level 2.49 t ha<sup>-1</sup>.

The yield level was determined by the formed mass of seeds in the head. The varieties of Emerald and Igolya, having more maximum weight of head seed, formed more high level of yield: 2.79 - 3.49 t ha<sup>-1</sup>.

It should be noted that in the conditions of the Steppe Ukraina, the sunflower samples of 52, 129, 44 with less yield 1.60; 1.70; 1.97 t ha<sup>-1</sup> had more the high content of feat in the seeds of 52.9; 52.3; 52.4%.

**Discussion**

A comparative analysis of the experimental data showed that, the newly obtained varieties of the sunflower Emelard, Igolya, and Orlik, with the minimum consumption of moisture from the soil in this stage of plant development – 12.4; 10.2, and 13.2 m<sup>-3</sup> ha, had low net productivity of photosynthetic of 11.9; 10.1, 12.6 g m<sup>-2</sup> per day, respectively.

The maximum net productivity of photosynthetic of 16.8 and 17.3 g m<sup>-2</sup> per day was characteristic of samples 33 and 65 with the average consumption of soil moisture of 20.9 and 21.9 m<sup>-3</sup> ha.

In the comprehensive assessment of plant development, the consumption indicators of soil moisture and net productivity of photosynthetic determined different levels of the ratio between these indicators. The high level consumption of soil moisture with low productivity of photosynthetic determined the maximum high ratio between these indicators of plant development at samples of sunflower 52 and 129.

Table 1. Traits indicators of of sunflower plants, (average for 2016-2023).

Sample	CSM	NPP	CSMPU	Weight of head seeds, g*
33	20.9	16.8	1.24	52.9
44	22.4	10.4	2.15	45.7
52	38.1	9.6	3.97	36.0
65	21.9	17.3	1.26	51.0
129	43.5	11.2	3.88	38.8
Malakhit	19.7	16.0	1.23	54.2
Emerald	12.4	11.9	1.04	60.2
Igolya	10.2	10.1	1.01	78.7
Orlik	13.2	12.6	1.05	58.7
Least significant difference 0.05	2.5	2.2	0.12	3.4

CSM: Consumption of soil moisture during the accounting period: phase of head formation - beginning of head flowering, m<sup>-3</sup> ha\*; NPP: Net productivity of photosynthesis (NPP) of plants: phase of head formation - beginning of head flowering, g m<sup>-2</sup> per day\*; CSMPU: Consumption of soil moisture per unit of NPP in the phase of head formation - beginning of head flowering, m<sup>-3</sup> g m<sup>-2</sup> per day\*; \*Significance level is p < 0.05. Values were calculated by taking the average of three replicates. No statistically significant difference was found between the years in the variance analysis.

Table 2. Yield of the created varieties and samples of sunflower by estimate the consumption of moisture per unit net productivity of photosynthesis, t ha<sup>-1</sup>, (average for 2016-2023).

Variety, sample	DPV	DIP	Plant height, cm*	Yield, t ha <sup>-1</sup> *	Content of seed fat, %*
Sur (control)	107	55	139.2	2.49	49.1
33	104	50	142.3	2.24	53.0
44	104	50	145.6	1.97	52.4
52	107	55	157.1	1.60	52.3
65	105	51	152.9	2.15	51.9
129	110	57	127.9	1.70	52.9
Malakhit	102	51	115.4	2.41	49.1
Emerald	102	50	98.1	2.79	50.4
Igolya	110	55	110.2	3.49	51.4
Orlik	102	50	107.8	2.68	49.4
Least significant difference, 0.05	1.1	1.0	4.7	0.12	0.6

DPV: Duration of period vegetation, day\*; DIP: Duration of interphase period "seedling-flowering", day\*; \*Significance level is p < 0.05. Values were calculated by taking the average of three replicates. No statistically significant difference was found between the years in the variance analysis



Table 3. Correlation matrix of the relationship between the developmental traits of sunflower plants, (average for 2016-2023).

Correlation coefficient	Value
Consumption of soil moisture and net productivity of photosynthesis	- 0.21
Consumption of soil moisture and consumption of soil moisture per unit of NPP	0.95
Consumption of soil moisture and weight of head seeds	- 0.87
Net productivity of photosynthesis and consumption of soil moisture per unit of NPP	- 0.50
Net productivity of photosynthesis and weight of head seeds	0.03
Consumption of soil moisture per unit of NPP and weight of head seeds	- 0.91

The ratio between the consumption moisture and net productivity of photosynthetic was  $3.97 \text{ m}^{-3} \text{ g m}^{-2}$  per day at sample 52 and  $3.88 \text{ m}^{-3} \text{ g m}^{-2}$  per day per day at sample 129. A decrease the productivity of the photosynthesis process to 9.6 and  $11.2 \text{ g m}^{-2}$  per day at samples 52 and 129 did not lead to the decrease in the consumption of soil moisture per unit of net productivity of photosynthetic due to the maximum consumption of the water by plants during the period of plant development from head formation to the beginning of flowering.

The lowest consumption of soil moisture per unit of net productivity of photosynthetic was 1.04; 1.01;  $1.05 \text{ m}^{-3} \text{ g m}^{-2}$  per day were characteristic for the varieties of the Emelard, Igolya, and Orlik, which consumed a minimal amount consumption of moisture from the soil and had minimal rates of photosynthesis in the arid conditions of the Steppe.

The sunflower varieties with lower consumption of soil moisture per unit of net productivity of the photosynthetic formed a greater weight of seeds in one head. The maximum weight of seeds in one head was noted at the varieties Emerald (60.2 g), Igolya (78.7 g), Orlik (58.7 g). Increase the consumption of soil moisture per unit of net productivity of the photosynthetic had a negative effect on the formation of the weight of seeds in the head and contributed to decrease of the seeds weight in the head. The sunflower samples 52 and 129 with the maximum consumption soil moisture per unit of NPP of 3.97 and  $3.88 \text{ m}^{-3} \text{ g m}^{-2}$  per day formed the minimum weight of seeds in one head of 36.9 g and 38.8 g in the droughty conditions. The varieties and samples of sunflower with a greater weight of seeds in the head had lower values of soil moisture consumption at stages 5-8 of ontogenesis and consumed less the soil moisture per unit of net productivity photosynthetic.

The conducted correlation analysis of the development indicators of sunflower plants did not show a correlation between the moisture consumption during the study period and the net productivity of photosynthesis, the correlation coefficient was  $r = -0.21$  (Table 3).

Increasing or decreasing in moisture consumption during the period of head formation – beginning of flowering did not contribute and not fully affect the increasing of the photosynthetic productivity. The strong positive correlation was found between the soil moisture consumption and soil moisture consumption per unit of net productivity of photosynthetic, the correlation coefficient  $r = 0.95$ . Correlation analysis shows a negative direct relationship between soil moisture consumption per unit of net productivity of photosynthetic and the weight of head seeds:  $r = -0.91$ . The increasing weight of head seeds in

sunflower genotypes was due the decreasing of the consumption of soil moisture per unit of net productivity of photosynthetic. Sunflower genotypes that form the maximum weight of head seeds in droughty conditions had less dependence from the consumption of soil moisture at the stage of plant development: head formation – beginning of flowering. The maximum weight of seeds in the head of 58.7-78.7 g at the varieties Emelard, Igolya, Orlik was determined the reduced consumption of the moisture from the soil:  $10.2-13.2 \text{ m}^{-3} \text{ ha}$  and the reduced consumption of the soil moisture per unit of net productivity of photosynthesis:  $1.01-1.05 \text{ m}^{-3} \text{ g m}^{-2}$  per day in the phase of head formation - the beginning of flowering.

The weight of the seeds of the head determined the level of formation of the yield in samples and varieties of sunflower. Over the years of research, the newly obtained varieties of sunflower Emelard, Igolya, Orlik in droughty conditions of the growing season with a minimum consumption of soil moisture per unit of net productivity of photosynthesis of  $1.01-1.05 \text{ m}^{-3} \text{ g m}^{-2}$  per day formed the highest yield of  $2.68-3.49 \text{ t ha}^{-1}$  with a fat content in the seeds of 49.4-51.4%.

The varieties that require the less quantity of moisture and consume the less quantity of moisture per unit of net productivity of the photosynthetic have the ability to forming the high levels of yield in relation to droughty conditions of vegetation periods.

Such varieties formed the yield on  $0.19-1.0 \text{ t ha}^{-1}$  higher than the control variety Sur. At the sunflower samples 52 and 129 with the highest amount of soil moisture consumption per unit of net productivity photosynthetic of 3.97 and  $3.88 \text{ m}^{-3} \text{ g m}^{-2}$  per day, the productivity in droughty conditions of steppe was formed at the lowest level of  $1.60-1.70 \text{ t ha}^{-1}$ . By the absence of a direct effect of net productivity of photosynthetic on the formation of the weight of seeds in the head and the yield of sunflower genotypes, the indicators of soil moisture consumption of plants and the consumption of soil moisture per unit of net productivity photosynthetic at the fifth stage of ontogenesis can be reliable criteria for assessing genotypes for drought resistance and adaptability of genotypes for growing in arid conditions of the Steppe of Ukraine.

By the absence of a direct effect of net productivity of photosynthetic on the formation of the weight of seeds in the head and the yield of sunflower genotypes, the indicators of soil moisture consumption of plants and the consumption of soil moisture per unit of net productivity photosynthetic at the fifth stage of ontogenesis can be reliable criteria for assessing genotypes for drought resistance and adaptability of genotypes for growing in arid conditions of the Steppe of Ukraine.

Thus, the level of formation of the yield of sunflower genotypes in the arid conditions of the Steppe of Ukraine is largely determined by a lower consumption of soil moisture per unit of net productivity of photosynthesis in the phase of plant development: formation of the head – the beginning of flowering. More highly productive varieties adapted to growing in arid conditions consume less moisture on the stage of formation of generative organs of plants.

## Conclusions

The net productivity of photosynthesis at the stage of formation of generative organs of sunflower plants does not have a direct effect on the formation of the weight of the seeds of the head. In the greater extent, the formation of the weight of the seeds of the head depends on the amount of consumed moisture from soil by plants during this period of development.

In the drought conditions of the steppe of Ukraine, the weight of the seeds of the sunflower head has strongly dependence from the consumption amount of soil moisture per unit of net productivity of photosynthesis in the phase of plant development: of the formation of the head - the beginning of flowering. The minimum consumption of soil moisture per unit of net productivity of photosynthesis objectively characterizes the ability of sunflower genotypes to form the maximum mass of seeds of the head, ultimately forming the maximum yield.

A negative correlation was established between the indicators of soil moisture consumption by plants per unit of net productivity of photosynthesis and the weight of the seeds of the head, the correlation coefficient was  $r = -0.91$ .

In the conditions of the Steppe of Ukraine, the decreasing of level of moisture consumption per unit of net productivity of photosynthesis at the fifth stage of plant ontogenesis ensures the formation of maximum yield of 2.68-3.49 t ha<sup>-1</sup> at sunflower varieties Emelard, Igolya, Orlik.

Determining the level of soil moisture consumption during the period of head formation - the beginning of flowering allows to evaluate and select the genotypes highly productive of sunflower that consume less moisture per unit of dry matter accumulation by plants in the phase of formation of generative organs of plants in the most critical period for water consumption.

Knowledge and understanding of the physiological and biological patterns of sunflower development at fifth stage of plant ontogenesis allows us to select and create genotypes with a high level of productivity, adapted to cultivation in the Steppe of Ukraine.

## Declarations

### Author Contribution Statement

Contributed to this work of authors.

Aksyonov Igor: development of an experimental project, project administration, analysis data, supervision, conceptualization, methodology, writing the end option of article, review and editing.

Koychenko Marina: Data collection, investigation, formal analysis, and writing the original draft.

### Conflict of Interest

The authors declare no conflict of interest.

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## Frequent Irrigation Under Increasing Doses of Stabilized Sewage Sludge in The Soil Increases the Yield and Quality of Silage Maize

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### ABSTRACT

Achieving higher efficiency and better quality production with appropriate irrigation regimes in silage maize cultivation in soils mixed with urban sludge is a requirement of sustainable agriculture. Therefore, a two-year field study was carried out with three replicates with four different sewage sludge doses (D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha, and D3: 90 t/ha), and three different irrigation regimes (S1, S2, and S3). In the S1, S2 and S3 regimes, when the sum of (Reference evapotranspiration - Effective rainfall) was 25 mm, 50 mm and 75 mm, respectively, irrigation was carried out and the soil moisture deficit was completed to the field capacity. Considering the two-year average, increasing sewage sludge dose and frequent irrigation significantly increased fresh and dry biomass yields and crude protein, while decreasing acid detergent fiber (ADF) and neutral detergent fiber (NDF). The fresh biomass yield in D3 treatment was 12.4%, 20.6%, and 42.1% higher than D2, D1, and D0, respectively. ADF in D3 was 5.6%, 2.1% and 1.7% lower than D0, D1 and D2, respectively, while NDF was 4.4%, 3.7% and 2.1% lower. D3 treatment increased the crude protein value by 27.3%, 15.5% and 7.7% compared to D0, D1 and D2 treatments, respectively. S1 provided 12.9% and 28.3% higher fresh biomass yield compared to S2 and S3. ADF value in S1 was 0.69% and 2.4% lower than S2 and S3, respectively, and NDF value was 0.86% lower compared to S3. There was a positive linear relationship with a high correlation between fresh and dry biomass yields. It could be concluded that D3-S1 treatment is the most effective practice for higher and quality yields, and followed by D3-S2 treatment.

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## Introduction

The sewage sludge produced on a global scale is increasing every year due to population growth, urbanization and continuous growths in wastewater treatment. Sludge consists of water, organic matter, and inorganic components commonly known as bio-solids (Fijalkowski et al., 2017). Disposal of sewage sludge is a technically, economically, and environmentally necessary procedure (Altun & Sahin, 2021).

The use of controlled and appropriate amounts of sewage sludge in agricultural production areas stands out as one of the simplest disposal methods of sludge (Mondal et al., 2015; Çakır & Çimrin, 2018). Stabilized sewage sludge is an important nutrient storage in addition to being a source of organic matter. The two main reasons for the use of sewage sludge, which is purified from pathogens and bad odor through the stabilization process, in agriculture are that they are a cost-effective fertilizer source and also serve as a good soil conditioner. Therefore, the soil is enriched in terms of macro and micro elements needed by

plants with the application of sewage sludge, while the addition of organic matter to the soil provides a sustainable contribution to soil fertility (Zuo et al., 2019). Vaca et al. (2011) stated that the addition of sewage sludge to the soil does not pose an environmental threat and offers a solution to the final disposal of organic waste. Skowrońska et al (2020) stated that the carbon and nitrogen levels in the soil increased significantly with the application of sewage sludge, especially at high doses, and that these changes had the potential to be permanent in the long term, and they also emphasized that it did not pose a risk in terms of heavy metal concentrations. However, increases in heavy metal content in soils with sewage sludge added may change depend on the dose of the sludge, the duration of its stay in the soil, and the type and concentration of the metal in it.

Maize is an important agricultural plant that can be successfully grown on almost every continent except Antarctica, can adapt to different climate and soil conditions, can grow at altitudes up to 4000 m from sea

level, and performs C4 photosynthesis (Özkan & Bayhan, 2022). It is one of the most produced plants in our country as well as in the world. According to 2023 plant production statistics in Türkiye, 28 653 531 tons of silage maize is produced in 524 860,9 ha of land. The total production amount of Erzurum province in 2023 is 93 649 tons in 1 901.1 ha of land (TSI, 2024). Silage maize is preferred as an important forage plant in nutrition of especially for dairy cattle due to its high dry matter content and green grass yield, and its suitability for silage production without the need for additives (Keskin et al., 2018). Çakır & Çimrin (2018) determined significant increases in dry and fresh biomass yields of maize plants with increasing sewage sludge doses. The product quality of silage maize is also important, in this context, crude protein, acid detergent fiber, neutral detergent fiber are the most known quality parameters (Ors et al., 2015).

Maize is highly sensitive to water stress and shows different responses to water deficit at different developmental stages (Ors et al. 2015; Yerli et al., 2023). Therefore, considerable decreases in soil moisture content is considered a limiting factor for maize growth (Peichl, 2018). Shahrabian and Soleymani (2011) determined that increased soil water consumption in silage maize cultivation by creating different soil moisture regimes at three different stress levels (low, medium and high) significantly reduced dry matter yield.

In this study, it was aimed to increase the yield and quality of silage maize by mixing stabilized sewage sludge including domestic wastes with soil at different doses and irrigation management. Within the scope of this research carried out in Erzurum semi-arid ecology, irrigation timing was arranged according to different water consumption levels with real-time water consumption approach and different moisture stress conditions between successive irrigations were created in the soil. Therefore, the hypothesis of this study was that frequent irrigation under high dose sewage sludge in soil would increase biomass yield and product quality in silage maize.

## Materials and Methods

### *Experimental Site and Experimental Processes*

The research was carried out at Atatürk University, Plant Production Application and Research Center in Erzurum, Turkey (39.933° N and 41.236° E, 1780 m a.s.l) using four stabilized sewage sludge doses and three irrigation regimes, in a 4x3 factorial design and with three replications. Thus, a trial consisting of a total of 36 plots was established. The trial region has a semi-arid climate with an annual precipitation of 395.7 mm. Average temperature, total precipitation and evaporation values in the vegetation periods were 18.2°C, 80.1 mm and 935.1 mm in 2021, respectively, and 17.6°C, 111.2 mm and 826.2 mm in 2022. Field soil was clay loam texture in surface layer of 0-30 cm, pH, EC, organic matter content was 7.61, 0.163 dS/m, 1.73%, respectively. Each plot 25.2 m<sup>2</sup> (3.5 m × 7.2 m) sizes with five rows. Irrigation was applied surface drip irrigation system using groundwater.

Sewage sludge obtained from Erzurum-İlca municipal wastewater treatment plant was used, which was stabilized by the activities of bacteria in anaerobic and mesophilic conditions. The stabilized sewage sludge with 29.9% dry

matter content was applied to the plots with the doses of D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha and D3: 90 t/ha and mixed to a soil depth of 15 cm with a hoeing machine in 22 September 2020. The field was plowed a vertical rotavator at a depth of 15 cm on May 7 in 2021 and on May 13 in 2022, and then the DKC6777 seed variety was planted with a pneumatic seeder at 15×70 cm intervals. Nitrogen and P fertilization considering region requirements to complete deficit amounts according to initial soil fertility analysis was applied in without sewage sludge plots in both years, but mineral fertilizer was not applied in the plots where sewage sludge was used. Hoeing was carried out in two stages where the plants reached 15-20 cm and 40-50 cm height.

Irrigation regimes were implemented by completing the moisture lost at the effective root depth to field capacity when the total of the (ET<sub>c</sub> – Peff) reached approximately 25 mm (S1), 50 mm (S2) and 75 mm (S3) levels. Crop evapotranspiration (ET<sub>c</sub>) values were estimated by calculation using the ET<sub>o</sub> × K<sub>c</sub> equation. Crop coefficient (K<sub>c</sub>) values were taken from the Evapotranspiration Guide for Irrigated Crops in Türkiye (TAGEM, 2017). Reference evapotranspiration (ET<sub>o</sub>) calculations were made with CROPWAT software according to the Penman-Monteith (FAO) method, the necessary climate data were obtained from the Erzurum Airport meteorology station close to the trial area, and the precipitation data were obtained from the pluviometer installed in the trial area. Since there was no deep flow and surface flow from precipitation, all were considered as effective precipitation (Peff). Soil moisture monitoring was performed before each irrigation using a TDR device (Trime-Pico, IPH/T3, IMKO) in the upper layer, and using the gravimetric method in the lower layers.

### *Crop Measurements and Statistical Analysis*

Harvesting was done manually on September 9 in 2021 and on September 10 in 2022. Fresh biomass yield was calculated by weighing 30 plants selected from the center of the plots on a precision scale, and these data were converted into biomass yield per unit area (kg/ha). To determine the dry matter ratio according to the method in Kacar (2014), the fresh weight of three randomly selected plants representing the plot was determined, then the plants were left to wither in greenhouse conditions for a while and oven dried at 78°C for 48 hours, then their dry weight was measured. The dry matter ratio was obtained by dividing the dry weight by the fresh weight, and the dry biomass yield was obtained by multiplying of dry matter ratio by the fresh biomass yield (kg/ha).

Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) analyses were carried out using the Fiber Bag System (Gerhardt), which was developed by modifying the Van Soest (1963) analysis method, and using the ANKOM 200 Fiber Analyzer. Crude protein content was calculated by multiplying the nitrogen amount determined by the Kjeldahl method by the coefficient of 6.25 (Yerli et al., 2023).

Data were analyzed with the general linear model in SPSS (ver. 22) statistics program. Duncan multiple comparison test was applied for significant differences and classification was made at 5% significance level. Binary relationships were investigated with Pearson correlation analysis.

## Results and discussion

### Fresh Biomass Yield

Sewage sludge and irrigation treatments had significant ( $p \leq 0.01$ ) effects on fresh biomass yield in both trial years and in the average of trial years. The interaction of dose and irrigation regime was also determined to be significant ( $p \leq 0.01$ ) in the second trial year only (Table 1). Increasing application dose of the sewage sludge and frequent irrigation practice provided significant increases in fresh biomass yield in both trial years. According to two-year averages, fresh biomass yield obtained with D3 treatment was 12.4% higher than D2, 20.6% higher than D1 and 42.1% higher than D0. Frequent irrigation treatment (S1) provided 12.9% higher fresh biomass yield compared to S2 and 28.3% higher biomass yield compared to S3 (Table 2). Considering the treatments interaction in the two-year average, the highest fresh biomass yield was determined as 130505.3 kg/ha in D3-S1 treatment, which provided 81% more yield than D0-S3, where the lowest value was determined. The fresh biomass yield of the D3-S1 treatment, in which the highest value was obtained (130505.3 kg/ha), corresponds to 2.39 times the 2023 silage maize Türkiye average of 54670 kg/ha, 2.51 times the Northeast Anatolia Region average of 52090 kg/ha, and 2.65 times the Erzurum province average of 49260 kg/ha (TSI, 2024).

The sewage sludge used in the experiment contained 38.6% organic matter, 4.13% total Kjeldahl nitrogen, 451.5 mg/kg  $\text{NO}_3\text{-N}$ , 1.32% P, 10.4 cmol/kg K, 41 cmol/kg Ca and 83.6 cmol/kg Mg, which could be seen as an important factor for the increase in yield at increasing doses due to increased soil fertility. Similarly, Yerli et al. (2023) stated that the increase in macro and micronutrient contents in the soil contributes the silage yield for a considerable level. Especially, the increase in soil N content caused significant increases in yield (Saracoglu, 2023; Norouzi et al., 2024). It was also reported by Motta and Maggiore (2013) that yield was increased together with increased plant N uptake. Da Silva et al. (2022) determined significant increases in maize yield in soils with sewage sludge. Černý et al. (2012) reported that sewage sludge application increased silage maize yield by 19% to 25% compared to without sludge. In addition, some other studies also have indicated that increases in N, P, K and other nutrients in the soil provide significant increases in the yield (Mousavi & Shahsavari 2014; Cakmakci & Sahin 2021; Gezer et al., 2023).

It is clear that in conditions where soil moisture is retained at low tensions, the low stress effect of both matrix and osmotic potential supports plant development by facilitating easily water and nutrient uptake by plants. Similarly, Ucak et al. (2016), Cakmakci & Sahin (2021), and Yerli et al. (2023) determined that increased durations of water stress in silage maize was obtained significant decreases in fresh biomasses yield. In addition, these findings were confirmed by many others examining the effect of water stress on yield loss (Tabatabaei et al., 2017; Zare et al., 2018; Akşit, 2020; Keten, 2020; Kamali et al., 2022; Balbaa et al., 2023).

Increasing sewage sludge doses and frequent irrigation regime supported yield by improving physiological indicators (SPAD, stomatal conductance, leaf relative water content, leaf area index, membrane damage index) in this study. The linear relationships between fresh biomass yield and leaf area index, the fresh biomass yield and the leaf relative water content, membrane damage and leaf relative water content, stomatal conductivity and fresh biomass yield, and SPAD and fresh biomass yield were found significant (Altun & Sahin, 2021, 2022, 2023). Similarly, Moreira et al. (2020) reported that sewage sludge application increased specific physiological traits such as photosynthetic activity and stomatal conductance in maize (Moreira et al., 2020). Karasu et al. (2015), Cakmakci & Sahin (2021), and Yerli et al. (2023) also reported significant correlations between the fresh biomass yield and the physiological parameters of the maize crop.

### Dry Biomass Yield

It is desired that the dry matter ratio of the green feed to be silage is at least between 25% and 30%. This value can be higher in silage maize (Koca, 2020). In this study, a dry matter ratio of around 28% was determined.

In both trial years and the average of the years, significant ( $p \leq 0.01$ ) effects of sewage sludge and irrigation treatments on dry matter yield were determined. In the second year of the trial and the average of the years, it was observed that the interaction between dose and irrigation regime was also significant ( $p \leq 0.01$ ) (Tablo 1). According to the two-year average, the highest dry biomass yield was determined in D3-S1 treatment as 37583.4 kg/ha (Table 3). This value corresponds to an 80% increase compared to D0-S3, which provides the lowest value. The changes in dry biomass yields were parallel to the fresh biomass yields, and were supported by a positive linear relationship with a high correlation between these two variables (Figure 1). Cakmakci & Sahin (2021) and Yerli et al. (2023) also reported positive linear relationships between fresh and dry biomass yields in silage maize.

Koutroubas et al. (2023) and Özgür et al. (2023) determined that increasing the sewage sludge dose had a positive effect on dry matter yield. Increasing water stress in the soil between irrigations can negatively affect photosynthetic efficiency by decreasing the metabolic activities of the maize plant and decreasing the chlorophyll content in the leaves. Decreased photosynthetic level with the decrease in the metabolic activity and the chlorophyll content of maize in the leaves reduces dry biomass yield (Gomaa et al., 2021). Moreover, it has been reported that the effect of water stress is associated with a decrease in the leaf area index, causing a decrease in dry matter yield (Bouazzama et al., 2012). The findings of this study also showed that the leaf area index decreased with the effect of increasing stress from S1 to S3 irrigation in the soil (Altun & Sahin, 2022). Some previous studies reported that increasing the level of water stress in the soil negatively affects dry matter yield, and that changes in dry matter yield are directly related to fresh biomass yield (Ucak et al., 2016; Kale et al., 2018; Cakmakci & Sahin 2021; Demir et al., 2021; Yerli et al., 2023).

Table 1. Variance analysis

Parameter	Year	Variance Sources	df	Mean Square	F	P
Fresh biomass yield	2021	Dose	3	1662926621	64.856	.000
		Irrigation	2	2215984225	86.426	.000
		Dose × Irrigation	6	1.72E+07	0.67	.675
		Error	24	2.56E+07		
	2022	Dose	3	2094483289	242.425	.000
		Irrigation	2	1509177369	174.679	.000
		Dose × Irrigation	6	3.92E+07	4.539	.003
		Error	24	8.64E+06		
	2021-2022	Dose	3	186688.3287	196.521426	.000
		Irrigation	2	183668.1549	193.342176	.000
		Dose × Irrigation	6	1.55E+03	1.62942133	.182
		Error	24	9.50E+02		
Dry biomass yield	2021	Dose	3	119087592.2	56.511	.000
		Irrigation	2	152068196.8	72.162	.000
		Dose × Irrigation	6	1.99E+06	0.942	.484
		Error	24	2.11E+06		
	2022	Dose	3	154143052.2	661.137	.000
		Irrigation	2	117896761.6	505.673	.000
		Dose × Irrigation	6	5.53E+06	23.74	.000
		Error	24	2.33E+05		
	2021-2022	Dose	3	135978174.3	195.86	.000
		Irrigation	2	134435177.3	193.638	.000
		Dose × Irrigation	6	2.63E+06	3.788	.009
		Error	24	6.94E+05		
ADF	2021	Dose	3	4.983	12.375	.000
		Irrigation	2	1.879	4.667	.019
		Dose × Irrigation	6	9.20E-02	0.228	.964
		Error	24	4.03E-01		
	2022	Dose	3	4.92	11.799	.000
		Irrigation	2	1.874	4.496	.022
		Dose × Irrigation	6	8.70E-02	0.21	.970
		Error	24	4.17E-01		
	2021-2022	Dose	3	4.983	12.375	.000
		Irrigation	2	1.879	4.667	.019
		Dose × Irrigation	6	9.20E-02	0.228	.964
		Error	24	4.03E-01		
NDF	2021	Dose	3	9.795	27.227	.000
		Irrigation	2	1.049	2.917	.073
		Dose × Irrigation	6	5.00E-02	0.14	.989
		Error	24	3.60E-01		
	2022	Dose	3	14.806	45.518	.000
		Irrigation	2	0.427	1.314	.287
		Dose × Irrigation	6	4.00E-02	0.123	.992
		Error	24	3.25E-01		
	2021-2022	Dose	3	11.916	70.554	.000
		Irrigation	2	0.646	3.824	.036
		Dose × Irrigation	6	3.90E-02	0.232	.962
		Error	24	1.69E-01		
Crude protein	2021	Dose	3	14.046	656.627	.000
		Irrigation	2	0.092	4.295	.025
		Dose × Irrigation	6	2.00E-03	0.075	.998
		Error	24	2.10E-02		
	2022	Dose	3	6.364	58.296	.000
		Irrigation	2	0.372	3.407	.050
		Dose × Irrigation	6	2.00E-02	0.184	.978
		Error	24	1.09E-01		
	2021-2022	Dose	3	9.879	298.868	.000
		Irrigation	2	0.189	5.706	.009
		Dose × Irrigation	6	7.00E-03	0.204	.972
		Error	24	3.30E-02		

Table 2. Fresh biomass yields (kg/ha) in silage maize at different sewage sludge doses and irrigation treatments

Year	IT	D0	D1	D2	D3	Mean
2021	S1	94606.4±4713.0	107513.3±2293.1	113932.3±3755.3	124142.9±1675.4	110048.7±3527.1A*
	S2	76804.2±3089.6	98211.7±1420.0	100095.2±3447.2	114555.5±1385.3	97416.7±4202.7C
	S3	67608.5±2432.6	79359.8±1463.0	85624.3±3372.5	98976.7±3658.6	82892.3±3624.0D
	Mean	79673.0±4340.4D	95028.2±4234.5C	99883.9±4450.9B	112558.4±3866.9A*	
2022	S1	95214.8±1835.2d	110333.3±1138.3c	115772.5±851.1b	136867.7±2220.6a*	114547.1±4552.3A*
	S2	81391.5±1057.5ef	99922.8±1739.3d	108012.7±1328.0c	116470.9±1828.0b	101449.5±1828.4B
	S3	76571.4±1479.6f	84888.9±3330.0e	99000.0±963.4d	108460.3±885.8c	92230.2±3808.8C
	Mean	84392.6±2891.4D	98381.7±3862.7C	107595.1±2481.2B	120599.6±4316.5A*	
2021-2022	S1	94910.6±2591.8	108923.3±1707.2	114852.4±1979.0	130505.3±1472.4	112297.9±3940.4A*
	S2	79097.9±1918.0	99067.2±1458.9	104054.0±1603.4	115513.2±677.3	99433.1±4020.5B
	S3	72089.9±1931.4	82124.3±1465.6	92312.2±1562.8	103718.5±2263.2	87561.2±3628.9C
	Mean	82032.8±3544.7D	96705.0±3988.8C	103739.5±3366.7B	116579±3957.9A*	

IT: Irrigation treatment; D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha, D3: 90 t/ha. S1, S2, and S3: irrigation when  $\Sigma$  (estimated evapotranspiration – effective precipitation) equals to 25 mm, 50 mm and 75 mm, respectively; \*: p < 0.05

Table 3. Dry biomass yields (kg/ha) in silage maize at different sewage sludge doses and irrigation treatments

Year	IT	D0	D1	D2	D3	Mean
2021	S1	26605.3±826.5	29021.7±440.2	31789.7±650.8	35810.7±1128.9	30806.8±1087.0A*
	S2	22254.3±202.5	27097.0±340.2	28301.0±1565.2	30379.0±812.3	27007.8±979.0B
	S3	19659.0±602.9	22451.0±667.9	24227.0±1312.9	28433.7±233.9	23692.7±1019.7C
	Mean	22839.6±1057.0D	26189.9±1006.9C	28105.9±1255.8B	31541.1±1176.4A*	
2022	S1	27099.9±100.2e	30796.2±173.5c	32341.7±76.4b	39356.2±366.6a*	32398.5±1343.5A*
	S2	23880.4±224.9f	28549.3±333.0d	30450.2±299.9c	33090.7±239.0b	28992.7±1021.0B
	S3	22102.9±421.4g	24028.3±487.0f	28402.8±75.1d	30016.7±152.8c	26137.7±974.6C
	Mean	24361.1±744.6D	27791.2±1010.8C	30398.2±576.1B	34154.6±1380.5A*	
2021-2022	S1	26852.6±363.2ef	29908.9±216.0c	32065.7±292.7b	37583.4±707.9a*	31602.7±1196.1A*
	S2	23067.4±24.2g	27823.2±336.6de	29375.6±912.2c	31734.9±520.1b	28000.2±984.4B
	S3	20880.9±482.5h	23239.6±275.0g	26314.9±672.0f	29225.2±187.6c	24915.2±967.8C
	Mean	23600.3±889.4D	26990.6±994.8C	29252.1±896.7B	32847.8±1264.9A*	

IT: Irrigation treatment; D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha, D3: 90 t/ha. S1, S2, and S3: irrigation when  $\Sigma$  (estimated evapotranspiration – effective precipitation) equals to 25 mm, 50 mm and 75 mm, respectively; \*: p < 0.05

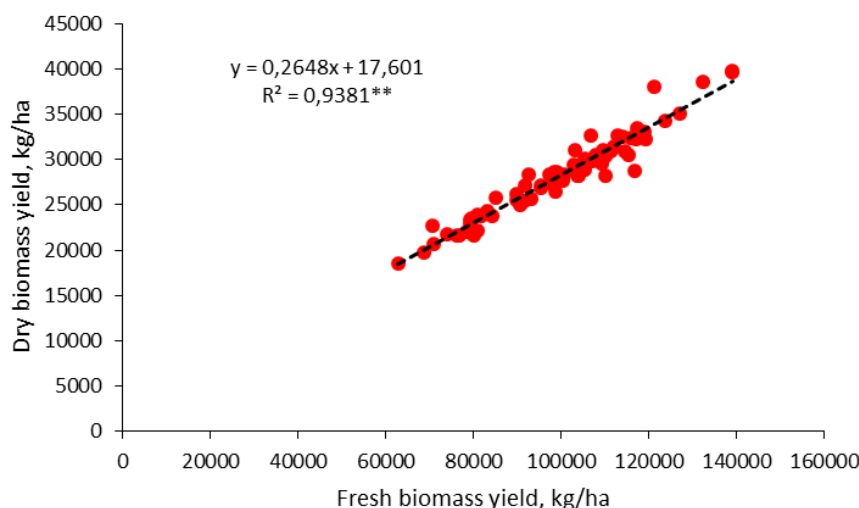


Figure 1. Relationship between fresh and dry biomass yields (n = 72, \*\*: p < 0.01)

**Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), And Crude Protein Content**

ADF and NDF are important quality parameters that allow the evaluation of digestibility and energy intake. High ADF indicates an excess of cellulose and lignin that are difficult to digest, high NDF indicates an excess of cellulose and fibrous carbohydrates of the cell wall such as hemicellulose, lignin, some proteins and silicon (Yerli et al., 2023). Therefore, it is reported that ADF is an indicator

of the digestibility of the plant, and NDF is an indicator of its uptake by animals (Mese & Gulumsar, 2021).

While significant (p ≤ 0.01) effects of sewage sludge treatments on ADF and NDF were determined in both trial years, significant (p ≤ 0.05) effect of irrigation regimes was determined only on ADF. However, significant effects of both sewage sludge doses (p ≤ 0.01) and irrigation regimes (p ≤ 0.05) were determined in the average of the trial years (Table 1).



Table 4. Acid detergent fiber (ADF) values (%) in silage maize at different sewage sludge doses and irrigation treatments

Year	IT	D0	D1	D2	D3	Mean
2021	S1	29.1±0.32	28.2±0.59	28.0±0.08	27.7±0.41	28.3±0.23B
	S2	29.6±0.37	28.3±0.41	28.3±0.35	27.8±0.26	28.5±0.25B
	S3	30.2±0.37	29.0±0.38	28.8±0.46	28.0±0.08	29.0±0.28A*
	Mean	29.6±0.24A*	28.5±0.27B	28.4±0.21B	27.9±0.15B	
2022	S1	30.1±0.32	29.2±0.59	29.0±0.08	28.7±0.41	29.3±0.23B
	S2	30.6±0.37	29.3±0.41	29.3±0.35	28.8±0.26	29.5±0.25B
	S3	31.2±0.37	30.0±0.38	29.8±0.46	29.0±0.08	30.0±0.28A*
	Mean	30.6±0.24A*	29.5±0.27B	29.4±0.21B	28.9±0.15B	
2021-2022	S1	29.6±0.32	28.7±0.59	28.5±0.08	28.2±0.41	28.8±0.23B
	S2	30.1±0.37	28.8±0.41	28.8±0.35	28.3±0.26	29.0±0.25B
	S3	30.7±0.37	29.5±0.38	29.3±0.46	28.5±0.08	29.5±0.28A*
	Mean	30.1±0.24A*	29.0±0.27B	28.9±0.21B	28.4±0.15B	

IT: Irrigation treatment; D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha, D3: 90 t/ha. S1, S2, and S3: irrigation when  $\Sigma$  (estimated evapotranspiration – effective precipitation) equals to 25 mm, 50 mm and 75 mm, respectively; \*:  $p < 0.05$

Table 5. Neutral detergent fiber (NDF) values (%) in silage maize at different sewage sludge doses and irrigation treatments

Year	IT	D0	D1	D2	D3	Mean
2021	S1	57.6±0.42	57.5±0.52	56.7±0.16	55.1±0.56	56.7±0.36
	S2	57.7±0.18	57.5±0.32	56.8±0.35	55.4±0.34	56.8±0.30
	S3	58.1±0.20	57.9±0.13	57.1±0.43	56.0±0.21	57.3±0.27
	Mean	57.8±0.16A*	57.7±0.19A	56.9±0.18B	55.5±0.24C	
2022	S1	60.2±0.26	59.7±0.22	58.2±0.23	57.2±0.57	58.8±0.39
	S2	60.3±0.24	59.7±0.36	58.5±0.34	57.7±0.30	59.1±0.34
	S3	60.6±0.28	59.8±0.21	58.7±0.55	57.7±0.21	59.2±0.36
	Mean	60.4±0.15A*	59.8±0.14B	58.5±0.21C	57.5±0.21D	
2021-2022	S1	58.9±0.32	58.6±0.29	57.5±0.09	56.2±0.04	57.8±0.34B
	S2	59.0±0.12	58.6±0.33	57.7±0.28	56.5±0.13	57.9±0.31AB
	S3	59.3±0.19	58.9±0.17	57.9±0.37	56.9±0.19	58.3±0.30A*
	Mean	59.1±0.13A*	58.7±0.14A	57.7±0.15B	56.5±0.13C	

IT: Irrigation treatment; D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha, D3: 90 t/ha. S1, S2, and S3: irrigation when  $\Sigma$  (estimated evapotranspiration – effective precipitation) equals to 25 mm, 50 mm and 75 mm, respectively; \*:  $p < 0.05$

The lowest ADF and NDF values were determined in D3 treatment among sewage sludge doses and S1 treatment among irrigation regimes in both trial years (Tables 4 and 5). When the two-year average is considered, in D3 treatment, ADF value was found to be 5.6%, 2.1% and 1.7% lower compared to D0, D1 and D2 treatments, respectively; NDF value was found to be 4.4%, 3.7% and 2.1% lower. For S1 treatment, ADF value was found to be 0.69% and 2.4% lower compared to S2 and S3, respectively, and NDF value was found to be 0.86% lower compared to S3.

Crude protein value was significantly affected by both sewage sludge doses ( $p \leq 0.01$ ) and irrigation regimes ( $p \leq 0.05$ ) in both experimental years and the average of the years ( $p \leq 0.01$ ) (Table 1). According to two-year averages, D3 treatment increased the crude protein value by 27.3%, 15.5% and 7.7% compared to D0, D1 and D2 treatments, respectively (Table 6). In the S1 treatment, the increase was 3.0% compared to the S3 treatment. It was evaluated that the main reason for the increase in the crude protein value with the ratio of sewage sludge application was due to the high nitrogen content of the sewage sludge. Kale et al. 2018 and Yerli et al. (2023) reported that there was an increase in the crude protein value in silage maize as the content of nitrogen in the soil and therefore in the plant increased. Therefore, in the S3 treatment, it was evaluated that the increased stress between irrigations caused lower crude protein value with less plant N uptake compared to

more frequent irrigations. Previous studies have reported that as water stress increases in silage maize, the protein content decreases because the plant cannot get enough nutrients (Aydınsakir et al., 2013; Cakmakçı, 2018, Yerli et al., 2023).

Yerli et al. (2023) indicated that lower ADF and NDF values in silage maize are associated with the higher crop N content, so crude protein content. Therefore, it was evaluated that the lower ADF and NDF values in D3 treatment were associated with the increase in crude protein values from the increase in sewage sludge dose (Table 6). Correlative relationships between crude protein and ADF and NDF also show that the increase in crude protein leads to a significant decrease in ADF and NDF values (Figures 2 and 3). Many previous studies have shown that increasing the protein content in silage maize via increasing N content reduces the ADF value (Kaplan et al., 2016; Kale et al., 2018; Cakmakçı, 2018; Yerli et al., 2023). Nitrogen is an essential nutrient for plant growth and development. Plants with high nitrogen content can increase their photosynthetic activity by remaining active for longer periods of time. This delays the maturation process of the plant and can therefore positively affect the health and productivity of the plant. Therefore, Safdarian et al. (2014) reported that the delay in the maturation process of the plant due to the effect of nitrogen caused the decrease of ADF and NDF, and indicated the negative relationship of N with ADF and NDF.

Table 6. Crude protein values (%) in silage maize at different sewage sludge doses and irrigation treatments

Year	IT	D0	D1	D2	D3	Mean
2021	S1	8.9±0.18	9.9±0.08	10.9±0.04	11.8±0.01	10.4±0.33A*
	S2	8.8±0.03	9.8±0.09	10.8±0.06	11.7±0.04	10.3±0.33AB
	S3	8.7±0.05	9.7±0.04	10.8±0.16	11.6±0.03	10.2±0.33B
	Mean	8.8±0.06D	9.8±0.05C	10.8±0.05B	11.7±0.03A*	
2022	S1	9.1±0.18	9.8±0.10	10.2±0.11	10.9±0.26	10.0±0.22A*
	S2	8.7±0.03	9.5±0.13	10.1±0.30	10.8±0.22	9.8±0.25AB
	S3	8.6±0.12	9.4±0.18	9.9±0.17	10.7±0.27	9.7±0.24 B
	Mean	8.8±0.09D	9.6±0.09C	10.0±0.11B	10.8±0.13A*	
2021-2022	S1	9.0±0.18	9.9±0.05	10.5±0.06	11.3±0.14	10.2±0.27A*
	S2	8.8±0.02	9.7±0.02	10.5±0.17	11.2±0.13	10.0±0.28AB
	S3	8.7±0.06	9.6±0.09	10.3±0.01	11.2±0.12	9.9±0.28B
	Mean	8.8±0.07D	9.7±0.05C	10.4±0.06B	11.2±0.07A*	

IT: Irrigation treatment; D0: 0 t/ha, D1: 30 t/ha, D2: 60 t/ha, D3: 90 t/ha. S1, S2, and S3: irrigation when  $\Sigma$  (estimated evapotranspiration – effective precipitation) equals to 25 mm, 50 mm and 75 mm, respectively; \*:  $p < 0.05$

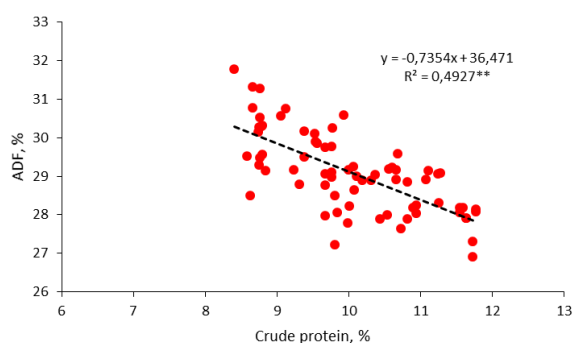


Figure 2. Relationship between crude protein and acid detergent fiber (ADF) (n = 72, \*\*:  $p < 0.01$ )

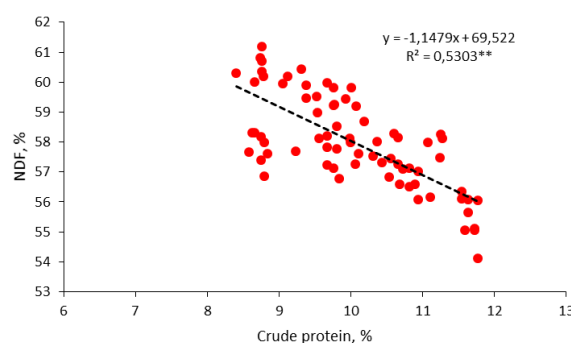


Figure 3. Relationship between crude protein and neutral detergent fiber (NDF) (n = 72, \*\*:  $p < 0.01$ )

## Conclusion

In this study, the effects of different doses of sewage sludge in soil on biomass yield and quality of biomass of silage maize under frequent and wider intervals irrigation regimes were investigated. Frequent irrigation and high dose of sewage sludge provided better finding for yield and quality. At 90 t/ha, 60 t/ha, and 30 t/ha sewage sludge doses, fresh and dry yields under frequent irrigation were approximately 1.4 times, 1.2 times, and 1.1 times higher than those under frequent irrigation without sewage sludge. Frequent irrigation at the highest dose decreased ADF and NDF approximately 5%, and increased crude protein 26% compared to the treatment without sewage sludge. Therefore, it can be concluded that frequent irrigation in sewage sludge dose of 90 t/ha can be the better practice to obtain higher yield and quality forage in silage maize cultivation. However, these findings need to enlargement with the findings under different climatic conditions and also higher doses.

## Declarations

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### Conflict of Interest

The authors declare no conflict of interest.

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## Mitigation of Flood Stress in Mazamort Pepper Variety through Manganese Application

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### ABSTRACT

This study was conducted to investigate the effect of Mn application on the resistance of pepper plants exposed to flood stress. The study was conducted in a climate-controlled room at Siirt University, utilizing the Mazamort three-lobed pepper variety as plant material. In the climate chamber (19 m<sup>2</sup>), conditions were established at 24±1°C during the day and 18±1°C at night, with a light/dark photoperiod of 16/8 hours. The growing medium consisted of a 2:1 (v) mixture of peat and perlite. Four treatment groups were established: control, flood stress, 2.5 mg/L manganese (Mn), and flood stress combined with 2.5 mg/L Mn. Sixty-day-old Mazamort pepper plants were subjected to continuous flooding and manganese application at each watering. The duration of flood stress was set at 0 days (control) and 10 days. The experiment was designed using a randomized complete block design with three replications, each containing 10 plants. Parameters evaluated at the end of the study included visual assessment, plant height, stem diameter, leaf number, leaf fresh and dry weight, leaf moisture content, root fresh and dry weight, root moisture content, chlorophyll content (SPAD value), ion leakage, relative water content (RWC), and turgor loss. The highest plant height was observed in the 2.5 mg/L manganese treatment (45.82 cm), while the greatest stem diameter was recorded in the control group. The highest leaf number (30.60) and SPAD value (35.34) were also noted in the control group. RWC was highest at 96.90% in the 2.5 mg/L manganese treatment. The maximum turgor loss was 5.606% in the control group, and the highest ion leakage (17.880%) was observed in the 2.5 mg/L manganese treatment. It was concluded that manganese application mitigated the negative effects of flood stress on various parameters; however, it did not fully restore the values to control group levels.

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## Introduction

Seasonal rainfall and flooding are recognized as natural phenomena in many ecosystems; however, they can lead to significant crop losses in agricultural contexts. In arid regions, both water scarcity and excess water act as major stress factors for plants (Nishiuchi et al., 2012; Erol, 2024). Climate change and increasing flood risks necessitate a deeper understanding of marginal crop cultivation and water tolerance in vulnerable areas. During flooding events, changes in soil concentrations of oxygen (O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), reactive oxygen species (ROS), and ethylene have been observed (Perata et al., 2011). Specifically, standing water in the soil for extended periods can lead to complete oxygen depletion, resulting in anoxia, while partial oxygen deficiency in roots is referred to as hypoxia (Drew & Lynch, 1980). Such conditions can cause significant damage to plant roots, prompting various morphological and physiological responses to the hypoxia induced by flooding.

Flood-sensitive plant species can develop adaptive structures, such as adventitious roots and aerenchyma, to cope with these conditions (Zhou et al., 2012; Akhtar & Nazir, 2013). However, the capacity for adaptation varies among plant species. For example, significant reductions in photosynthetic capacity have been observed in water-sensitive plants (Liao & Lin, 2001; Yavuz, 2024). During flooding, soil fills with water instead of air, which restricts oxygen uptake and leads to serious respiratory problems. The oxygen deficiency caused by flooding primarily affects the roots, hindering root development and potentially leading to root death. Plant responses can vary based on the timing of flooding, duration of soil saturation, and the plant species involved. Prolonged flooding often results in common adaptations such as the formation of adventitious roots and aerenchyma (Bradford & Hsiao, 1982; Campos et al., 2009; Haupt-Herting & Fock, 2000; Yavuz, 2024).

Oxygen deficiency can lead to nutrient deficiencies in plants, adversely affecting both root and shoot development. Reduced photosynthetic capacity may further increase plants' susceptibility to flooding. Decreased stomatal opening and the inhibition of specific plant hormones can negatively impact plant and leaf expansion. To survive in low-oxygen conditions, plants increase the production of ethylene in the root zone (Yavaş & Ünay, 2016). Consequently, the lack of oxygen in the root zone results in adverse outcomes that significantly affect plant growth (Dias-Filho & Dos Santos Lopes, 2011).

Plants may attempt to generate energy through anaerobic respiration by rapidly increasing protein synthesis. However, in flooded soils, gas diffusion rates decrease, leading to a reduction in root respiration—one of the earliest responses of plants to oxygen deficiency (Rajhi, 2011; Akhtar and Nazir, 2013). Oxygen deficiency weakens root growth by halting secondary metabolism and root development. Moreover, toxic compounds such as iron (Fe), manganese sulfate (MnSO<sub>4</sub>), and ammonia may accumulate in the soil (Visser et al., 1996).

Prolonged flooding can induce morphological changes in plants and promote the formation of aerenchyma, facilitating the transfer of oxygen from the shoots to the roots and enabling oxygen respiration in the roots (Yavaş & Ünay, 2016). In flood stress conditions, the formation of intercellular spaces in the root cortex increases, alongside lignification, which can heighten susceptibility to root rot. Plants unable to access sufficient nutrients from the soil may destroy harmful cells in their root tissues, leading to increased node numbers and internode lengths, as well as early senescence and yield losses (Yavaş & Ünay, 2016; Ekanayake, 1998).

Climate change models predict an increase in the frequency of flooding globally, turning flood stress into a significant environmental threat for plants. Annual crop damages resulting from non-seasonal and severe flooding events are estimated to amount to billions of dollars in yield losses (Pucciariello et al., 2014). With a growing global population and intensified agriculture, a reduction in arable land per capita is anticipated (FAO, 2006). Many crops are sensitive to flooding, and even a few days of inundation can cause serious damage. Identifying traits that enhance flood tolerance is therefore critical (Perata et al., 2011).

Manganese (Mn) is an essential micronutrient that serves various physiological functions in plants (Lidon et al., 2004; Ducic & Polle, 2005; Humphrie et al., 2007). While manganese can exhibit both synergistic and antagonistic effects on the uptake of other nutrients, such as iron, zinc, and copper, excessive levels may lead to nutrient imbalances and toxicity (Marschner, 1998; Shenker et al., 2004). In Turkey, peppers represent a significant agricultural product with extensive consumption both fresh and processed (Anonymous, 2018). A substantial portion of pepper production is processed industrially, providing a vital source of employment in the agricultural sector (Güvenç, 2017; Güvenç, 2020).

This study focused on the Mazamort pepper cultivar, which was subjected to flood stress for two different durations: 0 days (control) and 10 days. The experimental treatments included a control group, partial flood stress, 2.5 mg/L manganese (Mn), and a combination of partial flood

stress with 2.5 mg/L Mn. The objective of the present study was to investigate the effects of manganese application on the morphological and physiological resistance of pepper plants exposed to flood stress.

## Materials and Methods

The experiment was conducted in the climate chamber of the Plant Protection Department at Siirt University. The plant material used was the Mazamort three-lobed pepper variety, obtained from Sunagri Seed Company. This commercially available variety is suitable for both greenhouse cultivation and open-field production. The fruits are 10-12 cm long, crisp, sweet, uniformly shaped, dark green, and three-lobed, making them ideal for fresh consumption. The plant material was produced from seeds. In the climate chamber (19 m<sup>2</sup>), conditions were established at 24±1°C during the day and 18±1°C at night, with a light/dark photoperiod of 16/8 hours. A growing medium consisting of a 2:1 (v) ratio of peat to perlite was prepared for seed sowing and seedling cultivation. After transferring the prepared medium into 45-cell trays with an inner depth of 5 cm and outer dimensions of 33 cm x 54 cm, seeds were sown. Once the seeds germinated and the plants reached the 3-4 leaf stage, they were transplanted into larger pots. The transplanted pots had a top diameter of 160 mm, a height of 140 mm, and a volume of 2 liters.

### Application of Flood Stress to Plants

After the seedlings adapted to their environment and overcame transplanting stress, flood stress was applied as a partial flooding treatment. The plants subjected to flood stress were placed in deep plastic containers, where they remained until the end of the treatment. Partial flooding stress was implemented by submerging the plants above the root collar. The treatments included control, 2.5 mg/L manganese (Mn), partial flooding, and partial flooding combined with 2.5 mg/L Mn. Manganese was applied as a foliar spray, while control plants received a foliar spray of distilled water. The duration of flood stress was set to 0 days (control) and 10 days. The treatments were determined based on findings from previous studies (Dere et al., 2019; Zhen et al., 2021). The experiment was designed with three replications, each consisting of ten plants. The plants were provided with a nutrient solution (in ppm) containing: N (150), P (50), K (150), Ca (150), Mg (50), Fe (5.0), Mn (0.5), B (0.5), Zn (0.05), Cu (0.03), and Mo (0.02). The pH and electrical conductivity (EC) of the nutrient solution were maintained within the range of 6.0-6.5 and 1.8-2.0 dS/m, respectively. The plants were exposed to flood stress for the designated periods, after which the specified measurements and observations were conducted.

### Parameters to be Evaluated in the Experiment

#### Morphological Parameters

Visual Scale Assessment: A scale assessment was conducted to evaluate the damage resulting from flood stress in the plants. The damage was rated on a scale from 0 to 5 based on the condition of the plants (Kuşvuran, 2010):

- 0: No damage to pepper plants under flood stress
- 1: Yellowing and curling of pepper leaves

2: 25% necrosis and yellowing of pepper leaves  
 3: 25-50% necrosis and observed leaf drop in pepper plants  
 4: 50-75% necrosis in pepper leaves and onset of plant death

5: 75-100% necrosis in pepper plant leaves occurs rapidly and/or the plant death completely

*Plant Height (cm)*

The height of the plants was measured in centimeters from the root collar to the apex using a ruler (Dere et al., 2019).

*Stem Diameter (mm)*

The stem diameters of the plants were measured in millimeters at the midpoint using a caliper (Dere et al., 2019).

*Leaf Number (number/plant)*

At the end of the treatments, the number of leaves on each plant was counted and recorded (Yılmaz, 2020).

*Fresh Plant Weight (g)*

The fresh weights of the plant samples collected at the end of the treatment were recorded in grams using a precision scale (Yılmaz, 2020; Altuntaş et al., 2020).

*Dry Plant Weight (g)*

The fresh weights of the plants were placed in paper bags and dried in an oven at 75 °C until a constant weight was achieved. The dry weight was then recorded in grams (Yılmaz, 2020; Altuntaş et al., 2020).

*Moisture Content (%)*

Moisture content was determined using the following formula based on the fresh and dry weights of the plants (Köksal et al., 2016):

$$MCwb = (FW - DW / FW) \times 100 \quad (1)$$

Where:

MCwb: Moisture content (%)

FW: Fresh weight of the root (g)

DW: Dry weight of the root (g)

*Ion Leakage (%)*

To measure ion leakage, 1 cm diameter leaf disks were placed in deionized water for 5 hours, and the electrical conductivity (EC1) was measured. The same disks were then incubated at 75 °C for 24 hours, and the EC (EC2) was measured again. Ion leakage was calculated using the following formula (Arora et al., 1998):

$$Ion\ Leakage = (EC1 / EC2) \times 100 \quad (2)$$

*Relative Water Content of Leaves*

At the end of the treatments, 1 cm diameter leaf disks were taken as samples of equal age and size. The fresh weights (FW) of these disks were recorded using a precision scale. The disks were then placed in petri dishes containing distilled water and allowed to hydrate for 4 hours. Turgor weight (TW) was then measured, followed by drying the samples in an oven at 70 °C for 24 hours to determine dry weight (DW) (Kılıç, 2023):

$$RWC(\%) = (FW - DW / TW - DW) \times 100 \quad (3)$$

Where:

FW: Fresh weight

TW: Turgor weight

DW: Dry weight

*Turgor Loss (%)*

Turgor loss was calculated based on the fresh and turgor weights of the leaf disks:

$$Turgor\ Loss(\%) = (TW - FW / TW) \times 100 \quad (4)$$

Where:

FW: Fresh weight

TW: Turgor weight

*Chlorophyll Content of Leaves (SPAD)*

At the end of the treatments, SPAD readings were taken for samples of the same age and size using a Minolta SPAD 502 meter (Minolta 502, Osaka, Japan), with three replicates (Daşgan et al., 2010; Dere, 2019). These readings were used to assess the green shade of pepper plants, which varies according to the chlorophyll content in the fourth leaves.

*Statistical Analysis*

The obtained data were subjected to analysis of variance (ANOVA) using the JUMP 7.0 software package. Means that showed significant differences were grouped using the LSD multiple comparison test (Jump, 2007).

**Results and Discussion**

The effects of the treatments on plant height were statistically significant (Figure 1;  $p < 0.05$ ). This study examined the impact of various treatments on the height of the Mazamort pepper variety. The control group exhibited an average height of 45.08 cm. When a 2.5 mg/L manganese (Mn) treatment was applied, the plant height increased to 45.82 cm, indicating a slight enhancement in growth due to manganese. Conversely, under flooding stress, the plant height significantly decreased to 36.66 cm, demonstrating that flooding stress adversely affects plant growth. When Mn was applied in conjunction with flooding stress, the plant height measured 41.96 cm. These results suggest that Mn application partially alleviated the negative effects of flooding stress; however, it did not fully restore growth to the levels observed in the control group. Therefore, the influence of Mn on plant growth varies in the presence of water stress, highlighting the complex interactions between nutrient availability and abiotic stress conditions. Moderate waterlogging has been shown to significantly impair plant growth, leading primarily to stunting (Samad et al., 2001). For instance, in the case of *Sesamum indicum*, Mensah et al. (2006) demonstrated that plant height diminishes progressively with extended waterlogging duration. Furthermore, Collaku & Harrison (2002) reported that various wheat varieties and lines exhibited a marked reduction in height when subjected to waterlogging for 10, 20, and 30 days in controlled greenhouse conditions, reinforcing the notion that the impact of waterlogging is both duration-dependent and species-specific.

The effects of the treatments on stem diameter were statistically significant (Figure 2;  $p < 0.05$ ). This study evaluated the impact of various treatments on the stem diameters of the Mazamort pepper variety. The control group exhibited an average stem diameter of 7.10 mm.



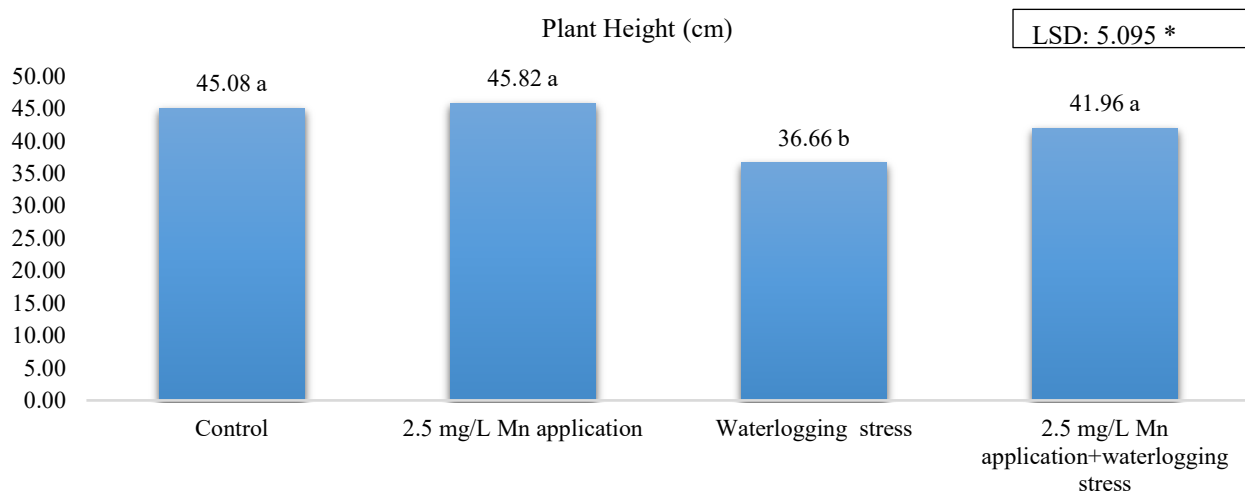


Figure 1. Effect of Manganese Application on Height of Pepper Plants under Waterlogging Stress Conditions

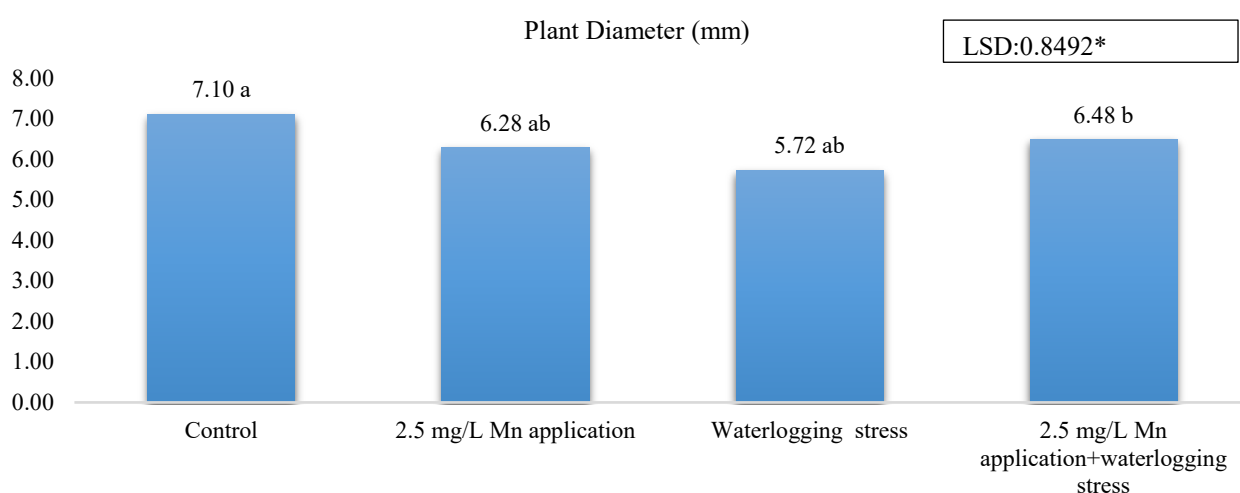


Figure 2. Effect of Manganese Application on Diameter of Pepper Plants under Waterlogging Stress Conditions

Following the application of 2.5 mg/L Mn, the stem diameter was measured at 6.28 mm, indicating that Mn application resulted in a reduction in stem diameter. Under flooding stress, the stem diameter further decreased to 5.72 mm, demonstrating the negative impact of flooding stress on plant growth. When Mn was applied in conjunction with flooding stress, the stem diameter was measured at 6.48 mm. These results suggest that Mn application partially mitigated the negative effects of water stress; however, it did not restore the stem diameter to the levels observed in the control group. Therefore, the effects of Mn on stem diameter vary in the presence of water stress, underscoring the complex interactions between nutrient application and abiotic stress conditions. This aligns with Villarreal-Navarrete et al. (2014), who reported that waterlogging stress negatively affected root growth parameters, including root collar diameter, in *Physalis peruviana*. Although they noted that waterlogging could initially increase root collar diameter in pepper plants, prolonged flooding led to a significant reduction. This highlights a complex relationship between water stress and plant growth, similar to your findings on the Mazamort pepper variety. Both studies underscore the detrimental effects of flooding on plant structural growth. Our results further emphasize how Mn application can influence these

parameters, though not entirely counteracting the adverse effects of water stress, reflecting the intricate dynamics between nutrient management and abiotic stress in agriculture.

The effects of the treatments on leaf number were found to be statistically insignificant (Figure 3;  $p < 0.05$ ). The highest leaf count was observed in the control group, with an average of 30.60 leaves. Following the application of 2.5 mg/L Mn, the leaf count decreased to 28.00, indicating that Mn application led to a reduction in leaf number. Under flooding stress, the leaf count further dropped to 27.00, demonstrating the adverse impact of flooding stress on the plant's leaf structure. When Mn was applied in conjunction with flooding stress, the leaf count measured 25.60. These results suggest that Mn application partially alleviated the negative effects of water stress; however, it did not restore leaf number to the levels observed in the control group. Therefore, the effects of Mn on leaf number vary in the presence of water stress conditions. Understanding these effects of Mn on plant growth and development, particularly under stress conditions, is crucial and highlights factors that should be considered in agricultural practices. This aligns with the findings of Nakayama & Komatsu (2008), Samad et al. (2001), Rao et al. (2002), Else et al. (2009), and Yetisir et al. (2006), all

of whom reported decreased leaf numbers in various crops due to flooding. Similarly, Çelik (2010) and Çelik & Turhan (2011) found that excess water reduced leaf counts in pigeon pea. Our research supports the broader consensus that flooding adversely affects leaf number across multiple species, reinforcing the idea that stress conditions negatively impact plant growth. Additionally, our findings on Mn suggest a potential for mitigation, although not enough to fully restore leaf numbers, highlighting the complexity of plant responses under stress and the importance of considering these factors in agricultural practices.

The effects of the treatments on the SPAD values of the plants were found to be statistically significant (Figure 4;  $p < 0.001$ ). The SPAD value for the control group was determined to be 35.34. Following the application of 2.5 mg/L Mn, the SPAD value decreased slightly to 33.82, indicating that Mn application resulted in a minor reduction in chlorophyll content in the plant leaves. Under flooding stress, the SPAD value significantly declined to 28.74, demonstrating that flooding stress considerably reduces chlorophyll content in the plant leaves. When 2.5 mg/L Mn was applied alongside flooding stress, the SPAD value measured 31.08. These results suggest that Mn application alleviated the negative effects of water stress, although it did not restore the SPAD values to those observed in the

control group. Consequently, the effects of Mn on SPAD values are contingent upon the presence of water stress conditions. These findings provide insights into the potential impacts of Mn use and water stress on plant productivity and chlorophyll content in agricultural practices. Huang et al. (1994) found that Bayles & Savannah wheat varieties subjected to flooding for 17 days experienced reduced photosynthesis and chlorophyll content. Other studies (Olgun et al., 2008; Zheng et al., 2009; Li et al., 2011) reported that chlorophyll content in wheat leaves did not change significantly immediately after flooding, but differences became apparent in the period following stress. These findings highlight that plant responses to flooding may vary based on species, growing conditions, and duration of flooding. This comparison reveals that both studies emphasize the detrimental impact of flooding on chlorophyll content. Additionally, our findings suggest that Mn application can partially mitigate these negative effects. However, the immediate decline in chlorophyll content observed in our study contrasts with literature indicating that significant changes may occur later. Overall, your research contributes to the understanding of how Mn interacts with flooding stress, pointing to the need for further investigation into its potential benefits in various species under different stress conditions.

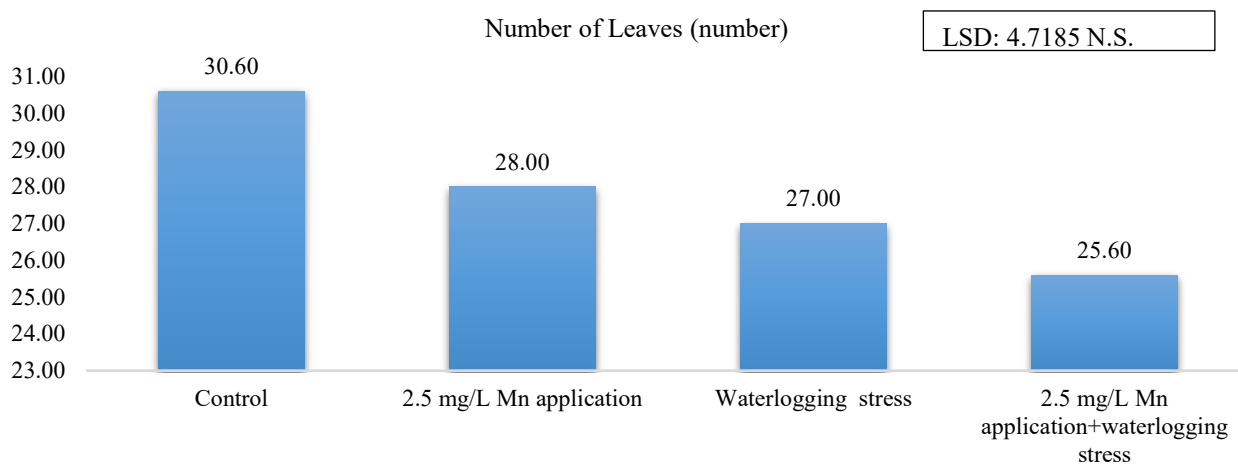


Figure 3. Effect of Manganese Application on Leaf Number of Pepper Plants under Waterlogging Stress Conditions

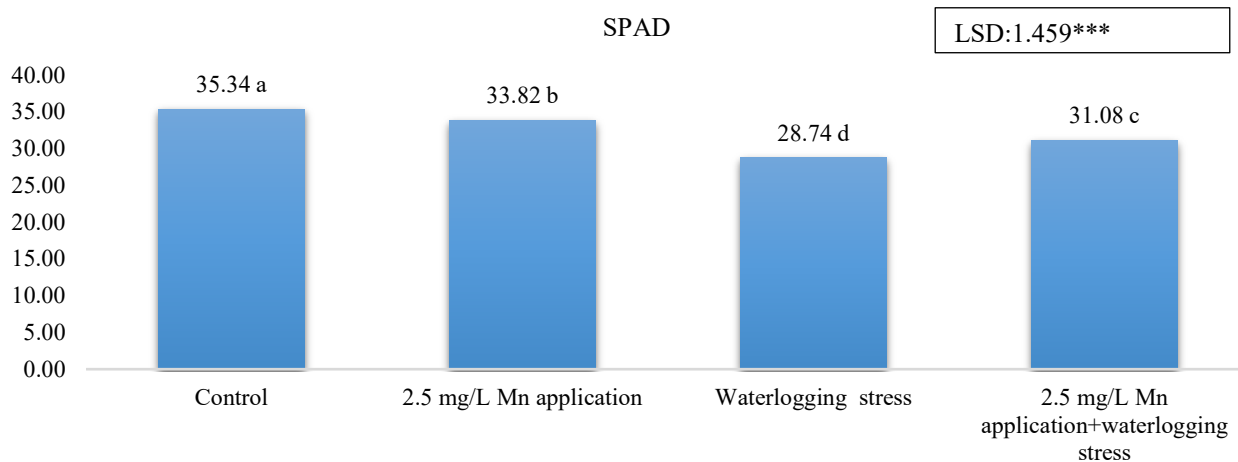


Figure 4. Effect of Manganese Application on SPAD Value of Pepper Plants under Waterlogging Stress Conditions

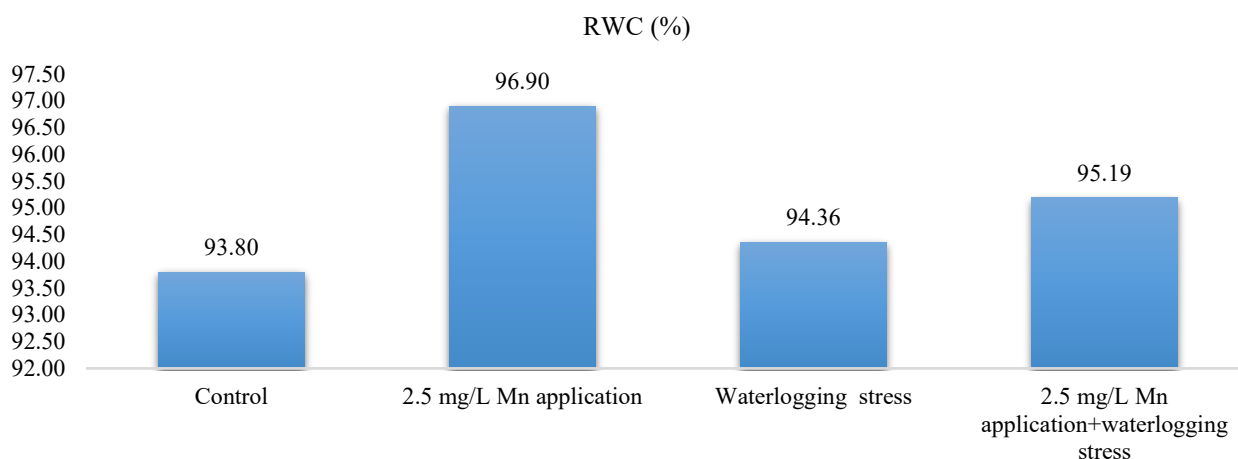


Figure 5. Effect of Manganese Application on RWC value of Pepper Plants under Waterlogging Stress Conditions

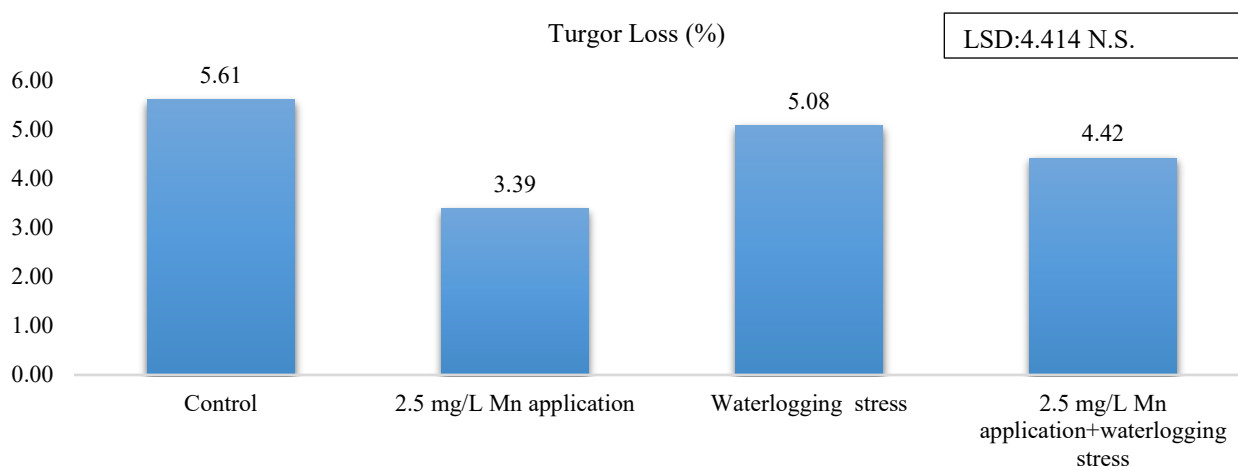


Figure 6. Effect of Manganese Application on Turgor Loss of Pepper Plants under Waterlogging Stress Conditions

The effects of the treatments on the relative water content (RWC) of the plants were not statistically significant (Figure 5;  $p < 0.05$ ). The RWC value for the control group was determined to be 93.80%. Following the application of 2.5 mg/L Mn, the RWC value increased to 96.90%, indicating that Mn application enhances water content in the plant leaves. Under flooding stress, the RWC value decreased to 94.36%, suggesting that flooding stress reduces water content in the leaves. When 2.5 mg/L Mn was applied in conjunction with flooding stress, the RWC value was measured at 95.19%. These results indicate that Mn application mitigated some of the negative effects of flooding stress, although it did not fully restore RWC values to those of the control group. Thus, the impact of Mn on RWC values is influenced by the presence of water stress conditions. These findings contribute to our understanding of the potential effects of Mn use on plant water status and resilience in agricultural practices. These findings align with the results reported by Kozłowski (1984), which indicated that waterlogging caused stomatal closure and reduced leaf water potential and turgor ratio in various tree seedlings. Similarly, Ashraf & Rehman (1999) noted that waterlogging reduced leaf turgor potential, reinforcing the idea that water stress adversely affects plant water status. In contrast, Ahmed et al. (2002) found that, in mung beans, the leaf water potential of control plants did

not differ significantly after 8 days of waterlogging, suggesting variability in responses among different species. Overall, our research contributes to understanding how Mn can influence plant water status under stress conditions, paralleling the findings in the literature regarding the negative effects of waterlogging on water potential and turgor. These comparisons emphasize the complexity of plant responses to water stress and highlight the potential for Mn to enhance resilience in agricultural practices, albeit not to the extent of fully restoring optimal water content.

The effects of the treatments on plant turgor loss were not statistically significant (Figure 6;  $p < 0.05$ ). In the control group of the rocket plant, a turgor loss of 5.606% was observed. Following the application of 2.5 mg/L Mn, the turgor loss decreased to 3.386%, indicating that Mn application reduces turgor loss in the plants. Under flooding stress, turgor loss increased to 5.075%, suggesting that flooding stress exacerbates turgor loss. When 2.5 mg/L Mn was applied in conjunction with flooding stress, the turgor loss was measured at 4.418%. These results indicate that Mn application mitigated some of the negative effects of water stress, although it did not fully restore turgor loss levels to those of the control group. Therefore, the effects of Mn on turgor loss vary depending on the presence of water stress conditions.

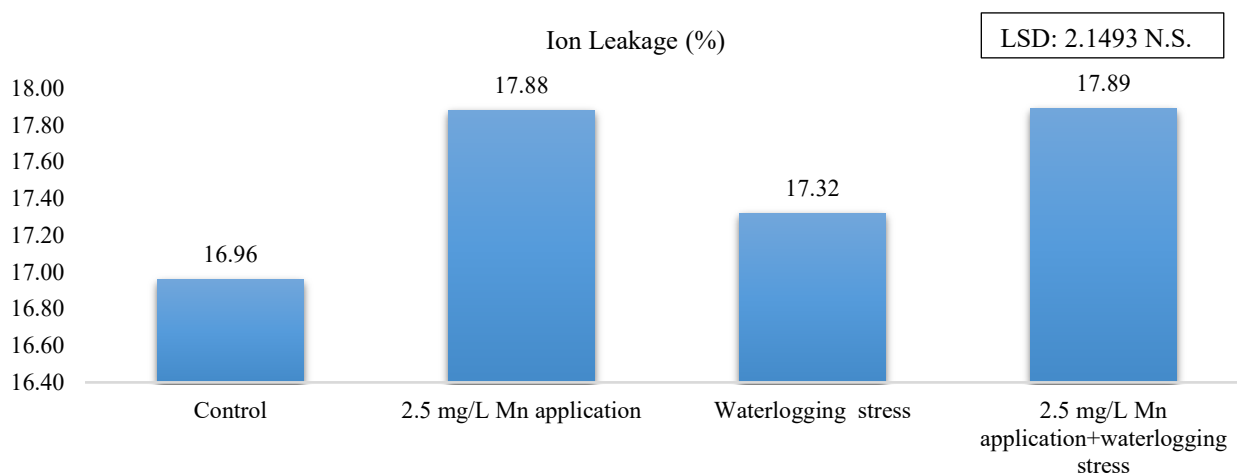


Figure 7. Effect of Manganese Application on Ion Leakage of Pepper Plants under Waterlogging Stress Conditions

These findings provide insights into the potential impacts of Mn use on plant water status and resilience in agricultural practices. This aligns with the findings of Ashraf & Rehman (1999), who reported that waterlogging reduced leaf turgor potential, reinforcing the idea that water stress negatively impacts plant turgor. The detrimental effects of waterlogging on plant water status and turgor, emphasizing how external stressors can significantly affect plant resilience. Our research adds a nuanced understanding by demonstrating that Mn can reduce turgor loss even under stress, though it does not fully counteract the effects of flooding. This interplay suggests that while Mn may enhance resilience in stressful conditions, further investigation is necessary to explore its full potential in agricultural practices.

In the control group, ion leakage was measured at 16.961%. Following the application of 2.5 mg/L Mn, the ion leakage value increased to 17.88%, indicating that Mn application raises ion leakage in the plants. Under flooding stress, ion leakage was recorded at 17.32%, suggesting that flooding stress also elevates ion leakage in the plants. When 2.5 mg/L Mn was applied alongside flooding stress, ion leakage reached 17.89%. These results demonstrate that while Mn application alleviates some of the negative effects of water stress, it does not fully restore ion leakage levels to those of the control group. Thus, the impact of Mn on plant ion leakage varies with the presence of water stress conditions. These findings enhance our understanding of the potential effects of Mn use on plant health and ion balance in agricultural practices. This is consistent with Aydoğan & Turhan (2012), who reported increased ion leakage rates in all green bean genotypes after waterlogging, noting that these rates were higher than those observed during recovery applications. Both studies highlight the adverse effects of waterlogging on ion leakage, demonstrating a common response across different plant species. Our findings expand on this by showing that while Mn can help mitigate some impacts of water stress, it still results in elevated ion leakage compared to control conditions. This suggests that the effects of Mn on ion balance are complex and influenced by stress conditions, underscoring the need for further research to optimize Mn use in agricultural practices for improved plant health and resilience.

## Conclusion

This study evaluated the effects of manganese application and flooding stress on the growth and physiology of pepper plants. The impacts of manganese on plant height, diameter, leaf number, SPAD value, relative water content (RWC), turgor loss, and ion leakage were assessed in comparison to the control group. The effects of manganese on plant height varied depending on the presence of flooding stress. While the average height of plants in the control group was 45.08 cm, manganese application increased this value to 45.82 cm. However, under flooding stress, plant height significantly decreased to 36.66 cm. With the combined application of manganese and flooding stress, plant height was measured at 41.96 cm. These results indicate that manganese has a growth-promoting effect, although water stress partially mitigates this impact. Similarly, the assessment of plant diameter revealed that manganese application had a reducing effect compared to the control group. The average diameter in the control group was 7.10 mm, while this value dropped to 6.28 mm following manganese application. Under flooding stress, the diameter further decreased to 5.72 mm. However, after applying manganese alongside flooding stress, the diameter was recorded at 6.48 mm. These findings demonstrate that manganese reduces plant diameter, with flooding stress exacerbating this effect. Analysis of leaf number, SPAD value, RWC, turgor loss, and ion leakage also indicated that manganese generally exhibited reducing effects compared to the control group, while flooding stress intensified these effects. In conclusion, the influence of manganese application on growth and physiological parameters in pepper plants varies significantly under stress conditions such as flooding. These findings underscore the importance of carefully evaluating manganese use in agricultural practices and understanding its potential effects on plant health and productivity.

## Declarations

### *Ethical Approval Certificate*

There is no need for an ethics committee report for the study.

**Author Contribution Statement**

M.E.D.: Data collection, investigation, formal analysis, and writing the original draft.

S.D.: Project administration, supervision, conceptualization, methodology, review and editing.

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**Conflict of Interest**

The authors have no conflicts of interest.

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## Effect of Different Nitrogen Doses on Yield and Some Quality Elements in Some Barley Varieties (*Hordeum vulgare L.*)

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 08.10.2024 Accepted : 05.11.2024</p> <p><b>Keywords:</b> Malt Crude Protein Rate Grain Largness Ratio Hectoliter weight Grain yield</p>	<p>It was aimed to determine the effect of nitrogen applied at different doses on grain yield and some quality characteristics of four barley cultivars. In this study, which was conducted in Tokat during the 2018-19 and 2019-20 growing periods, according to the Factorial Trial Plan in Random Blocks with 3 replications, four different nitrogen doses (0, 3, 6, 9 and 12 kg/da) were used in Bolayır, Harvest, Hazar and Ünver barley varieties were used. In the study, the number of ears per m<sup>2</sup>, grain yield, 1000 grain weight, hectoliter weight, crude protein ratio and grain largness ratio were examined. Climatic factors significantly affected the investigated characteristics of the cultivars. In the study, the differences between varieties were found to be significant in terms of crude protein ratio (insignificant in the first year), number of spikes per square meter, grain yield, 1000 grain weight, hectoliter weight and grain largness ratio. The applied nitrogen generally increased the grain yield up to a certain dose, but decreased the malt quality characteristics. It was determined that Bolayır variety in terms of yield and malting characteristics and Ünver variety in terms of forage characteristics are in better than other varieties for the region. It has been determined that 3 kg N/da dose is sufficient in terms of malt characteristics, however, 6 kg N/da dose is appropriate if Bolayır and Ünver varieties with the highest grain yield are grown as feed. It was determined that the Bolayır variety was more suitable for the region in terms of grain yield and malting properties, and the Ünver variety was more suitable for the region than other varieties in terms of yield and forage properties.</p>

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## Farklı Azot Dozlarının Bazı Arpa (*Hordeum vulgare L.*) Çeşitlerinde Verim ve Bazı Kalite Öğelerine Etkisi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 08.10.2024 Kabul : 05.11.2024</p> <p><b>Anahtar Kelimeler:</b> Malt Ham Protein Oranı Tane İrilik Oranı Hektolitire ağırlığı Tane verimi</p>	<p>Farklı dozlarda uygulanan azotun dört arpa çeşidinde tane verimi ve bazı kalite özelliklerine etkisinin belirlenmesi amaçlanmıştır. Tokat'ta 2018-19 ve 2019-20 yetiştirme dönemlerinde Tesadüf Bloklarında Faktöriyel Deneme planına göre 3 tekerrürlü olarak yapılan bu çalışmada, dört farklı azot dozu (0, 3, 6, 9 ve 12 kg/da) ile Bolayır, Hasat, Hazar ve Ünver arpa çeşitleri kullanılmıştır. Araştırmada m<sup>2</sup>'deki başak sayısı, tane verimi, 1000 tane ağırlığı, hektolitire ağırlığı, ham protein oranı ve tane irilik oranları incelenmiştir. Ortalamaların karşılaştırılmasında LSD testi kullanılmıştır. İklim faktörleri çeşitlerin incelenen özelliklerini önemli derecede etkilemiştir. Araştırmada, ham protein oranı (İlk yıl önemsiz) metrekaredeki başak sayısı, tane verimi, 1000 tane ağırlığı, hektolitire ağırlığı ve tane irilik oranı açısından çeşitler arasında farklar önemli bulunmuştur. Uygulanan azot genelde belli bir doza kadar tane verimini artırmış, fakat malt kalite özelliklerini düşürmüştür. Verim ve malthık özelliği bakımından Bolayır, yemlik özelliği bakımından Ünver çeşidinin bölge için diğer çeşitlere göre daha iyi durumda oldukları belirlenmiştir. Malt özelliği bakımından 3 kg N/da dozunun yeterli olduğu, bununla beraber, en yüksek tane verimine sahip Bolayır ve Ünver çeşitlerinin yemlik olarak yetiştirilmesi durumunda 6 kg N/da dozunun uygun olduğu belirlenmiştir. Tane verimi ve malthık özelliği bakımından Bolayır, yine verim ve yemlik özellik bakımından uygun çeşidinin bölge için diğer çeşitlere göre daha uygun oldukları belirlenmiştir.</p>

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## Giriş

Genelde hayvan beslenmesinde ve malt sanayisinde kullanılan arpa bazen insan gıdası olarak tüketilmiştir. Ham protein oranı (%7,5-15,0) ve sindirilebilir besin maddesi oranının (%75) yüksek olması nedeniyle hayvan beslenmesinde önemli yer tutmaktadır (Kün, 1996). Türkiye’de yetiştirilen arpanın yaklaşık %95’lik hayvan beslenmesinde kullanılırken geriye kalan kısmı (%5) ise malt ve bira endüstrisinde değerlendirilmektedir (Anonim, 2022a). Bu nedenle arpa Türkiye tarımında ve malt sanayinde önemli bir yer tutmaktadır. Bununla beraber, Türkiye de ortalama 2,68 ton/ha arpa verimi halen dünya ortalamasından (3,0 ton/ha) azdır (Anonim, 2022a). Tokat ilinde de ortalama tane verimi (307 kg/da) düşük olarak kabul edilebilecek düzeydedir (TÜİK, 2022). Bölgede verimi artırmak için uygun çeşitlerin seçilmesi yanında diğer yetiştirme teknikleri ile beraber uygun gübrelemenin de yapılması gerekir. Bitkilerin ihtiyaç duydukları besin elementi seviyeleri bitkide hem vejetatif hem de generatif dönem arasında olumlu ilişki söz konusudur. Bitki besin elementinin tam yeterli olduğu düzey bitki için kritik yeterlilik seviyesidir.

Besin elementi miktarı bu kritik seviyeden düşük ise bu durumda besin elementi noksan demektir (Karaman M, 2012). Türkiye’de toprakta noksanlığı en çok görülen bitki besin maddesi azottur (Kınacı ve ark., 2008). Proteinin temelini oluşturan azot diğerine benzer olarak arpada da büyüme ve gelişme bakımından önemli bir etkiye sahiptir. Maltlık olarak yetiştirilecek arpalarda proteinin yüksek olması kalite açısından istenmemektedir. Bu nedenle maltlık arpalara azot uygulanırken dikkat edilmesi gereklidir (Kartal ve ark., 2003). Yemlik arpalarda ise protein oranının yüksek olması arzulanmaktadır. Bununla beraber yatmaya yol açmayacak dozda, azotlu gübreleme yapılması tane verimi açısından da oldukça önemlidir. Bu nedenle maltlık ve yemlik olarak yetiştirilen arpalara farklı azot gübreleme programına gerek vardır.

Bu araştırmada, Özdemir ve ark. (2022) tarafından yapılan çalışması neticesinde verim bakımından ümit var görülen çeşitlerden Bolayır, Hasat, Hazar ve Ünver’in tane verimi ve bazı kalite öğelerine azotun etkisi incelenmek suretiyle tane verimi ve kalite açısından uygun çeşit ve azot miktarının belirlenmesi amaçlanmıştır.

## Materyal ve Yöntem

Bu araştırma, 2018-19 ve 2019-20 yıllarında Tokat Gaziosmanpaşa Üniversitesi Tarımsal Uygulama ve

Araştırma Merkezi deneme alanlarında 2 yıl süreyle yürütülmüştür. Denemenin yürütüldüğü alanın toprakları killi-tınlı tekstür yapısında, organik madde (1,73) ve fosfor (4,12 P<sub>2</sub>O<sub>5</sub> kg/da) bakımından fakir, pH’sı kuvvetli alkali (8,7) karakterdedir. Araştırmanın yürütüldüğü Ekim-Haziran dönemindeki toplam yağış miktarı uzun yıllar ortalamasında 394,6 mm iken 2018-19 ve 2019-20 yetiştirme yıllarında sırasıyla 359,5 ve 354,6 mm olmuştur (Çizelge 1). Her iki yetiştirme döneminde uzun yıllara ait değerlere oranla daha az yağış olmuştur. Bununla beraber ikinci yetiştirme döneminde generatif gelişmenin gerçekleştiği Mart-Mayıs aylarındaki yağış miktarının ilk yıla oranla düşük olması dikkat çekmektedir. Deneme yıllarındaki aylık ortalama sıcaklık değerleri ile uzun yılların sıcaklık değerleri büyük ölçüde benzerdir

Şansa Bağlı Bloklarda Faktöriyel Deneme planına göre 3 tekerrürlü olarak kurulan bu çalışmada (Düzgüneş ve ark., 1987) 4 arpa çeşidi ve 5 azot dozu kullanılmıştır. Tarla Bitkileri Bölümü tarafından yürütülen çalışmada Tokat için ümit var olarak görülen çeşitlerden Bolayır, Hasat, Hazar ve Ünver (Özdemir ve ark., 2022) bu araştırmada materyal olarak kullanılmıştır. Çeşitlerden Hazar altı sıralı diğerleri ise 2 sıralı çeşitlerdir. İkinci faktör olarak 0, 3, 6, 9 ve 12 kg N/da dozları (Sağlam, 2001) kullanılmıştır. Bloklar arasında 2,5 m mesafe ve parseller arasında 0,5 m olacak şekilde, ekimler m<sup>2</sup>’de 500 bitki olacak şekilde her iki yılda da kasım ayında elle yapılmıştır. Parseller 7,2 m<sup>2</sup> (6,0 m × 1,2 m) ve 6 sıralıdır (Kıral ve Çelik, 2012). Uygulanan azotlu gübrenin yarısı ekimle, kalan kısmı ise kardeşlenme dönemi sonunda uygulanmıştır. Ayrıca, 5 kg P<sub>2</sub>O<sub>5</sub>/da (Triple süper Fosfat) dozunda ekimde fosfor verilmiştir (Sade ve ark., 1999). Hasat olgunluğuna ulaşan bitkilerin hasadı orakla yapılmıştır (Naneli ve ark., 2015).

Daha önce yapılan araştırmalarda (Düzgüneş ve ark., 1987; Genç ve ark., 1987; Kırtok ve ark., 1988; Kandemir ve ark., 2000; Sade ve ark., 1999; Sirat ve Sezer, 2017) kullanılan yöntemler izlenerek m<sup>2</sup>’deki başak sayısı, tane verimi, 1000 tane ağırlığı, hektolitre ağırlığı, ham protein oranı ve tane irilik oranları belirlenmiştir. Verilerin istatistiksel analizi Costat (Anonim, 2004) programı ile yapılmıştır. Bartlet testine göre incelenen özellikler bakımından yıllar arasında varyanslar önemli bulunmuş ve bu nedenle varyans analizi yıllar birleştirilmeden ayrı ayrı yapılmıştır. Ortalamaların karşılaştırılmasında LSD testi kullanılmıştır (Düzgüneş ve ark., 1987).

Çizelge 1. Araştırma yerinin yağış, sıcaklık ve nispi nem değerleri\*

Table 1. Precipitation, temperature and relative humidity values of the research area

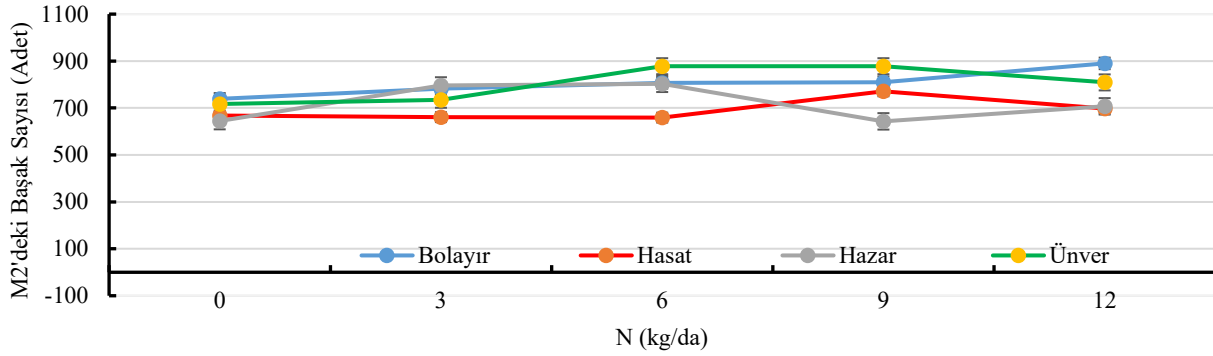
Aylar	Yağış (mm)			Sıcaklık (C)			Nispi Nem (%)		
	Uzun Yıllar	2018/19	2019/20	Uzun Yıllar	2018/19	2019/20	Uzun Yıllar	2018/19	2019/20
Ekim	35,9	39,6	3,7	13,8	15,7	17,4	65,0	69,3	60,9
Kasım	41,5	8,2	25,0	8,0	9,2	9,0	70,1	73,8	65,9
Aralık	44,5	49,4	37,0	3,8	4,9	5,8	72,2	80,7	76,0
Ocak	43,2	71,6	52,9	0,9	2,3	3,0	69,4	75,5	74,0
Şubat	34,1	14,7	66,1	3,6	5,9	4,0	64,6	67,0	70,9
Mart	43,1	36,8	32,9	7,3	7,3	9,8	61,2	61,8	62,7
Nisan	52,6	63,5	19,9	12,5	11,5	11,1	58,8	64,4	56,8
Mayıs	58,7	49,1	35,5	16,4	19,1	17,0	61,5	58,9	59,1
Haziran	41,0	26,6	81,6	19,7	23,1	20,7	60,5	63,4	63,8
Ort./Top.	394,6	359,5	354,6	9,6	11,0	10,9	64,8	68,3	65,6

\* Tokat Meteoroloji Müdürlüğü kayıtlarından alınmıştır.

Çizelge 2. Azotun bazı arpa çeşitlerinde metrekarede başak sayısı, tane verimi ve 1000 tane ağırlığına etkisine ait değerler  
Table 2. Values of the effect of nitrogen on the number of ears per square meter, grain yield and 1000 grain weight in some barley varieties.

	M <sup>2</sup> 'de Başak Sayısı (adet)		Tane Verimi (kg/da)		Bin Tane Ağırlığı (g)	
	1.Yıl	2.Yıl	1.Yıl	2.Yıl	1.Yıl	2.Yıl
Çeşit (Ç)						
Bolayır	805,8 a	762,1 ab	409,9 a	247,3 c	43,5 a	43,9 a
Hasat	692,5 b	680,3 bc	347,1 b	264,6 c	44,3 a	44,3 a
Hazar	718,3 b	648,3 c	328,0 b	318,2 b	29,4 c	31,4 b
Ünver	803,2 a	777,7 a	313,3 b	384,6 a	41,0 b	45,2 a
Azot (kg N/da)						
0	691,9 b	585,0 c	308,3 c	193,2 c	37,1 b	43,9 a
3	744,9 ab	578,6 c	329,4 bc	302,4 b	40,8 a	42,2 ab
6	786,9 a	735,0 b	348,3 abc	338,4 ab	40,3 a	40,6 b
9	775,2 a	762,5 b	390,4 a	343,7 a	39,8 a	41,4 b
12	775,9 a	923,8 a	370,7 ab	340,7 a	39,8 a	37,8 c
Ort.	754,9	717,1	349,6	303,8	39,6	41,2
Ç	**	**	**	**	**	**
N	**	**	**	**	**	**
Ç × N	**	öd	öd	*	öd	*
Var. Kat.	7,5	12,9	13,9	10,8	5,5	4,7

Aynı harf ile gösterilen ortalamalar arasında fark yoktur, \* ve \*\*: F değerleri P<0,05 ve 0,01 olasılık düzeyinde önemlidir, öd: Önemli değil



Şekil 1. 2018-19 Sezonunda azot uygulamasının çeşitlerin m<sup>2</sup>'deki başak sayısına etkisi  
Figure 1. Effect of nitrogen application on the number of spikes per m<sup>2</sup> of varieties in the 2018-19 Season

## Bulgular ve Tartışma

### Metrekarede Başak Sayısı (adet)

Araştırmanın her iki yılında da metrekaredeki başak sayısı bakımından çeşitler arasında önemli (P<0,01) farklar meydana gelmiştir (Çizelge 2). Metrekaredeki başak sayısı, nispeten yağışın daha yüksek olduğu birinci yetiştirme yılında fazla olmuştur. Ortalama metrekaredeki başak sayısının daha yüksek olduğu birinci yılda Bolayır ve Ünver çeşitlerinde diğerlerine göre önemli derecede fazla metrekarede başak sayısı elde edilmiştir. Ayrıca, çeşit×azot (Ç × A) interaksyonu da önemli olmuştur. İkinci yılda da genotipler benzer bir tepki vermiş olup, Bolayır ve Ünver çeşitleri ilk sıralarda yer alırken Hazar çeşidi son sırada yer almıştır. Bolayır ve Ünver çeşitleri ile Hazar çeşidi arasındaki farklarda önemli (P<0,01) olmuştur.

Genotipin kardeşlenme yeteneği ile ilişkili olan metrekaredeki başak sayısı tahıllarda tane verimini önemli derecede etkilemektedir. Metrekaredeki başak sayısı ve kardeşlenme çeşit özelliği yanında iklim (yağış, nem, sıcaklık) ekim zamanı, bitki sıklığı ve uygulanan azot miktarı gibi faktörlerinde etkisi altındadır (Gouis ve ark., 1999; Ülker ve ark., 1999; Ergün ve ark., 2017).

Birinci deneme yılında azotun metrekaredeki başak sayısına etkisi genotiplere göre değişmiş ve bu farklı etki önemli (P<0,01) bulunmuştur (Çizelge 2). Şekil 1'de görüldüğü üzere azot uygulaması bütün çeşitlerde metrekaredeki başak sayısını genel olarak pozitif yönde etkilemiştir. Bolayır çeşidinde metrekaredeki başak sayısı azot miktarına bağlı olarak düzenli şekilde artmış ve 12 kg N/da dozunda 889,8 adet olmuştur. Ünver ve Hazar çeşitlerinde metrekaredeki başak sayısı 6 kg N/da dozuna (878,3 ve 803,3 adet) kadar artmış, daha sonraki azot dozlarında ise metrekaredeki başak sayısı bu iki çeşitte de azalmıştır. Diğer taraftan Hasat çeşidi uygulanan azot dozlarından farklı şekilde etkilenmiştir. Bu çeşit de metrekaredeki başak sayısı uygulanan 3 ve 6 kg N/da dozlarında önemli bir düzeyde etkilenmemiş fakat 9 kg N/da dozunda önemli derecede artmıştır. Metrekaredeki başak sayısı 12 kg N/da dozunda ise azalmıştır.

İkinci yılda azot uygulaması ile metrekaredeki başak sayısında düzenli artışlar meydana gelmiş ve en yüksek değere 12 kg N/da dozunda ulaşılmış ve diğer dozlardaki değerler ile aralarındaki farklarda istatistiksel olarak (P<0,01) önemli bulunmuştur. Tahılların büyümesi ve

kardeşlenmesi ve sap yapısı üzerinde azotun önemli bir etkisi bulunmakta, azotun yetersiz olması durumunda bu karakterler olumsuz yönde etkilenmekte ve bu nedenle de birim alandaki başak sayısı düşük olmaktadır (Zabunoğlu, 1983). Daha önce yapılan araştırmalarda da (Abledo ve ark., 2003; Ergün ve ark., 2017), uygulanan azotun belli bir doza kadar metrekaredeki başak sayısını artırdığı, birim alandaki başak sayısının genotiplere göre önemli derecede değişkenlik gösterdiği bildirilmiştir.

### Tane Verimi (kg/da)

Ortalama tane verimi, metrekaredeki başak sayısında olduğu gibi 2018-19 yılında daha yüksek olmuştur (Çizelge 2). Ortalama tane verimi birinci yıl 349,6 kg/da iken, ikinci yılda 303,8 kg/da'ya düşmüştür (Çizelge 2). Yetiştirme dönemleri arasında toplam yağış bakımından önemli bir fark olmamasına rağmen ikinci yılda özellikle Mart, Nisan ve Mayıs aylarındaki yağış miktarı belirgin bir şekilde az olmuştur (Çizelge 1). Birinci yılda gerçekleşen daha uygun iklim koşulları nedeniyle metrekaredeki başak sayısı daha yüksek olmuş ve bunun neticesi olarak tane verimi de ilk yıl daha fazla olmuştur. Tane verimi ile metrekaredeki başak sayısı arasında pozitif ilişkili mevcuttur (Feil, 1992; Doğan ve ark., 2014; Koca ve ark., 2015). 2018-19 Yılında elde edilen tane verimine göre çeşitler arasında meydana gelen farklılıklar istatistiksel olarak da önemli bulunmuştur (Çizelge 2). Tane verimi bakımından 409,2 kg/da ile Bolayır ilk sırada yer alırken bu çeşidi azalan sırayla Hasat, Hazar ve Ünver çeşitleri izlenmiş ve Bolayır çeşidinin diğer çeşitlerden önemli derecede ( $P<0,01$ ) daha yüksek tane verimi vermiştir. Çalışmadaki diğer üç çeşit arasındaki farkın ise önemsiz olduğu görülmüştür. İkinci deneme yılında ise çeşitlerin tane verimi bakımından sıralamaları değişmiş ve ilk sırada Ünver (384,6 kg/da) yer almıştır. Ünver çeşidinin, sırasıyla Hazar, Hasat ve Bolayır çeşitleri izlenmiştir. Bu çeşitler ile Ünver çeşidi arasında belirlenen farklarda önemli bulunmuştur. Her ne kadar çeşitlerin ortalama tane verimleri arasındaki farklar önemli bulunsun da çeşitlerin azot'a verdikleri tepkilerde önemli farklılık olduğu ( $\text{Ç} \times \text{N}$  interaksyonu önemli) gözlenmiştir (Çizelge 2). Elde edilen tane verimleri açısından çeşitler arasında gözlemlenen farklar ya da benzerlikler genotiplerin çevre faktörleri ile olan ilişkilerden kaynaklanmaktadır (Feil, 1992). Arpa tane verimi çeşitlere, çevre şartlarına ve yetiştirme tekniklerine göre değişiklik gösterdiğini bildirmişlerdir (Ertürk 2014; Yılkan & Arpalı, 2019).

Uygulanan azot, araştırmanın her iki yılında da tane verimini genel olarak olumlu yönde etkilemiştir (Çizelge 2). Birinci yılda kontrol parsellerinde ortalama tane verimi 308.3 kg/da iken verilen azotun etkisiyle artış göstermiş ve

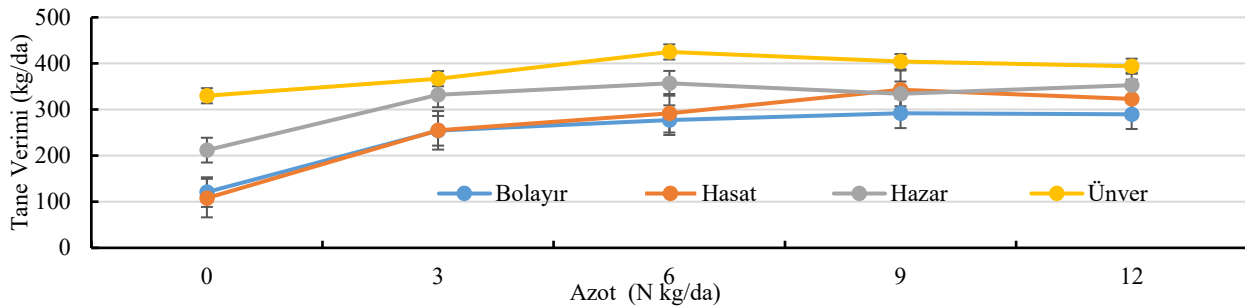
en yüksek verim (390,4 kg/da) 9 kg N/da dozunda elde edilmiş fakat bu artış 6 kg N/da dozuna göre önemli ( $P<0,01$ ) olmamıştır.

Azotun 12 kg N/da dozuna çıkması halinde ise tane verimi önemsiz olsa da azalmıştır. İkinci yılda da azotun benzer bir etkisi olmuş ve artan azot uygulaması genel olarak tane verimini artırmış fakat daha önce ifade edildiği üzere azotun etkisi çeşit bazında farklılık göstermiştir. Şekil 2'ün incelenmesinde görüldüğü üzere tane verimi Bolayır ve Hasat çeşitlerinde 9 kg N/da dozuna kadar artış göstermiş, 12 kg N/da dozunda azalış, Hazar ve Ünver çeşitlerinde ise 6 kg N/da'a kadar artış, daha sonra ise azalış göstermiştir. Çeşitlerin azota verdikleri bu farklı tepkiler ( $\text{Ç} \times \text{N}$  int.) istatistiksel olarak da önemli ( $P<0,05$ ) bulunmuştur (Çizelge 2). Bu durum deneme yılları arasında görülen yağış farklılığından ve çeşitlerin çevre faktörlerine tepkilerinin farklı olmasından kaynaklandığı düşünülmektedir (Doğan ve ark., 2014; Koca ve ark., 2015). İkinci yılda başaklanma ve tane dolununun gerçekleştiği Nisan ve Mayıs aylarındaki yağış toplamı birinci yılın aynı dönemine göre oldukça düşük kalmış (Çizelge 1) ve bu durum doğal olarak da tane veriminde etkisini hissettirmiştir.

Tahıllarda tane verimini önemli derecede etkileyen metrekaredeki başak sayısı (Sönmez ve ark., 1999) üzerine azotun yapmış olduğu olumlu etki, doğal olarak da tane veriminin artmasını sağlamıştır. Bunun yanında azot Hazar ve Ünver çeşitlerinde 6 kg N/da, Bolayır ve Hasat çeşitlerinde 9 kg N/da dozundan sonra başak tane sayısı ve başak tane verimi gibi verim unsurlarını muhtemelen olumsuz yönde etkilemiş ve bunun sonucunda tane verimi genel olarak 12 kg N/da dozunda azalmıştır.

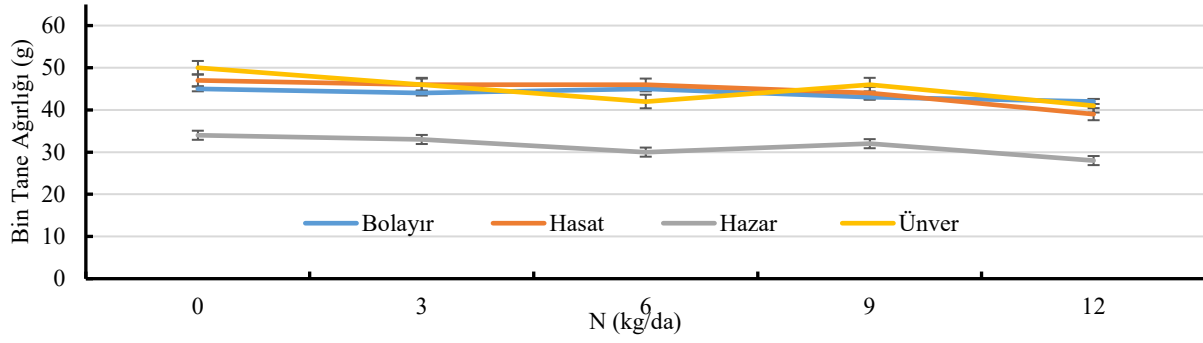
### Bin Tane Ağırlığı (g)

Çeşitlerin bin tane ağırlığı yönünden her iki deneme yılında da önemli fark olduğu belirlenmiştir (Çizelge 2). Bununla beraber 2019-20 yılında  $\text{Ç} \times \text{N}$  interaksyonu da önemli ( $P<0,05$ ) bulunmuştur (Şekil 3). 2018-19 Yılında çeşitler arasında Hasat çeşidi 44,3 g bin tane ağırlığı ile ilk sırada yer almış ve bu çeşidi azalan sırayla Bolayır, Ünver ve Hazar çeşitleri izlenmiştir (Çizelge 2). Bin tane ağırlığı bakımından ilk sıradaki Hasat ve Bolayır çeşitleri arasında belirlenen fark istatistiksel olarak anlamlı bulunmamıştır. Buna karşın Hasat çeşidi ile diğer çeşitler arasında önemli ( $P<0,01$ ) fark bulunmuştur. Son sırada ise altı sıralı olan Hazar çeşidi yer almıştır. 2019-20 Yılında  $\text{Ç} \times \text{N}$  interaksyonu önemli olmakla beraber Ünver (45,2 g) çeşidi ilk sırada, altı sıralı olan Hazar çeşidi yine son sırada yer almıştır. Bin tane ağırlığı fiziki kalite ölçütlerinden bir tanesi olup bitkinin genetik yapısına göre değişen bir karakterdir (Kılıç, 2005).



Şekil 2. 2019-20 Sezonunda azot uygulamasının arpa çeşitlerin tane verimine etkisi.

Figure 2. Effect of nitrogen application on grain yield of barley varieties in the 2019-20 Season.



Şekil 3. 2019-20 Sezonunda azot uygulamasının çeşitlerin bin tane ağırlığına etkisi.

Figure 3. Effect of nitrogen application on thousand grain weight of varieties in 2019-20 Season.

Çizelge 3. Azotun bazı arpa çeşitlerinde hektolitreye, ham protein oranı ve tane irilik oranına etkisine ait değerler.

Table 3. Values of the effect of nitrogen on hectolitre, crude protein content and grain size content in some barley varieties.

	Hektolitreye (kg)		Ham Protein (%)		Tane irilik Oranı (%)	
	1.Yıl	2.Yıl	1.Yıl	2.Yıl	1.Yıl	2.Yıl
Çeşit (C)						
Bolayır	66,7 a	64,6 a	11,0	12,6 b	83,1 a	77,7 a
Hasat	65,5 a	63,1 b	11,7	13,4 a	83,9 a	85,0 a
Hazar	58,9 b	59,4 c	11,6	11,6 c	23,9 c	33,0 c
Ünver	58,8 b	63,9 ab	11,1	12,9 ab	39,8 b	58,9 b
Azot (kg N/da)						
0	60,3 b	65,0 a	10,6	11,8 c	49,9 c	80,4 a
3	62,5 ab	63,2 b	11,4	12,1 c	65,5 a	69,5 ab
6	63,4 a	62,8 b	11,8	12,2 c	59,3 ab	64,9 bc
9	62,9 a	61,9 bc	11,9	13,1 b	55,5 bc	57,5 c
12	63,3 a	60,9 c	11,0	13,9 a	58,1 abc	46,0 d
Ort.	62,5	62,6	11,4	12,6	57,6	63,6
Ç	**	**	öd	**	**	**
N	**	**	öd	**	*	**
ÇxN	öd	öd	öd	öd	öd	öd
Var. Kat.	3,3	10,4	10,2	5,7	19,0	15,8

Aynı harf ile gösterilen ortalamalar arasında fark yoktur, \* ve \*\*: F değerleri P<0,05 ve 0,01 olasılık düzeyinde önemlidir, öd: Önemli değil.

Azotun bin tane ağırlığına yaptığı etki 2018-19 yılında istatistiksel olarak önemli (P<0,01) olmuştur (Çizelge 2). Kontrol parsellerinde bin tane ağırlığı ortalaması 37,0 g olup, 3 kg N/da azot uygulandığında olumlu yönde etkilenecek 40,8 g'a yükselmiştir. Fakat verilen azotun daha yüksek seviyelerinde bin tane ağırlığı önemsiz de olsa azalma eğiliminde olmuştur (Çizelge 2). Bu durum muhtemelen metrekaresindeki başak sayısının azotun etkisi ile artmasından ileri gelmektedir. Diğer taraftan azotun bin tane ağırlığı üzerine yıllara göre kararlı bir etkisi olmaması yanında bu kararsızlık ikinci deneme yılında çeşitler üzerinde de saptanmıştır. Bolayır ve Hasat çeşitlerinde uygulanan azot dozları genel olarak bin tane ağırlığını azaltmış ve en düşük değerler 12 kg N/da dozunda elde edilmiştir (Şekil 3). Azotun etkisi Hazar ve Ünver çeşitlerinde biraz daha farklı seyir izlemiştir; şöyle ki, bin tane ağırlığı artan azot miktarına bağlı olarak 3 ve 6 kg N/da dozlarında azalış, 9 kg N/da dozunda ise genel eğilimin aksine bir artış, daha sonraki dozda yine bir azalış göstererek en düşük değere ulaşmıştır. Azotun bin tane ağırlığına etkisinin incelendiği birçok araştırmada birbirinden farklı sonuçlar elde edilmiş olup; Sairam ve Singh 1989, Kılıç, 2005, Kartal ve ark., 2003 gibi araştırmacılar azotun bin tane ağırlığını artırdığını, Eagles ve ark., 1995 ile Kon, 2011 ise etkilemediğini saptamışlardır.

#### Hektolitreye Ağırlığı (kg)

Hektolitreye ağırlığı bakımından çeşitler arasında belirlenen farklar iki yılda da önemli bulunmuştur (P<0,01). Benzer şekilde azotun etkisi de önemli (P<0,01) bulunmuştur (Çizelge 3). Birinci deneme yılında hektolitreye ağırlığı bakımından en yüksek değer 66,7 kg ile Bolayır çeşidinde elde edilmiş ve bu çeşidi, azalan sırayla Hasat (65,4 kg), Hazar (58,9 kg) ve Ünver (58,7 kg) izlemiştir (Çizelge 3). Yapılan LSD testine göre Hasat ve Bolayır çeşitleri arasındaki fark önemsiz, bu çeşitler ile Ünver ve Hazar çeşitleri arasında belirlenen farklar ise istatistiksel olarak önemli (P<0,01) bulunmuştur. 2019-20 Yılında da Bolayır çeşidi 64,6 kg ile ilk sırada yer almıştır. Bolayır çeşidini Ünver (63,9 kg), Hasat (63,1 kg) ve Hazar (59,4 kg) çeşitleri izlemiştir. Ortalamalar bakımından Bolayır ile Ünver çeşitleri arasındaki fark önemsiz, Hasat ve Hazar ile olan farklar ise önemli bulunmuştur (P<0,01). Altı sıralı olan Hazar çeşidi beklenildiği gibi özellikle ikinci yılda çok düşük değer vermiştir.

Bira ve malt sanayinde kullanılan arpalarda hektolitreye ağırlığı önemli bir kalite kriteridir ve 65 kg hL/1'den az olmaması istenir (Mut ve ark., 2014). Çünkü hektolitreye ağırlığı ile tanedeki nişasta arasında olumlu bir ilişki vardır. Bu nedenle nişasta oranı yüksek olan arpa çeşitlerinin ekstrakt değerleri de daha fazladır (Engin, 1989). Bu açıdan çeşitler değerlendirildiğinde ilk yıl

Bolayır ve Hasat, ikinci yıl Bolayır standarda yakın değerler vermiştir. Daha önce yapılan araştırmalarda, başta çeşit özelliği olmak üzere çevre faktörleri ve tane yapısı (tane tekdüzeliği, kavuz oranı, endosperm yapısı) hektolitreye ağırlığını etkilemektedir (Özdemir ve Yüksel, 2007; Kendal ve ark., 2010; İmamoğlu ve Yılmaz, 2015; Çöken ve Akman, 2016).

Azotun hektolitreye ağırlığına etkisi yıllara göre farklılık göstermiştir (Çizelge 3). İlk yıl ortalama hektolitreye ağırlığı 3 kg/da azot uygulamasıyla kontrole göre önemli ( $P<0,01$ ) artış göstermiş fakat daha sonraki dozlarda görülen değişimler önemsiz olmuştur (Çizelge 3). İkinci yılda ise azotun hektolitreye ağırlığı üzerine etkisi ise daha farklı bir seyir izlemiş ve azot miktarı arttıkça hektolitreye ağırlığı da kontrole göre önemli düzeyde düşüş göstermiştir. Hektolitreye ağırlığına ikinci yıldaki etkisi, bin tane ağırlığına olan etkiye benzer olması dikkat çekicidir. İkinci deneme yılında azot artışına bağlı olarak metrekaredeki başak sayısı artmış fakat tane dolm döneminde daha az yağış olmuştur (Çizelge 1). Bu durum tanelerin daha cılız olmasına dolayısıyla da hektolitreye ağırlığının düşmesine neden olmuştur. Benzer konuda yapılan araştırmalarda hektolitreye ağırlığının değişen iklim ve çere koşullarından etkilendiği (Kün ve ark., 1992; Mut ve ark., 2014), azotun hektolitreye ağırlığı üzerine etkisi hususunda ise farklı sonuçlar elde edilmiş olup; Kartal ve ark. (2003) yaptığı çalışmada kontrol ve 2 kg N/da göre hektolitreye ağırlığının 4 kg/da N dozunda arttığını, daha sonraki dozların önemli bir etkisinin olmadığını, yine Petrie ark. (2003) ve Akkaya (1986), artan azotun hektolitreye ağırlığını azalttığını, Clancy ve ark. (1991), Sönmez, F. & Yılmaz N. (2000) ve Kon (2019) ise azotun hektolitreye ağırlığını etkilemediğini bildirmişlerdir.

#### **Ham Protein Oranı (%)**

2018-19 Yılında ham protein oranı bakımından çeşitler arasında belirlenen farklar önemli olmamış ( $P>0,01$ ) ve çeşitlerin ortalama ham protein oranları %11,0 ile %11,7 arasında değişmiştir (Çizelge 3). En yüksek değer %11,7 ile Hasat çeşidinden elde edilmiş ve bu çeşidi azalan protein oranı değerleri ile Hazar (%11,6), Ünver (%11,1) ve Bolayır (%11,0) çeşitleri izlemiştir. İkinci yılda ise çeşitler arasında gözlemlenen farklar önemli ( $P<0,01$ ) olmuş ve ilk yıla oranla daha yüksek değerler elde edilmiştir. Bu durum çevre koşulları açısından deneme yılları arasındaki farklılıktan kaynaklanmıştır. İkinci yılda en yüksek ham protein oranı değeri (%13,4) Hasat çeşidinden elde edilmiş ve bu çeşidi Ünver (%12,9) çeşidi izlemiş ve aralarındaki fark önemsiz bulunmuştur. Bolayır ve Hazar çeşitlerine ait ham protein oranları Hasat çeşidinin değerine göre önemli düzeyde düşük olduğu belirlenmiştir. Hasat ve Ünver diğer çeşitlere oranla yemlik olarak değerlendirilebilecek çeşitler olarak öne çıkmaktadır. Oscarsson ve ark.(1998), İmamoğlu & Yılmaz (2015), Koca ve ark. (2015), Çöken ve Akman (2016) da protein oranını bakımından genotipler arasında önemli farklılıklar olduğunu bildirmişlerdir.

Her iki yılda da azot miktarı arttıkça ham protein oranı da artmasına rağmen azotun etkisi ilk yıl önemsiz, ikinci yıl ise önemli ( $P<0,01$ ) bulunmuştur (Çizelge 3). İlk yılda hiç azot uygulanmayan kontrol parsellerinde en düşük ham protein oranı ortalaması (%10,6) elde edilmiş olup azot miktarındaki artışla ham protein oranı da yükselmiş en

yüksek orana 9 kg N/da dozunda (%11,9) ulaşılmıştır. İkinci yılda ham protein oranı azot dozundaki artışa bağlı olarak 12 kg N/da'ya kadar düzenli artış göstermiştir (Çizelge 3) ve 12 kg N/da dozundaki ham protein oranı ile diğer bütün dozlar arasındaki farklar önemli ( $P<0,01$ ) bulunmuştur. Azotun ham protein oranına etkisinin yıllara göre değişmesi çevre ve genotip etkisinin bir sonucudur. Çünkü, arpada protein oranı çevre şartlarından çok etkilenen aynı zamanda kalıtım derecesi düşük ve bir karakterdir (Atlı ve ark., 1989). Daha önceden yapılmış çalışmalarda, birbirinden farklı sonuçlar elde edilmiş olup; Conry (1994) artan azotun protein oranı üzerinde önemli etkisinin olmadığını, Petrie ve ark. (2003) ise artan azotun ham protein oranına olumlu etkisinin olduğunu bildirmişlerdir.

#### **Tane İriliği Oranı (%)**

Çeşitlerin tane iriliği oranı (2,8+2,5 mm) değerleri arasında hem 2018-19 ve hem de 2019-20 dönemlerinde önemli ( $P<0,01$ ) farklar bulunmuştur (Çizelge 3). Birinci yıl en yüksek tane iriliği oranı Hasat (%83,9) çeşidinde belirlenmiş ve bu çeşidi Bolayır (%83,1), Ünver (%39,8) ve Hazar (%23,9) çeşitleri izlemiştir. Tane irilik oranı bakımından Hasat çeşidi ile Bolayır çeşidi arasındaki fark önemsiz, bu çeşitler ile diğer genotipler arasında oluşan farklar ise önemli ( $P<0,01$ ) olmuştur. İkinci yıldaki tane irilik oranları ilk yıla nazaran daha yüksek olmuş ve çeşitler sıralanışı da benzer bulunmuştur (Çizelge 3). Tane iriliği hem çeşidin genetik yapısından ve hem de yetiştirme ortamından etkilenmektedir (Fox ve ark., 2003). Bir çok araştırma da tane iriliğinin çeşitlere göre önemli derecede değiştiği bildirilmiştir (Weston ve ark., 1993; Eagles ve ark., 1995; Kartal ve ark., 2003; Kon, 2019).

Farklı delik çaplı eleklerle (2,2, 2,5 ve 2,8 mm) yapılan analizlerde oluşan sınıflar tanelerin fiziki yapıları (dolgunluk ve zayıf olmasını) hakkında fikir vermektedir. Bu analizler ayrıca homojenlik durumu hakkında da bilgi vermektedir. Şöyleki; iki elek üstü (2,2 ile 2,5 veya 2,5 ile 2,8) toplamı %75'ten yüksek ise örneğin irilik açısından homojen olduğu kabul edilmektedir (Elgün ve Certel, 1987). Bu açıdan çeşitler değerlendirildiğinde Hasat ve Bolayır çeşitlerinin tanelerinin daha dolgun (%75 üzeri) olduğu ve malt kriterlerine uygun homojen çeşitler olduğu söylenebilir. Bununla beraber özellikle ikinci yılda Hasat çeşidinde ham protein oranının yüksek olması malt açısından olumsuz bir durum olarak ortaya çıkmakta fakat bu çeşidin protein oranının olması, yemlik olarak da kaliteli bir çeşit olabileceğini göstermektedir. Daha önce yapılan birçok araştırmada da tane irilik oranı bakımından çeşitler arasında önemli farklılık olduğu ortaya konmuştur (Mut ve ark., 2014; Kon, 2019).

Azotun araştırmada kullanılan çeşitlerin tane iriliği üzerine etkisi birinci yıl  $P<0,05$ , ikinci yıl  $P<0,01$  düzeyinde önemli olmuştur (Çizelge 3). Tane iriliği oranı birinci yıl kontrol uygulamasında %49,9 iken, 3 kg N/da dozunda önemli bir artış göstererek %65,5 yükselmiş fakat 6 kg N/da dozundan itibaren azalmıştır. İkinci deneme yılında değişen iklim koşullarının etkisiyle azot tane irilik oranını daha farklı bir şekilde etkilemiştir (Çizelge 3). Çizelge 3'den de görüleceği üzere en yüksek tane iriliği oranı kontrol parselinde %80,4 olarak elde edilmiştir. Uygulanan azot miktarındaki artışa karşın tane iriliği oranı da düzenli azalma göstererek 12 kg N/da en düşük orana

ulaşmıştır. Bu azalmalar 3 kg N/da dozu hariç diğer bütün dozlarda önemli ( $P<0,01$ ) bulunmuştur. Benzer konuda yapılan araştırmalarda; Kartal ve ark. (2003) önemsiz olmakla birlikte azotun tane iriliğini 6 kg N/da dozuna kadar artırdığı ve daha fazla uygulanan azotun ise tane iriliğini düşürdüğünü, Kon (2019) önemli bir etkisinin olmadığını bildirmişlerdir.

## Sonuç

Yapılan bu çalışma sonucunda; araştırmada ele alınan çeşitlerin incelenen özelliklerinin değişen iklim koşullarından önemli derecede etkilendiği, tane verimi açısından çeşitlerin kararlı bir davranış göstermediği; 1000 tane ağırlığı, hektolitreye ağırlığı ve tane iriliği oranı bakımından Bolayır çeşidinin ön plana çıktığı ve malt özelliği bakımından Bolayır'ın standartlara daha yakın olduğu, Ünver çeşidinin yemlik olarak daha iyi durumda olduğu görülmüştür. Sonuç olarak, tane verimi ve maltlık özelliği bakımından Bolayır, yine verim ve yemlik özellik bakımından Ünver çeşidinin bölge için diğer çeşitlere göre daha uygun oldukları belirlenmiştir.

Artan miktarlarda uygulanan azot belli bir doza kadar tane veriminde artış sağlarken, diğer yandan malt kalitesini etkileyen tane irilik oranı, hektolitreye ve bin tane ağırlığı gibi karakterlerde azalmaya neden olduğu saptanmıştır. Yılların ortalaması da dikkate alındığında, çalışmadaki deneme koşullarına benzer çevre koşulları için malt özelliği bakımından 3 kg N/da dozu, yemlik yetiştiricilik açısından ise Bolayır ve Ünver çeşidinde 6 kg N/da dozunun uygun olacağı sonucuna varılmıştır.

Not: Bu çalışmanın birinci yılı sonuçları Tokat Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsünde Büşra DEMİR'in Yüksek Lisans Tezi olarak sunulmuştur.

## Bilgi

Bu çalışmanın birinci yıl sonuçları Tokat Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsünde Büşra DEMİR'in Yüksek Lisans Tezi olarak sunulmuştur.

### Çıkar çatışması

Çıkar çatışması Yazarlar arasında herhangi bir çıkar çatışması yoktur

BY: Araştırmanın; denemelerin kurulması, tarla gözlemlerinin alınması ve yazım aşamasında katkı sağlamıştır

ME: Araştırmanın; denemelerin kurulması, tarla gözlemlerinin alınması ve yazım aşamasında katkı sağlamıştır

FS: Araştırmanın; denemelerinin kurulması, tarla gözlemlerinin alınması, istatistik analizlerin yapılması ve makalenin yazım aşamasına katkı sağlamıştır.

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## Agronomic Management of Faba Bean (*Vicia faba* L.): A Review

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### ABSTRACT

The faba bean (*Vicia faba* L.) is a winter crop that can be cultivated as a versatile crop. Its yield and quality being strongly influenced by environmental and agronomic factors, nutritional content, medicinal properties, and ability to fix nitrogen biologically. Therefore, to maximize advantages of faba bean cultivation, choosing the appropriate varieties, planting times, techniques, plant density, depth of sowing, and ensuring proper crop nutrients and irrigation is essential. For successful faba bean production in subtropical climates, it's important to assess the performance of different varieties under these specific conditions. Planting dates and soil temperature are crucial for germination, growth, and yield. At the same time, the crop's performance is also influenced by sowing methods, plant density, sowing depth, and water and fertilizer management. Integrating faba beans into cropping systems is expected to offer various ecological benefits. This paper reviews the existing literature on the agronomic practices of faba beans.

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## Introduction

The faba bean (*Vicia faba* L.) is a widely consumed pulse that is a significant basis of protein to both human and animal nutrition. (Cazzato et al., 2012) and as a major supply of nitrogen for the biosphere (Rubiales, 2010). It is originated in the Middle East in the prehistoric era (Multari et al., 2015; Yadav et al., 2017). The genus *Vicia* belongs to the family *Fabaceae*, which contains a wide range of species and is distributed around the world. Approximately 750 genera include 16,000–19,000 species in this family, based on current estimates (Chakraverty et al., 2013). Over sixty countries across the globe, including Australia, South and North America, West and South Asia, East and North Africa, and Southern Europe, are growing it (Merga et al., 2019; Paul and Gupta, 2021; Roy et al., 2022; Meital et al., 2023). After chickpeas, dry-beans, and dry-peas, faba beans are the fourth-most significant pulse grain worldly cultivated, which are used as animal and human feed (Gasim et al., 2015). Because it may be consumed both raw and prepared, it can also be utilized year-round. The crop enhances the quality of the soil by biological nitrogen-fixation (Rubiales and Mikic, 2015). Soil fertility is increased when faba beans are incorporated into agricultural systems (Karkanis et al., 2018). It has been reported that 50–330 kg nitrogen  $\text{hm}^{-2}$  can be fixed by faba beans depending on climatic situations and cultivation

management (Galloway et al., 2004). In recent cropping, it has been largely disregarded as an effective part of crop-cycle, which is vital for reducing the environmental impact of chemical fertilizers. As a result of its diverse applications, high nutritious rate, and capability to flourish in a wide range of agroclimatic environments, the faba bean is a strong fit for sustainable cropping in many underutilized zones. These factors have led to increased international attention for the crop in recent years (Gasim et al., 2015). The faba bean, which is cultivated in the centre and northern regions of Bangladesh, is regarded as an underutilized pulse crop (Paul et al., 2022) and regionally familiar as *Kalimotor*, *Baklakalai*, or *Bhograkalai* (Sheikh et al., 2020; Bhomik et al., 2022). This crop usually planted in the winter after the harvest of *T. aman* rice and sown directly as a relay crop in low-lying areas with little to no tillage (Biswas, 1988). Still, there is no available data on its cultivation area or production in Bangladesh. Pulse crops in general are grown on 0.37 million hectares in Bangladesh, producing 0.39 million tons, with faba bean contributing only a small fraction to this total (BBS, 2017). For the people of Bangladesh, pulses are a significant protein source. Faba beans, in particular, are a unique type of legume crop because they are high in minerals, dietary fibre, complex carbohydrates,

lecithin, choline, and secondary metabolites like phenolics (Paul and Gupta, 2022). According to Ramirez-Moreno et al. (2015), L-dopa can be found in faba beans, which is known to be pharmacologically active when managing Parkinson disease patients and to improve motor function in humans by raising plasma levels of the drug. Faba beans are well-liked globally as a result of their high in essential nutrients and numerous uses. While it is fed to humans in developing countries, it is mainly fed to hogs, steeds, chickens, and pigeons in advanced country (Singh and Bhatt, 2012a). It is typically served as a human dal (soup) in Bangladesh. It can be eaten fresh, canned, dried, or green (Gasim and Link, 2007). A crop with multiple uses, faba beans can be produced in several climate zones (Singh et al., 2013). The yield of faba beans and their nutritional value are affected by agronomic methods such as timely sowing, managing nutrients and water (Huthily et al., 2020). So, suitable management techniques like water management, soil health, and planting time can greatly lessen the quantity of flowers that fail, increasing the amount of seeds or pods produced in the end.

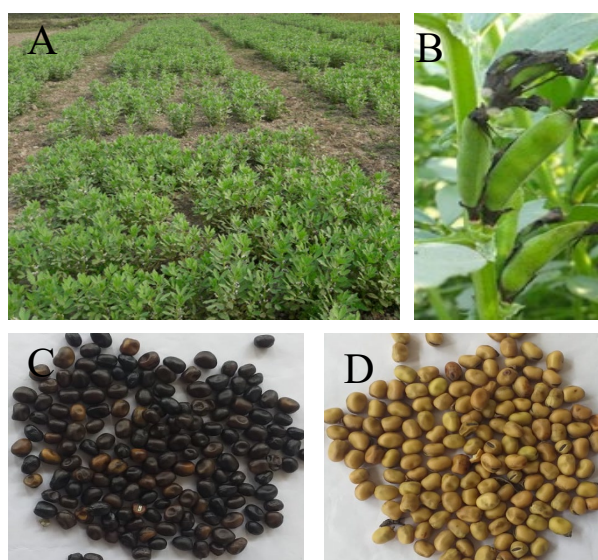


Figure 1. Field of faba bean (A), green pods (B), black seeds (C) and brown seeds (D)

Most legumes are generally sensitive to low soil temperature during seed germination. But unlike most grain legumes, faba beans are one of the few cool-season varieties whose germination can withstand chilly soil temperatures. The goal of improving seed germination in soil that is colder than 15 °C has been partially achieved by selection (Singh and Jauhar, 2005). Due to its remarkable nutritional attributes, such as elevated carbohydrate, protein, B vitamin, and mineral content (Crepon et al., 2010), the faba bean is regarded globally a important pulse crop. The faba bean has various uses, is highly nutritious, and can adjust to a wide range of edaphic and agro-climatic environment, making it an excellent choice for environmentally friendly farming in numerous peripheral areas (Nadal et al., 2003). The crop has drawn more and more attention from around the world in recent years. Therefore, this manuscript focused on agronomical techniques for improving faba bean quality and productivity.

## Species Diversity

Species diversity can play a crucial role in enhancing the yield of faba beans by improving pest and disease management, soil health, pollination, microclimate regulation, and overall resilience. Yield and agronomic traits of faba bean varies greatly among different cultivated species due to environmental conditions and their interactions, as well as genetic factors. Comparable to other crops, selecting a cultivar or variety involves finding a stability between adjustability to a particular environment, disease resistance, farming goals, and suitability of a product (Zandvakili et al., 2018). Faba bean varieties differ widely in terms of seed size and color. Small-seeded types, known as tick beans (*Vicia faba* L. subsp. minor), are typically used for animal feed and smother crop. In contrast, medium and large-seeded, referred to as broad beans (*V. faba* subsp. major Harz), are usually used as both green and dry beans (Crépon et al., 2010). The species exhibits considerable genetic diversity, often described through phenotypic traits such as seed size, weight, shape, and color (Cilesiz et al., 2023). According to Karkanis et al. (2018), Depending on seed size, faba bean genotypes are typically divided into three main botanical varieties: (a) Large seeds- *Vicia faba* var. *major* (b) Medium seeds- *Vicia faba* var. *equine* (c) Small seeds- *Vicia faba* var. *minor*, (d) *Vicia faba* sp. *paucijuga*. However, faba bean genotypes are also classified based on frost tolerance, agro-climatic zone, sowing time (spring and winter types), and capability to adjust to aquatic or terrestrial (i.e., drought-prone) conditions.

## Soil and Climate

Faba beans grow optimally in fine-textured soils but are versatile enough to thrive in various soil types (Jensen et al., 2010). Though, it can withstand a wide pH extent of 6–9 and sandy-loam soils, particularly in humid areas, faba beans prefer clay-lime, chalky, permeable, medium-textured soils with a neutral pH. Legumes need neutral to alkaline soil to maximize N-fixation by nodules bacteria. However, faba beans can also adapt to more acidic soil conditions (Singh et al., 2013). Growing seasons should be as cool as possible; 18 to 27°C (65–85°F) is the ideal temperature range for productivity (Duke, 1981). According to Gasim and Link (2007), faba beans grow best in a cool season with moderate annual rainfall of 650 to 1000 mm. Although they are thought to withstand frost, they are vulnerable to drought and water logging (Subash and Priya, 2012). The maturation duration varies (90 to 220 days) based on the climate and cultivars (Bond et al., 1985). Most legumes are vulnerable to low soil temperatures during seed germination. Nevertheless, faba beans are unique among cool-season grain legumes in that their germination can withstand lower soil temperatures than that of most other grain legumes. There has been some progress in selecting better seed germination in soil that is colder than 15 °C (Singh and Jauhar, 2005). Large-seeded cultivars have been observed to have a higher germination rate at 12.5 °C than small-seeded beans (Kang et al., 2008). During blossoming, it cannot withstand high temperatures. Some faba bean cultivars seem to be day length-neutral, however, germplasm exchanges between latitudes show that many require lengthy days to flower and mature (Singh et al., 2013).

Table 1. Pod and Seed Characteristics of the Botanical Groups of *Vicia faba* L. (Fouad et al., 2013, Duc et al., 2015a, Etemadi et al., 2019).

Botanical Group	Seed Shape	Seed Weight	Pod Characteristics	Uses
Major	Very plate	>1g	Small to large (from 2 to 10 seeds)	Dry and green beans
Equina	Plate	0.45–1.1 g	Plate, thick, non-dehiscent pods Medium size, 3–5 seeds	Dry and green beans
Minor	Cylindrical to rounded form	0.2–0.5 g	Small with 3–4seeds	Animal feeding and cover cropping
Paucijuga	Rounded to an elliptical form	0.2-0.3g	Very small, dehiscent or non-dehiscent types	Nitrogen fixing cover crop

Table 2. Impact of seed size on the biological yield, harvest index, and seed output of local faba beans.

Treatments	Seed yield (kg/ha)		Biological yield (kg/ha)		Harvest index (%)	
	1997/98	1996/97	1997/98	1996/97	1997/98	1996/97
Seed size						
Very small	4298	4298	7856	2326	55.3	48.4
Small	4479	4479	8982	2879	50.2	52.1
Medium	4118	4118	8842	3232	47.3	38.3
Large	4513	4513	9528	3675	48.1	25.6
LSD (0.05)	NS	NS	738	524	3	10

(LSD, least significant difference (P = 0.05); NS, not significant) (Source: Al-Rifae et al., 2004)

### Seeding Rate

The population selection of most legumes is a crucial agronomic decision that impacts both the amount of biologically fixed nitrogen (N) and the plant's ultimate output. According to Parr et al. (2011), a legume utilized as a cover crop must yield a minimum of 4,000–4,500 kg hm<sup>-2</sup> of biomass in order to supply enough readily accessible nitrogen to meet the crop's subsequent nitrogen requirements. It is unclear what the ideal faba bean plant population is for both N fixation and maximum pod and grain yield. Depending on the variety (size of seed) and the area, faba bean densities ranging from 45 000 to 60 000 plants hm<sup>-2</sup> are frequently utilized (Parr et al., 2011).

### Seed Size

The faba bean is typically a large-seeded pulse, but different cultivars and types can differ significantly in seed size. Even within the same cultivar, seed size can differ significantly based on the pod's position. Seeds are categorized into four size groups by weight: large (>1500 mg seed<sup>-1</sup>), medium (1001-1500 mg seed<sup>-1</sup>), small (700-1000 mg seed<sup>-1</sup>), and very small (<700 mg seed<sup>-1</sup>) (Al-Rifae et al., 2004). Traditionally the farmers are use the seeding rate based on seed quantity per kilogramme, to establish the target population density (Gezahegn et al., 2016). Plant breeders have long emphasized the significance of larger seeds for crop yield, as they are believed to have more food reserves, which can lead to faster germination, better vigor, and higher yields compared to smaller seeds of the similar cultivars (Harker et al., 2015; Patel et al., 2016). Tomaszewski et al. (1978) observed that larger seeds produced more fresh matter and seeds in faba bean crops. However, Al-Rifae et al. (2004) found that plants from very small and small seeds emerged more quickly, flowered earlier, podded sooner, matured faster, and had the higher harvest index, which definitely impacted seed yield. In contrast, larger seeds were more

susceptible to unfavourable circumstances like low temperatures and dryness. Thus, using smaller seeds is recommended to achieve high yields, decline seeding rates, and improve resilience to environmental stresses like low moisture and temperature (Al-Rifae et al., 2004). Additionally, smaller seeds can decrease production costs by reducing the seed quantity required for a area (Etemadi et al., 2017).

### Seed Treatment and Sowing

Rhizobia are able to survive for a period of years in the soil, but they may not contain the best N-fixing bacteria. For optimal nitrogen fixation, inoculation of seeds is advised annually, even though rhizobium bacteria can persist in soil for three to five years. Abd-Alla et al. (2014) suggest that faba bean seeds ought to be injected with *R. leguminosarum* cv. *viciae*. Rodelas et al. (1999) emphasize the importance of proper storage of inoculants, keeping them dry and fresh. To maximize the availability of rhizobia for initiating the process of infection as soon as roots show up, seeds need to be infected soon before planting (Etemadi et al., 2015). These rhizobia have a fast ability to infect roots and trigger nodulation. Direct seeding is the usual method for faba beans. Etemadi et al. (2019) suggest that in places with shorter growing seasons, transplanting might be a more effective method of ensuring early sowing than direct seeding. Moving seedlings may also provide the following additional advantages: double cropping, earlier harvest, early flowering, increased yield, and improved survival rate as well as preventing incident disorders like chocolate spots (Lee et al., 2018, Etemadi et al., 2019). Faba beans take 20 to 25 days to sprout, and it's important to keep the seeds moist until the seedlings are well established. Due to the hard, dry seed's slower water absorption and germination compared to regular beans, planting depth is crucial. To prevent the seed from drying out, planting depth should be between 6 and 10 cm (Singh et al., 2013).

### Sowing Time and Spacing

The faba bean productivity is greatly influenced by spacing, which also affects plant growth, resource utilization, and total productivity. Proper spacing allows plants to develop stronger root systems, which are crucial for nutrient uptake and stability. In contrast, overcrowding can result in shallow or tangled roots, limiting nutrient absorption. Plant density, which is regulated by spacing, and yield may not always have a direct relationship. There is an optimal plant density that maximizes yield per unit area. Too few plants (wide spacing) can reduce total yield, while too many plants (narrow spacing) can cause competition and lower yield plant<sup>-1</sup>. Field trials were carried out by Sharaan et al. (2004) using four cultivars of faba beans (G.2, G.429, G.843, and Misr 1) planted at three distinct dates (October 15, November 5, and November 25) and at three intra-row spacings (15, 20, and 25 cm). The study aimed to find the best sowing date and spacing combination for high yields and quality, considering both environmental and genetic factors. Delay in planting from October 15 to November 25 resulted in a 16.14% increase in seed and pod counts during the first season. During the two seasons, there was a drop in both the number of seeds plant<sup>-1</sup> and the seed yield faddan<sup>-1</sup>, by 6.88% and 24.84% and 28.85% and 15.84%, respectively. For the first and second seasons, the intermediate and earliest sowing dates yielded the largest seed weight plant<sup>-1</sup>, respectively. The greatest harvest index was achieved in both seasons by sowing on November 5. Reducing intra-row spacing from 25 to 15 cm decreased seeds pod<sup>-1</sup> (by 9.66% in the first season and 6.64% in the second), seeds plant<sup>-1</sup> (by 31.08% in the first season and 6.01% in the second), and seed weight plant<sup>-1</sup> (by 34.40% in the first season and 10.67% in the second). Increased plant density in the first season, however, led to a 14.29% increase in seed yield faddan<sup>-1</sup>. Sheikh et al. (2020) conducted tests on four different plant spacings: and found that 30 cm × 20 cm spacing produced the highest seeds 1.23 t ha<sup>-1</sup> followed by 30 cm × 15 cm (.14 t ha<sup>-1</sup>) (Figure 2). The highest values of crude fat (3.16%) and ash (4.79%) were similarly obtained with a spacing of 30 cm × 20 cm. On the other hand, 30 cm × 25 cm had the highest fibre content (15.49%) and the highest crude protein (33.62%). Furthermore, with the 30 cm × 20 cm spacing, the maximum stover output of 1.86 t ha<sup>-1</sup> was observed (Figure 3).

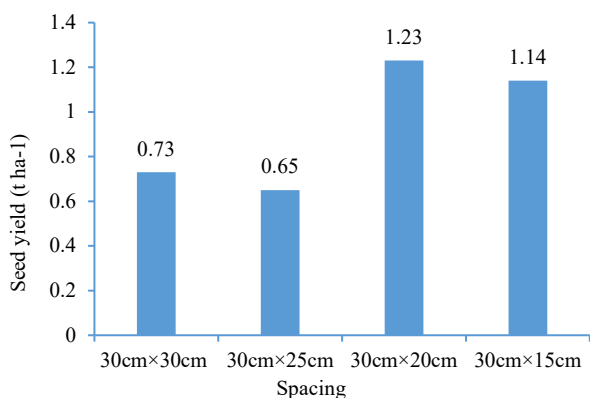


Figure 2. Impact of spacing on seed yield of faba bean (Reproduced from Sheikh et al., 2020)

### Irrigation

Faba beans generally require about 400-600 mm of water throughout the growing season, with the highest demand during the development of pods and flowering. The irrigation schedule should be adjusted based on soil type, climate, and growth stage to ensure optimal plant health and yield. According to Khan et al. (2010), faba beans are very responsive to water scarcity than other grain legumes. Although water shortage significantly limits crop yields, faba beans can still somewhat adapt to low water availability. This ability is particularly important in countries with low rainfall, such as Ethiopia, Sudan, Egypt, and Palestine, where faba beans are a major crop (Multari et al., 2015). According to Ouda et al. (2010), water stress during the early podding stage can reduce yield around 50%. The ideal annual rainfall for faba beans ranges from 650 to 1000 mm, and it should be evenly distributed. In standing water, faba beans do not grow well. Therefore, a consistent water supply is essential for optimal yield. The most crucial period for moisture is 9 to 12 weeks after the crop has been established (Torres et al., 2012; Singh et al., 2013). Drought stress, which increases transpiration, negatively affects both the yield and seed quality of faba bean. Paul et al. (2021) observed that faba bean produced higher seed yield (1.49 t ha<sup>-1</sup>) when irrigated twice at early branching and pod formation stages while the control produced the lowest values (Figure 4).

### Fertilizer management:

Leguminous crops need a sufficient amount of available nutrients for optimum growth and yield. Choudhary (2014) stated that the yield of faba beans can be greatly increased by adding 100 quintals of Farm Yard Manure (FYM) or compost, 15-20 kg of N, 40-50 kg of P<sub>2</sub>O<sub>5</sub>, and 30-40 kg of K<sub>2</sub>O per hectare during the final ploughing. The macronutrients that are important for the faba bean crop's maximum growth and productivity are provided by this balanced approach to nutrient management. Choudhary, 2014). The application of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O-S @ 10-40-40-10 kg ha<sup>-1</sup>, respectively resulted in a greater seed production (1.70 t ha<sup>-1</sup>), according to Paul et al. (2021), while the control produced the lowest values (Figure 5).

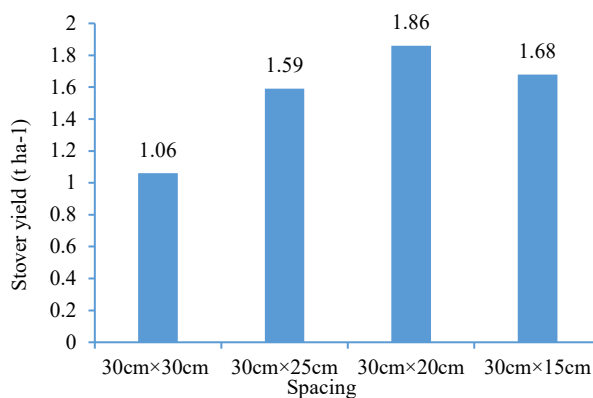


Figure 3. Impact of spacing on stover yield of faba bean (Reproduced from Sheikh et al., 2020)



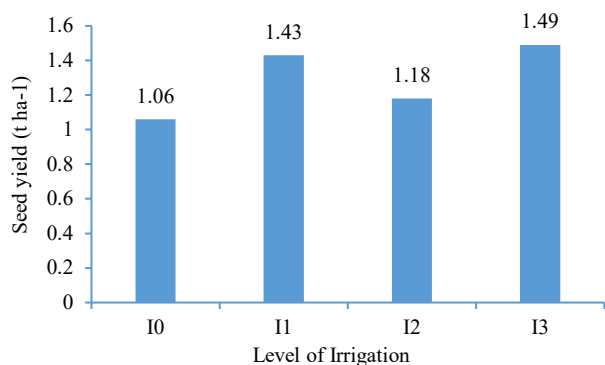


Figure 4. Impact of Irrigation on seed yield of faba bean (Reproduced from Paul et al., 2021). Here, I<sub>0</sub>= no irrigation, I<sub>1</sub>= one irrigation at early branching stage, I<sub>2</sub>= one irrigation at pod formation stage and I<sub>3</sub>= two irrigations at early branching and pod formation stages.

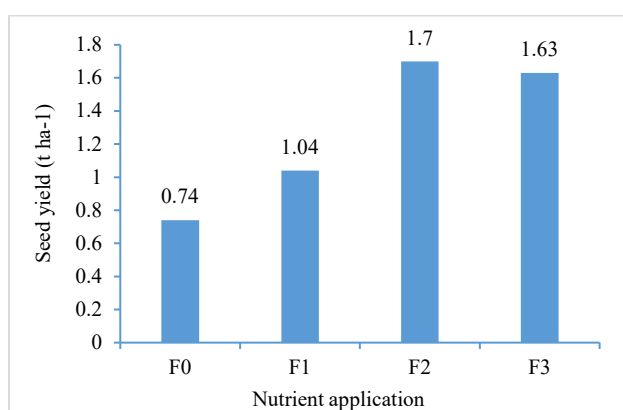


Figure 5. Impact of nutrient application on seed yield (Reproduced from Paul et al., 2021). Here, F<sub>0</sub> = 0-0-0-0, F<sub>1</sub> = 5-20-20-5, F<sub>2</sub> = 10-40-40-10 and F<sub>3</sub> = 15-60-60-15 @ N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O-S kg ha<sup>-1</sup>, respectively.

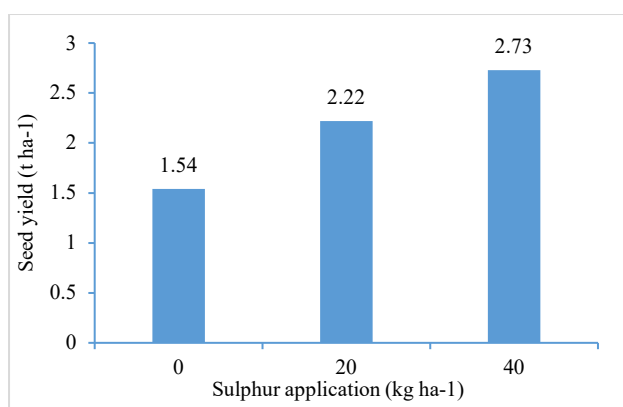


Figure 6. Impact of sulphur on seed yield of faba beans (Reproduced from Miah et al., 2023)

Legume crops often respond well to phosphorus fertilization, especially in soils where phosphorus is limited. Phosphorus is an essential nutrient element for legume crops (Kumar et al., 2019). For faba beans, phosphorus fertilizer applications typically ranges 20 - 30 kg P ha<sup>-1</sup> (FAO, 2000). Increasing the rate of phosphorus fertilization to 45-46.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> has been shown to significantly boost faba bean yield and its contributing characteristics (El-Habbasha et al., 2007). Furthermore, Yasmin et al. (2020) found that applying 40 kg P ha<sup>-1</sup> led

to the greatest numbers for the branches plant<sup>-1</sup>, pods plant<sup>-1</sup>, 1000-seed weight, seed and stover yield, and protein percent in seeds (Figure 7).

Sulphur has unique physiological and biochemical functions, making it an essential component of the legume-rhizobium system. Following nitrogen, phosphorus, and potassium, it is frequently recognised as the fourth primary nutrient (Marschner 1995). According to Cazzato et al. (2012) and Głowacka et al. (2019), sulphur fertilization has an influence on grain yield, seed quality, and N-fixation. According to Miah et al. (2023) the highest results for branches plant<sup>-1</sup>, pods plant<sup>-1</sup>, 1000-seed weight, seed and stover yields were obtained by applying 40 kg S ha<sup>-1</sup> (Figure 6).

### Biofertilizer application

A biofertilizer, also known as a biological fertilizer, is a substance that contains live or dormant microorganisms. These microorganisms either colonize the rhizosphere or reside within plants to promote growth directly or indirectly by supplying essential nutrients (Fasusi et al., 2021; Timofeeva et al., 2023). Biofertilizers improve soil fertility by including the microorganisms like *Anabaena*, *Nostoc*, *Rhizobia*, *Burkholderia*, *Clostridium* and *Tolypothrix*. These microorganisms settle in the rhizosphere or plant tissues when treated to seeds, surfaces of crops, or soil. They aid in the growth of the plant by transforming essential nutrients, like phosphorus and nitrogen, into forms that are soluble through procedures like phosphate solubilisation and nitrogen fixation (Rokhzadi et al., 2004; Ramasamy et al., 2020). Incorporating biofertilizers into faba bean cultivation enhances growth, yield, and quality by optimizing nutrient availability, improving soil health, and reducing the environmental impact of farming practices (Sheikh et al. 2020; Ding et al. 2021; Bhardwaj et al. 2024). El-Yazid et al. (2007), observed that beneficial microorganisms in biofertilizers also promote growth of plants and provide defense against infections and pests. According to Sheikh et al. (2020) the use of biofertilizer improved seed quality and yield when compared to a control group. While *Rhizobium* inoculum 120 g kg<sup>-1</sup> seeds provided the highest levels of crude protein (33.54%) and crude fat (2.82%), *Rhizobium* inoculum 80 g kg<sup>-1</sup> seed application produced the highest levels of seed yield (1.04 t ha<sup>-1</sup>), stover yield (1.75 t ha<sup>-1</sup>), ash (4.88%), and fibre (15.28%). According to Badr et al. (2013) In order to fix nitrogen, faba beans symbiotically partner with *Rhizobium* bacteria, more especially *Rhizobium leguminosarum* Frank bv. *viciae*. To achieve the maximum amount of nitrogen fixation, which is usually between 60 and 250 kg ha<sup>-1</sup> year<sup>-1</sup>, farmers are normally encouraged to inoculate their seed with the appropriate culture (Torres et al., 2012). When compared to the non-inoculation control, faba bean cultivars treated with VA-mycorrhizal demonstrated increases in seed yield ha<sup>-1</sup> of 20.7% and 23.2% (Figure 8). According to Bhomik et al. (2022), brown seeded faba bean with rhizobial strain FM-1a had the highest number of nodules plant<sup>-1</sup> (85.67), nodules dry weight ha<sup>-1</sup> (8.27 mg), dry weight ha<sup>-1</sup> (198 g), number of pods ha<sup>-1</sup> (47.33), 100-seed weight (23.48 g), seed (2.66 t ha<sup>-1</sup>) and stover (3.26 t ha<sup>-1</sup>) yields compared to other strains and controls.

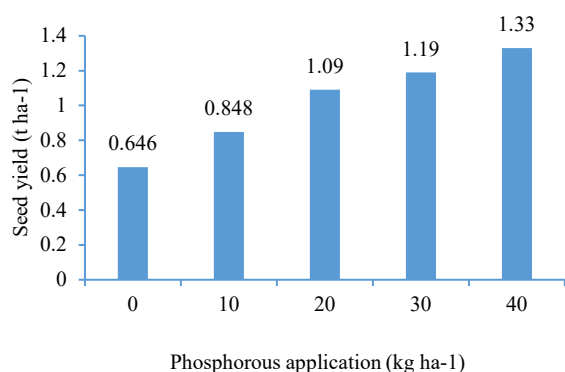


Figure 7. Impact of sulphur on seed yield of faba beans (Reproduced from Miah et al., 2023)

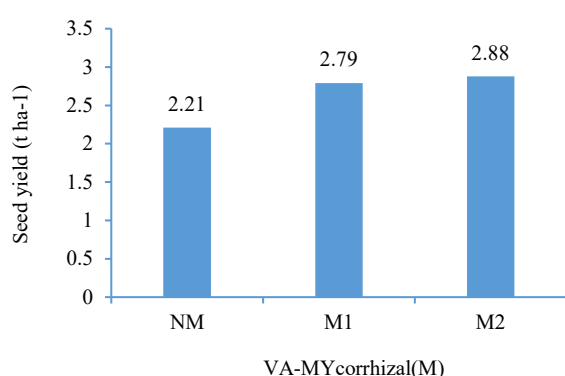


Figure 8. Impact of VA-MYcorrhzial (M) inoculation on seed yield of faba bean (Reproduced from Badr et al., 2013). Here, (a) inoculation with strain of VA-mycorrhizal fungi *Glomus macrocarpium* (M<sub>1</sub>) *Glomus manihitis* (M<sub>2</sub>) and (b) without inoculation (NM).

### Crop rotation

Crop rotation, an essential practice for boosting the yield through successive production of agronomic crops, which provide sustainability of faba bean production by improving soil fertility, managing pests and diseases, controlling weeds, and enhancing overall soil health. These benefits lead to higher and more consistent yields. Establishing an effective crop rotation strategy is crucial for ensuring long-term productivity in faba bean farming through soil biological and physical properties improvement (Aschi et al., 2017) and also offers a range of ecological benefits by including faba bean into a cropping system (Köpke and Nemecek, 2010). Traditionally a legume into a crop rotation system were incorporated due to their ability to supply nitrogen for subsequent crops (Ntatsi et al., 2018). It is a effective approach for organic farming, where nitrogen supply are heavily depends on the sources that are not derived from fossil fuels. Growing economic and environmental concerns regarding synthetic nitrogen fertilizers have revived interest in the role of legumes within crop rotations. Although the exact nitrogen-fixing capacity of faba beans is still being studied, they are acknowledged as a reliable nitrogen source. As

such, faba beans can be integrated into rotational systems as a cash, cover, or multi-purpose agronomic crop. Additional advantages of faba bean incorporated in a rotational system are: role as break crop (Abera et al., 2015), resources for beneficial and pollinator insects (Etemadi et al., 2015), boosting soil microbes (Wahbi et al., 2016), reducing soil-borne pathogen (Jensen et al., 2010), and provide L-DOPA (L-3, 4-dihydroxyphenylalanine) sources.

### Mulching

Mulching is a highly beneficial practice for faba bean cultivation, offering advantages such as moisture conservation, temperature regulation, weed suppression, soil health improvement, and disease prevention. These benefits contribute to improved plant growth, higher yields, and more sustainable farming practices. Implementing mulching (artificial and natural) can be a valuable strategy for improving the yield and resilience of faba bean. Artificial mulching refers to the practice of covering soil around the plants with organic or inorganic materials that can provide several purposes. Natural mulching helps in retaining soil moisture by reducing evaporation. As the soil crust breaks down, it creates a loose, porous layer on the surface that traps moisture and reduces water loss. Both are highly beneficial practice for faba bean cultivation, offering advantages such as soil moisture conservation through evaporation reduction, temperature regulation, weed suppression, soil health improvement, minimizing fertilizer leaching and disease prevention which improves crop resilience and final yield. The benefits of using mulching materials in cultivation also include early harvesting through early flowering and increasing flower number (Lee et al., 2018).

### Weed Management

Weed competition is most critical when the crop is young or actively growing. During this stage, it is essential to remove weeds, as this is the brief period in the crop's development when weeding yields the highest economic benefits, the most intense competition in faba beans with weeds occurs within the first 20-30 days after sowing. This emphasizes the importance of timely weed management in faba bean productivity. Frenda et al. (2013) noticed that weed infestation is a significant obstacle to the production of faba bean, which can cut yield by up to 50%. To achieve a high yield, weeds must be removed as soon as possible, ideally between 25 and 75 days after sowing (Tawaha and Turk, 2001).

A weed-free environment in faba bean throughout the growth period till 60 DAS can resulted in increasing of plant height, branching, pod and seed numbers, seed weight and finally yield (Miah et al., 2023). Weed management methods that have been practiced in faba bean field are prevention, cultural control, chemical control and integrated management. An integrated weed management strategy in include ploughing, sowing improved variety, fertilizer application, pre-emergence herbicide spray and/or manual weeding in such sequential order with proper timing of the activities (Table 3).

Table 3. Integrated management of weeds in faba bean (Source: Hundessa et al., 2022)

Season	Weed control methods
Off-season	<ul style="list-style-type: none"> <li>clearing farm land by cutting weeds before seed setting and removing them from the field</li> <li>cleaning farm tools and machineries before entering and starting operation in the crop field</li> </ul>
Before planting	<ul style="list-style-type: none"> <li>twice ploughing</li> </ul>
At planting	<ul style="list-style-type: none"> <li>sowing improved variety weed free seed at recommended rate, time, pattern and spacing</li> <li>application of recommended NPS</li> </ul>
Before emergence	<ul style="list-style-type: none"> <li>Pendimethalin or S-metolachlor application at 1 L ha<sup>-1</sup> rate in 200 L ha<sup>-1</sup> water</li> </ul>
After emergence	<ul style="list-style-type: none"> <li>manual assisted hoeing and hand weeding activities on 30 to 35 and 50 to 55 DAS</li> <li>removal of weeds left from the control practices</li> </ul>

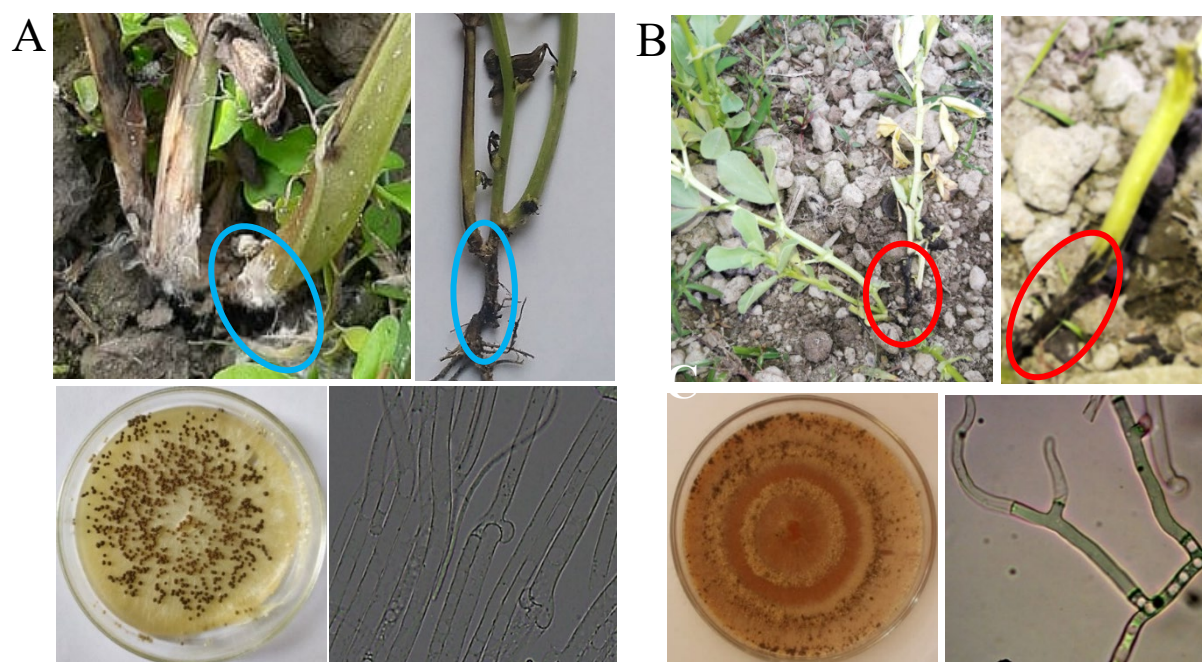


Figure 9. A. Collar rot disease of faba beans caused by *Sclerotium rolfsii* (Paul et al., 2023) B. Collar and root rot disease caused by *Rhizoctonia solani* (Paul et al., 2022) observed in Bangladesh field.

### Diseases and their management

Faba beans is susceptible to several diseases that can significantly impact their yield and quality. Particularly in humid weather, faba bean crops are mostly vulnerable to fungal infections. The three primary diseases affecting faba bean crops worldwide are rust, chocolate spot, and ascochyta blight (Torres et al., 2006; Stoddard et al., 2010). However, root rot of fababean is a major problem in china, Bangladesh, Egypt, Ethiopia and some other tropical countries (Abdel-Razik et al. 2012; Yu et al. 2023; Paul et al. 22, 23). Many pathogens, such as *Fusarium* spp., *Rhizoctonia* spp., *Sclerotium rolfsii*, *Pythium* spp., *Phoma* spp., and *Aphanomyces* spp., can cause the root rot disease of faba beans (Heyman et al. 2013; Rubiales and Khazaei, 2022; Paul et al. 22, 23). In china, root rot and wilt disease associated with *Fusarium* spp., *Rhizoctonia solani*, and *Pythium debaryanum* are a major challenge are the major concern to yield losses of 5%–30% and even up to 100% due to advantageous environmental situations (Dong et al., 2014; Zhang et al., 2018). Additionally, Yu et al. (2023) documented that a large amount of root and stem rot disease were identified with 10% to 30% of plants dying and up to 40% in fields with severe infection (Yu et al., 2023). In Bangladesh, collar rot (Figure 9A), caused by *Sclerotium rolfsii*, has been reported with an incidence

ranging from 11% to 19%, while collar and root rot (Figure 9B), attributed to *Rhizoctonia solani*, showed an average disease incidence of 7.16% (Paul et al., 2022; Paul et al., 2023). Sahile et al. (2010) and Emeran et al. (2011) have indicated that yield losses from rust and chocolate spot infections can vary significantly, from 22% to 42% and 36% to 68%, respectively. Synthetic fungicides are commonly used for managing faba bean diseases, alongside practices such as crop rotation and residue removal from fields after harvest (Harveson and Schwartz, 2007). Biofungicides are also employed as a biorational approach to disease management (El-Mougy and Abdel-Kader. 2008; Sahile et al. 2011). An overview of prevalent diseases affecting faba beans and their corresponding management strategies is provided in Table 4.

### Insects

Aphids, beetles, and hoppers are regarded as major pests of faba beans. Though these pests occasionally appear on faba beans, they can rarely reach levels that cause significant economic harm. Aphids and leafhoppers also role as a vector for viruses, with leafhoppers specifically transmitting aster yellows.



Table 4. List of faba beans diseases, their causal agents, symptoms and possible management

Disease name and causal organism	Symptoms	Management
<i>Chocolate spot (Botrytis fabae)</i>	Small reddish-brown lesions appear on the plant's leaves and sometimes on the stems and pods. (Haile et al., 2016).	The best management technique is prevention. It is advised to plant early to avoid late spring and early summer's high humidity and heat. Application of <i>Trichoderma</i> spp. have also shown effectiveness to control the disease ( Sahile et al. 2011; Kora et al., 2017).
<i>Leaf blight (Xanthomonas campestris syn. Xanthomonas axonopodis)</i>	Small, expanding necrotic water spots develop on the leaves, with lesions that can merge, giving the plant a scorched appearance. The spots may be surrounded by a yellow discoloration. Round, deep reddish-brown lesions can also appear on the pods, and dead leaves may remain attached. Buruchara et al., 2010)	Effective treatment methods include using clean seeds (Tar'an et al., 2001; Gillard et al., 2009), planting resistant varieties, treating seeds before planting, and spraying plants with a suitable copper-based fungicide as a preventive measure before symptoms appear (Buruchara et al., 2010 ).
<i>Bacterial brown spot (Pseudomonas syringae)</i>	Small, dark-brown necrotic patches are frequently encircled by a yellow tissue patch (Schwartz et al., 2005).	Preventive measures include using clean seeds, implementing crop rotation, and burning or cleaning residues from crop field after harvesting (Harveson and Schwartz, 2007).
<i>Powdery mildew (Erysiphe cichoracearum)</i>	Yellow dots appear on the tops of the leaves, and areas of powdery grey-white coating cover the entire plant. Plants that are heavily infected may exhibit slight gray and blue discoloration. (Trabanco et al., 2012).	Using resistant varieties and, overhead irrigation to wash fungus spores from the leaves. Early planting to avoid high temperatures and humidity. Spraying sulfur pesticides is also advised for chemical control. (Van Emden et al., 1988).
<i>Fusarium root rot (Fusarium solani)</i>	Stunted growth, yellowing of leaves, necrotic basal leaves, and brown, black or red streaks on mature plant roots. (Abdel-Kader et al., 2011).	Use resistant varieties, proper crop rotation, improve soil drainage and structure. If the disease is severe, apply appropriate soil fungicides (benomyl or carbendazim) before planting. Furthermore, seed priming with biofungicides reduce the disease severity (El-Mougy and Abdel-Kader. 2008)
<i>Collar and root rot (Rhizoctonia solani)</i>	Dark brown to black lesions on infected plant roots extending upon collar region (Paul et al., 2022)	Intercropping with garlic, application of Rizolex-T, chitosan and biocontrol agents such as Arbuscular mycorrhizae (AM), yeast significantly decreased the incidence and disease index of root rot diseases El-Mehy et al., 2022; Gazzar et al., 2023).
<i>Collar rot (Sclerotium rolfsii)</i>	Water-soaked sunken lesions appeared on the collar region followed by the expansion of rotten areas along with white mycelial mass (Paul et al., 2023).	Implementing crop rotation, and burning or cleaning residues from crop field. Provax (Carboxin 17.5% + Thiram 17.5% FF) shows strongest mycelia growth inhibition.
<i>Rust (Uromyces viciae-fabae (Pers.) J. Schröt)</i>	Rust usually occurs late in the growing season during podding, resulting in leaf damage and premature leaf drop (Sillero et al., 2011)	Rust can be successfully controlled by foliar spraying fungicides such as chlorothalonil, thiram, maneb, or mancozeb, and dithiocarbamates (difenoconazole, epoxiconazole, or tebuconazole) (Emeran et al., 2011).

Shoots and buds of faba beans are consumed through Blister beetles and grasshoppers, and localized damage symptom appear on the infected plant. Blister beetles pose a concern when faba beans are cultivated for livestock feed because of the toxin cantharidin. Early in the season, inspect plants for tissue damage caused by cutworms. It's important to note that insecticide seed treatments do not protect against cutworms, but faba bean seedlings can regrow from the growing point at the seed after damage. Additionally, pea leaf weevils (Tkachuk, et al., 2020), and black bean aphid (Webster et al., 2010) causes significant

damage to faba beans. Visual inspection before transplanting can reduce the aphid infestation, and a reflective mulch (silver-colored plastic) can prevent aphids to reaching on plants. A mechanical way like strong water jet can dislodge aphids from sturdy plant leaves. Plants can usually tolerate low to medium levels of aphid presence, otherwise insecticides are typically needed for severe infestations. Neem or canola soap, extract or oil are generally applied as the most effective biocontrol. (Birch, 1985). Faba bean's insects are listed in Table 5.

Table 5. Insects found feeding on leaves, stems, flowers and pods of faba beans (Source: Nuessly et al., 2004).

Order	Family	Insect	Plant part	
Orthoptera	Acrididae	<i>Chortophaga australion</i>	Leaf	
	Tettigoniidae	<i>Microcentrum rhombifolium</i>	Leaf	
Thysanoptera	Thripidae	<i>Frankliniella bispinosa</i>	Flower	
		<i>Frankliniella insularis</i>	Flower	
		<i>Frankliniella kelliae</i>	Flower	
Hemiptera	Miridae	<i>Creontiades rubinervis</i>	Leaf	
	Lygaeidae	<i>Oncopeltus cayensis</i>	Stem /pod	
		<i>Ozophora trinotata</i>	Leaf	
	Pyrrhocoridae	<i>Dysdercus mimulus</i>	Pod	
	Coreidae	<i>Acanthocephala femorata</i>	Pod	
		<i>Anasa scorbatica</i>	Pod	
		<i>Leptoglossus phyllopus</i>	Pod	
		<i>Zicca taeniola</i>	Pod	
	Pentatomidae	<i>Acrosternum hilare</i>	Pod	
		<i>Acrosternum marginatum</i>	Pod	
		<i>Edessa bifida</i>	Pod	
		<i>Euschistus ictericus</i>	Pod	
		<i>Euschistus quatrator</i>	Pod	
		<i>Nezara viridula</i>	Pod	
		<i>Thyanta perditor</i>	Pod	
		Cicadellidae	<i>Draeculocephala mollipes</i>	Leaf
			<i>Gypona</i> sp.	Leaf
		Aphidae	<i>Acyrtosiphon pisum</i>	Leaf
	<i>Aphis craccivora</i>		Leaf and stem	
	Coleoptera	Pseudococcidae	<i>Planococcus citri</i>	Root and stem
Scarabaeidae		<i>Anomala marginata</i>	Pollen/nectar	
		<i>Euphoria sepulcralis</i>	Pollen/nectar	
		<i>Trigonopeltastes delta</i> Forster	Pollen/nectar	
Cantharidae		<i>Chauliognathus marginatus</i>	Pollen/nectar	
Chrysomelidae		<i>Diabrotica balteata</i>	Leaf	
		<i>Diabrotica undecimpunctata howardi</i>	Leaf	
Lepidoptera		Curculionidae	<i>Diaprepes abbreviatus</i>	Leaf
		Arctiidae	<i>Spilosoma virginica</i>	Leaf
		Noctuidae	<i>Feltia subterranea</i>	Seedling stem
	<i>Spodoptera eridania</i>		Leaf	
	Saturniidae	<i>Automeris io io</i>	Leaf	
	Hesperiidae	<i>Lerema accius</i>	Flower	
Diptera	Agromyzidae	<i>Liriomyza trifolii</i>	Leaf	
Hymenoptera	Chrysididae	<i>Chrysis</i> sp.	Nectar	
	Halictidae	<i>Agapostemon splendens</i>	Nectar	
		<i>Halictus</i> sp.	Nectar	
	Anthophoridae	<i>Xylocopa micans</i>	Nectar	
Apidae	<i>Apis mellifera</i> L.	Pollen/nectar		

(Nuessly et al., 2004)

### Harvesting and Yield

Harvesting is important for faba beans because it maximizes yield, preserves quality, ensures proper storage, and has significant economic, environmental, and sustainability implications. Timely and efficient harvesting practices are essential for producing high-quality faba beans that meet market demands and involve to sustainable agronomic practices. The time required for faba beans to extent harvest maturity depends on the variety and environmental conditions, typically ranging from 80 to 120 days after planting. Harvesting should commence when the stems are still slightly green. As the beans mature, the stems become black or dry, and the lower pods start to darken or when most seeds can be easily separated from the hilum. At this stage, the beans usually have a moisture content between 30 and 35%. Generally, a moisture level of 15 to 18% in the seeds at harvest is adequate to reduce

the risk of shattering. However, drying too quickly at high temperatures often leads to stress cracks (Singh et al., 2013). Typically, about 70-100 quintals per hectare of green pods can be obtained (Choudhary, 2014). One tonne of raw fava beans produces around 240 kg of protein, 520 kg of starch, 200 kg of fiber, and a small amount of oil (Tsolakis et al., 2019).

### Limitations and Future Directions for Faba Bean Cultivation

Faba bean cultivation faces several limitations, including susceptibility to diseases like rust, chocolate spot, and collar rot, which can significantly reduce yield (Torres et al., 2006; Stoddard et al., 2010; Paul et al., 2022, 2023). The reliance on synthetic fungicides raises concerns

about environmental impact and the development of resistant pathogen strains. Additionally, abiotic stresses such as drought, poor soil fertility, and extreme temperatures can further limit productivity (Khan et al., 2010). Therefore research should focus on developing disease-resistant and stress-tolerant faba bean varieties through advanced breeding techniques, including marker-assisted selection and CRISPR-Cas technology. Integrating sustainable farming practices, such as biofungicides, crop rotation, and organic soil amendments, can also improve disease management. Moreover, increasing research on faba bean's nutritional value and promoting its use as a protein-rich crop in sustainable food systems could enhance its global significance.

## Conclusion

The faba bean is a versatile and highly valued crop, renowned for its nutritious and medicinal properties. Its seeds, pods, and leaves are packed with protein and contain

nearly all the essential nutrients required in human diets. Additionally, faba beans are renowned for their high biological nitrogen fixation capabilities among grain legumes. It is appreciated for its nitrogen-fixing ability, which improves soil fertility and minimizes the reliance on synthetic fertilizers. This makes them an excellent choice for crop rotations, particularly in sustainable agricultural systems. With proper management, including the right choice of variety, planting time, and soil management, faba beans can produce high yields. However, the yield can be significantly influenced by environmental factors including soil type, available moisture, and temperature. Effective management of pests and diseases is crucial for maximizing faba bean productivity. Faba beans are a versatile and valuable crop in agronomy, offering numerous benefits to farmers, the environment, and food systems. Ongoing research and development are essential to optimize their production and address challenges such as disease resistance and climate adaptability.

### Limitations

- Susceptible to various disease and pests.
- Insufficient management strategies.
- Sensitive to extreme temperatures (both high and low), drought and salinity.
- Limited genetic diversity.
- Lower cultivation area compared to other pulses.
- Limited market demand can discourage farmers in faba bean cultivation.

### Future directions

- Breeding and genetic modification to develop pest and disease-resistant varieties.
- Develop integrated management strategies.
- Breeding for varieties that are more tolerant to temperature extremes, drought and salinity conditions.
- Collection faba beans genotypes and use genomic tools to explore and incorporate genetic diversity from wild relatives and landraces to broaden the genetic base.
- Integration into diverse cropping systems, such as intercropping and crop rotations.
- Develop new markets (poultry and livestock feed) and value-added products to increase demand for faba beans.

Figure 10. Limitations and future directions for faba bean cultivation

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## Hot Water Treatments in The Production of Healthy Vine Saplings

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ARTICLE INFO	ABSTRACT
<p>Review Article</p> <p>Received : 03.09.2024 Accepted : 03.11.2024</p> <p><b>Keywords:</b> Grapevine trunk diseases Pathogens Clean propagation material Grapevine seedling quality Sustainable viticulture</p>	<p>Grapevine trunk diseases (GTD) include <i>Esca</i> syndrome (<i>Phaeoconiella chlamydospora</i> and <i>Phaeoacremonium oleophilum</i>, <i>Botryosphaeria</i> spp., <i>Eutypa lata</i>, <i>Phomopsis viticola</i>, <i>Cylindrocarpon</i> spp.), Petri disease (<i>Phaeoconiella chlamydospora</i>, <i>Phaeoacremonium</i> spp., <i>Cadophora luteo-olivacea</i> and <i>Pleurostoma richardsiae</i>), Blackfoot (<i>Dactylonectria</i>, <i>Ilyonectria</i>, <i>Campylocarpon</i>, <i>Cylindrocladiella</i> and <i>Neonectria</i>), <i>Botryosphaeria</i> dieback (<i>Lasiodiplodia theobromae</i>, <i>Neofusicoccum parvum</i> and <i>Botryosphaeria dothidea</i>), <i>Eutypa</i> dieback (<i>Eutypa lata</i> and <i>Diatrypaeaceae</i> spp.), <i>Phomopsis</i> dieback (<i>Phomopsis viticola</i>). GTD infections cause the death of grapevines in the short or long term. On a global scale, it has been considered the most devastating disease of grapevines for the last thirty years, as it affects the sustainability of viticulture and spreads rapidly in all wine-growing countries. Hot water treatment (HWT), agricultural chemicals and disinfectants are used to control GTD. Young vines, dormant bud or rootstock cuttings, rooted or grafted rooted vine seedlings and <i>Vitis vinifera</i> L. cultivars may show varying levels of sensitivity to HWT. This sensitivity may be affected by the seasonal temperatures in which the cuttings or saplings are growing and the temperature range to be treated may vary according to the pathogens to be controlled. HWTs (45-54°C for 30-45 minutes) are recommended at varying intervals depending on the variety to suppress GTD in vine cuttings. HWT is an effective control method for phylloxera, nematode and phytoplasma pathogens that may be transmitted by grapevine propagation materials. A quality grapevine sapling should be healthy, true name, have a good plant form, be well united, be free from viruses and pathogens and not be exposed to environmental stress. In this review, a summary of HWT studies used in the control of pathogenic bacterial, fungal, nematode, phytoplasma and phylloxera infections of grapevine propagation materials is presented.</p>

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## Sağlıklı Asma Fidanı Üretiminde Sıcak Su Uygulamaları

MAKALE BİLGİSİ	ÖZ
<p>Derleme Makalesi</p> <p>Geliş : 03.09.2024 Kabul : 03.11.2024</p> <p><b>Anahtar Kelimeler:</b> Asma gövde hastalıkları Patojenler Temiz çoğaltma materyali Asma fidanı kalitesi Sürdürülebilir bağcılık</p>	<p>Asmalarda gövde hastalıkları (AGH) arasında, <i>Esca</i> sendromu (<i>Phaeoconiella chlamydospora</i> ve <i>Phaeoacremonium oleophilum</i>, <i>Botryosphaeria</i> spp., <i>Eutypa lata</i>, <i>Phomopsis viticola</i>, <i>Cylindrocarpon</i> spp.), Petri hastalığı (<i>Phaeoconiella chlamydospora</i>, <i>Phaeoacremonium</i> spp., <i>Cadophora luteo-olivacea</i> ve <i>Pleurostoma richardsiae</i>), Siyah ayak (<i>Dactylonectria</i>, <i>Ilyonectria</i>, <i>Campylocarpon</i>, <i>Cylindrocladiella</i> veya <i>Neonectria</i>), <i>Botryosphaeria</i> dieback (<i>Lasiodiplodia theobromae</i>, <i>Neofusicoccum parvum</i> ve <i>Botryosphaeria dothidea</i>) <i>Eutypa</i> dieback, (<i>Eutypa lata</i> ve <i>Diatrypaeaceae</i> spp.) <i>Phomopsis</i> dieback, (<i>Phomopsis viticola</i>) yer almaktadır. AGH enfeksiyonları kısa veya uzun vadede asmaların ölümüne neden olur. Global ölçekte, bağcılığın sürdürülebilirliğini etkilediğinden ve tüm bağcı ülkelerde hızla yayıldığından, son otuz yıldır asmanın en yıkıcı hastalıkları olarak kabul edilmektedir. AGH'nın kontrolünde sıcak su uygulaması (SSU), tarım kimyasalları ve dezenfektanlar kullanılmaktadır. SSU'na genç asmalar, dinlenme halindeki aşı gözü veya anaç çelikleri, köklü veya aşı köklü asma fidanları ve <i>Vitis vinifera</i> çeşitleri farklı düzeyde hassasiyet gösterebilirler. Bu hassasiyet çeliklerin veya fidanların büyüdüğü mevsim sıcaklıklarından etkilenebilir ve uygulanacak sıcaklık aralığı, kontrol edilecek patojenlere göre değişebilir. Asma çeliklerindeki AGH'ni baskılamak için çeşidine göre değişen aralıklarda SSU'ları (30-45 dakika süreyle 45-54°C) önerilmektedir. SSU, asma çoğaltma materyalleri ile taşınabilecek filoksera, nematod ve fitoplazma patojenleri için etkin bir kontrol yöntemidir. Kaliteli bir asma fidanı, sağlıklı, ismine doğru, iyi bir bitki formuna sahip, iyi kaynaşmış, virüsler ve patojenlerden arındırılmış, çevresel strese maruz kalmamış olmalıdır. Bu derlemede, asma çoğaltma materyallerinin patojenik bakteri, mantar, nematod, fitoplazma ve filoksera enfeksiyonlarının kontrolünde kullanılan SSU çalışmalarının bir özeti sunulmuştur.</p>

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## Giriş

Çoğaltma ve dikimde kullanılacak asma materyalinin bazı kalite özellikleri görsel incelemeyle kolayca ayırt edilebilir (Morton ve Waite, 2007). Satışa sunulan asma fidanları genellikle uyku döneminde ve çıplak köklü olarak satıldığından, sağlık ve kalite özellikleri (Baker ve Linderman, 1979), bağda büyümeye başladığından sonra fark edilebilir (Morton ve Waite, 2007). Fidanlarda makine veya haşerelerin neden olduğu dış yaralar istenmez. Çünkü bu yaralar yapısal zayıflığa neden olabilir ve dokuyu enfeksiyona maruz bırakabilir. Aşı yerleri tamamen kaynaşmalı, orta düzeyde basınç uygulandığında kırılmamalı ve aşı yerindeki kallus çapı gövde çapının dışına 2-2,5 mm'den fazla taşmamalıdır (Morton ve Waite, 2007). Dinlenme halindeki, aşısız ve aşılı asmalar, en az 3 iyi gelişmiş kök ve iyi gelişmiş sağlıklı kışık tomurcukları olan 2 sürgüne sahip olmalıdır (Nicholas ve ark., 1992). Yüksek kaliteli dikim materyali, aynı zamanda kaynağına (ana-asma bloğu ve fidanlık) kadar izlenebilir olmalı ve kökenleri ve hastalık durumunu belgeleyen sertifikalara sahip olmalıdır (Morton ve Waite, 2007).

AGH, global ölçekte bağcılıkta önemli ekonomik bir sorundur. Son otuz yıldır asmanın en yıkıcı hastalıkları olarak kabul edilmekte olup bağcılığın yapıldığı tüm ülkelerde hızla artan bir endişe kaynağıdır. Dünya çapındaki AGH etkisiyle ölen asmaların yenilenmesi ekonomik maliyetinin kabaca yılda 1,5 milyar doları aştığı tahmin edilmektedir (Hofstetter ve ark., 2012). AGH'dan sorumlu patojenler, çok yıllık organları enfekte ederek asmaların kısa veya uzun vadede ölümüne neden olur. AGH'na çok çeşitli mikro organizmalar neden olabilir ve bu nedenle birkaç gruba ayrılır (Travadon ve ark., 2013).

Bugüne kadar, dünya genelinde 34 cinsle ait 133 mantar türü AGH ile ilişkilendirilmiştir. Bununla birlikte, AGH mantarları, asmaları enfekte ettiği bilinen en büyük patojen grubunu oluşturmaktadır (Mugnai ve ark., 1999; Mostert ve ark., 2006a; Christen ve ark., 2007; Agusti-Brisach ve Armengol, 2013; Carlucci ve ark., 2015; Da Silva ve ark., 2017; Lawrence ve ark., 2017; Gramaje ve ark., 2018). Farklı mantarların neden olduğu dört ana asma gövde hastalığı vardır. Bu hastalıklara *Esca*, *Eutypa dieback*, *Botryosphaeria dieback* ve *Phomopsis dieback* 'excoriosis' adı verilir. *Esca*, Avrupa'da büyük bir sorundur ve *Eutypa* tüm dünyada görülmektedir. *Botryosphaeria* da küreseldir, ancak pek çok üretici tarafından çok iyi anlaşılmamış veya tanınmamıştır (Smart, 2015). Bunlara Petri hastalığı, Siyah ayak hastalığı, *Esca* hastalığı kompleksi, *Phomopsis*, *Eutypa* ve *Botryosphaeria* patojenlerinin neden olduğu geriye doğru ölüm dahildir (Gramaje ve ark., 2018). Ayrıca bu patojenler, odun kanserleri, sürgün gerilemesi, tomurcuk nekrozu, gecikmiş tomurcuk patlaması ve tane tutumunun azalması gibi çok çeşitli semptomlara neden olabilir (Aroca ve ark., 2006; Amponsah ve ark., 2011; Gramaje ve ark., 2018).

AGH nedenleri arasında çoklu enfeksiyonlar da bildirilmiştir (Bertsch ve ark., 2013). Genç bağlarda, Siyah ayak veya Petri hastalığından etkilenen bodur büyüme, azalan büyüme gücü, sürgün büyümesinde gecikme veya hiç sürgün gelişmemesi, boğum aralarında kılma, yaprak kenarlarında nekrotik lekeler, solma, sürgünlerde geriye doğru çökme ve ölüm gibi dış belirtiler ortaya çıkmakla birlikte çoğunlukla ayırt edilememektedirler (Gramaje ve Armengol, 2011).

AGH'dan etkilenen bir bitkide, enfekte olan dokuyu ortadan kaldırmanın tek yöntemi, etkilenen organları fiziksel olarak kesmektir. En kötü durumda asmanın tamamının çıkarılması önerilir (Billones-Baaijens ve ark., 2018). Hem asmaları AGH enfeksiyonundan korumak hem de inokulum seviyelerini azaltmak için kültürel, biyolojik ve kimyasal yöntemlerin değerlendirilmesi geniş çapta çalışılmış olmakla birlikte bunların etkin bir şekilde yönetilmesi zor olmaya devam etmektedir (Gramaje ve ark., 2018).

Fidanlıktaki bitki sağlığı önlemlerinin, AGH patojenlerinin neden olduğu enfeksiyonları azaltmak için en iyi yaklaşım olduğu kabul edilir (Surico ve ark., 2008). Petri hastalığına neden olan *P. chlamydospora* ve birçok *Phaeoacremonium* spp.'nin yönetimi, dahili patojenleri ortadan kaldırmak yerine, depolama ve kallus gelişimi sürecinde, çoğaltma materyali üzerindeki yüzeysel büyümeyi sınırlamak için anaç ve aşı gözü çeliklerinin kimyasal formülasyonların süspansiyonlarıyla ıslatılmasıyla başarılmıştır (Fourie ve Halleen, 2004a). 1.5 ml L<sup>-1</sup> Sporekill (bir didesildimetil amonyum klorür formülasyonu olarak patentli bir ürün) ve 10 ml L<sup>-1</sup> Captan, çıplak köklü asma fidanlarının kök uçları ve aşı yerlerinde patojen oluşumunu azaltmış ve aşılı fidanlarda büyüme parametrelerini olumsuz etkilememiştir (Fourie ve Halleen, 2004a).

Asma dikim materyalinde patojenik bakteri, mantar ve nematod çıkışı ve enfeksiyonunu azaltmak için fidanlıklarda SSU yapılır (Waite ve ark., 2013; Gramaje ve ark., 2018). Patojenler bitki dokularında asemptomatik olabilirler (van Niekerk ve ark., 2004; Billones-Baaijens ve ark., 2013) ve bu tip materyallerin kullanılması patojenlerin bağda yayılmasına neden olabilir (Billones-Baaijens ve ark., 2018).

Fidanlık materyallerine SSU'nun *Botryosphaeria dieback* (Crous ve ark., 2001; Bleach ve ark., 2013; Elena ve ark., 2015; Bruez ve ark., 2017), fitoplazmalar ve nematodlar dahil olmak üzere AGH patojenlerinin kontrolünde kullanılmaktadır (Waite ve May, 2005). Bununla birlikte SSU asma çoğaltma materyallerini olumsuz etkileyebilir (Waite ve May, 2005; Halaly ve ark., 2008; Songy ve ark., 2019) fakat, bu etkiler çeşitler, bölgeler ve bitkinin spesifik koşullarına göre değişebilmektedir (Paniagua-Madrigal, 2020). SSU ve depolama sürecinin de çoğaltma materyali sağlığını etkilediğinden kallus gelişiminden önce uygulanması ve ayrıca, SSU protokolünün sıcaklık ve zamanlamasının asma çoğaltma materyallerinin çeşidine göre belirlenmesi tavsiye edilmiştir (Waite ve May, 2005).

Çalışmalar, 65°C ve üzerindeki bir sıcak buhar en az 30 dakika uygulandığında bitki patojenleri ve böcek zararlılarının hayatta kalmadığını göstermiştir (Baker ve Chandler, 1957; Samtani ve ark., 2012). Basınçlı sprey olarak buhar kullanılarak yapılan ilaçlama ve SSU etkili olup ticari çiftlik ekipmanlarının dezenfeksiyonu için kolaylıkla uygulanabilir (Bollen, 1969; van Loenen ve ark., 2003). Isıl işlem genellikle kimyasal işlemden daha güvenlidir ve minimum çevresel ve sağlık etkilerine sahiptir. Özellikle haşereleri veya patojenleri öldürmek için gereken maruziyet süresinin nispeten kısa olması, ısıl işlemlerle karantina zararlılarının kontrolünde maliyet açısından etkili olabilir (Hansen ve ark., 2006; Hansen ve ark., 2011).

Buhar halinde uygulanan ısıtma işlemi, bitki patojenlerini, böcek zararlılarını ve yabancı otları kontrol etmek için fidanlıklarda ve sera bitki üretim sistemlerinde rutin olarak kullanılmaktadır (Shellie ve Mangan, 2000; Neven ve ark., 2012; Samtani ve ark., 2012). Bununla birlikte tüm yüzeyler tamamen buharla kaplandığında buhar etkinliği elde edildiğinden, yakıt, işçilik ve zaman açısından maliyetli olabilir (Samtani ve ark., 2012).

### Bağ Alanlarında Etkili Asma Gövde Hastalıkları

AGH son yıllarda dünya çapında artmıştır. Bu artışa başlangıçta kendi kökleri üzerinde yetiştirilen *V. vinifera* çeşitlerine göre bazı gövde patojenlerine karşı daha duyarlı, filokseraya dirençli asma anaçları kullanımının artmasıyla ilişkili olduğuna inanılmaktadır (Gutter ve ark., 2004). AGH etmenleri genellikle, olgun asmada büyüyen, ksilemde ve odunsu dokularda yaşayan fungal patojenler olup (Pascoe, 1998), burada su ve besin taşıma sistemleri ile bağlantılı hücrelerin bozulmasına neden olabilirler. Bu nedenle, hastalıkların dış semptomları, bodur sürgünler, klorotik yapraklar ve bazen asmanın ölümünü takip eden gerileme gibi su ve besin eksiklikleri belirtilerine benzer (Pascoe ve Cottrall, 2000). Kaliforniya’da, Munkvold ve ark. (1994), hastalık şiddetine bağlı olarak bağlarda AGH’dan kaynaklanan verim kaybının %30-%62 aralığında değiştiğini tahmin etmişlerdir. Bununla birlikte, bu hastalıklardan sorumlu patojenler, görünüşte sağlıklı bitkilerden de izole edilmiştir (Aroca ve ark., 2006) bu durum, mantarların gizli enfeksiyonlar olarak var olabileceği ve asmalar strese girdiğinde patojenik hale gelebileceği öngörülerini desteklemektedir (Gubler ve ark., 2005). AGH olarak kabul edilen başlıca hastalıklar aşağıda sıralanmıştır.

#### **Petri Hastalığı**

‘Siyah yapışkan’ veya ‘Genç asma çöküşü’ olarak da adlandırılan Petri hastalığı, genç asmaların zayıflamasıyla ilişkili bir damar hastalığıdır. Bu hastalıkla ilişkili başlıca mantar ajanları, *Phaeoconiella chlamydospora* ve birkaç *Phaeoacremonium* spp., *Pleurostoma richardsiae* ve altı *Cadophora* türüdür (Gramaje ve Armengol, 2011; Travadon ve ark., 2015; Da Silva ve ark., 2017; Gramaje ve ark., 2018). Hastalık öncelikle genç asmaları etkiler ve yeni bağ tesislerinde önemli bir sorun olabilir (Edwards ve Pascoe, 2004; Gubler ve ark., 2005). Dünyanın birçok ülkesinde zayıflayan asmalarda teşhis edilmiştir (Mugnai ve ark., 1999; Pascoe ve Cottrall, 2000).

Dış belirtiler arasında bodur büyüme, boğum aralarında kısılma, yaprak damar aralarında kloroz/nekroz, zayıflamış veya daha az gelişmiş bitki tacı ve muhtemelen sürgünlerde geriye doğru çökme ve aşı başarısızlığı yer alır (Scheck ve ark., 1998; Fourie ve Halleen, 2002; Edwards ve Pascoe, 2004). Gövdede, ksilem damarları içi ve çevresinde gelişen mantara yanıt olarak ksilem tarafından bu damarlarda oluşan tilozlar, sakızlar ve fenolik bileşiklerin bir sonucu olarak tipik bir kahverengi çizgilenme ve kahverengi kırmızı/kahverengi nekroz gözlemlenebilir (Gramaje ve Armengol, 2011).

#### **Siyah Ayak**

Asma Siyah ayak hastalığı, çeşitli ülkelerde iyi belgelenmiş bir hastalıktır ve daha önce *Cylindrocarpon* spp. ve *Campylocarpon* spp. (Gramaje ve Armengol,

2011)’nin, ancak günümüzde *Dactylonectria*, *Ilyonectria*, *Campylocarpon*, *Cylindrocladiella*, *Neonectria* ve *Thelonectria* cinslerindeki 24 kadar türün siyah ayak hastalığına neden olduğu bildirilmiştir (Agusti-Brisach ve Armengol, 2013; Lombard ve ark., 2014; Carlucci ve ark., 2017). Siyah ayak hastalığı, fidanlıklar ve genç bağlarda asmaları etkileyen ve sonuçta asma ölümlerine neden olan en önemli gövde hastalıklarından biri olarak kabul edilir (Halleen ve ark., 2007). Patojenler toprak kaynaklı olup enfekte bir bitkinin çıkarılmasından sonra toprakta kalabilir (Bleach ve ark., 2008). Patojen propagülleri, patojenle bulaşık alanlara dikilen asma fidanlarının kökleri veya asma anacı çeliklerinin taban uçlarından enfekte eder (Halleen ve ark., 2003; Agusti-Brisach ve Armengol, 2013). Enfekte asmalarda, çökmüş nekrotik kök lezyonlarının yanı sıra kök biyokütlesinde azalma ve kökte saçaklanma görülür ve telafi için ikinci bir kök katmanı gelişebilir (Alaniz ve ark., 2007). Anaçlar enine kesildiğinde özünde siyah nekroz görülür (Alaniz ve ark., 2007; Bleach ve ark., 2008).

#### **Esca**

Esca hastalığı, dünya genelinde yayılım gösteren ve AGH kümesine ait en karmaşık ve önemli damar hastalıklardan biridir (Kovács ve Sándor, 2016; Bortolami ve ark., 2019). Bu hastalık, özellikle yaygınlığı, doğrudan üretim kayıplarına neden olması (üzüm miktarını ve kalitesini düşürmesi), bağların ömrünü önemli ölçüde kısaltması ve hastalığı kontrol veya sınırlamak için yeterli önlemlerin bulunmaması nedeniyle zararlıdır (Choueiri ve ark., 2014; Bortolami ve ark., 2019; Foglia ve ark., 2022; Muntean ve ark., 2022).

Esca hastalığına yakalanmış asmaların odun dokularından, taksonomik olarak birbiriyle ilişkili olmayan geniş bir yelpazede mantar gövde patojenleri (Kubátová ve ark., 2004; Mostert ve ark., 2006b; Essakhi ve ark., 2008; Gramaje ve Armengol, 2011; Fischer ve Ashnaei, 2019; Foglia ve ark., 2022) ve hatta endofitik bakteriler izole edilmiştir (Hofstetter ve ark., 2012). *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum* ve *Fomitiporia mediterranea* hastalığın başlıca etkenleri olarak kabul edilmektedir (Bertsch ve ark., 2013).

Esca’nın yaprak semptomları ilk önce sürgün tabanlarında ortaya çıkar ve yaprak damarları arasında klorotik lekelerle yol açan yapısal ve biyokimyasal değişikliklerle ilişkilidir. Bunlar daha sonra yavaş yavaş genişleyip sarı kahverengi veya kırmızı kahverengiyeye döner ve sadece dar bir yeşil doku şeridi bırakır. Kaliforniya’da genellikle tane yüzeyinde mordan siyaha kadar lekeler oluşur ve buna ‘Siyah kızamık’ denir. Siyah kızamık ABD’de çok yaygındır ve *Phaeoconiella chlamydospora* odun dokusu enfeksiyonu ile ilişkili toksinler, taneler üzerinde sistemik bir etkiyle lekeler oluşturur ve bu taneler çatlar (Gutter ve ark., 2004).

Eski bağlarda iki Esca rapor edilmiş, yakın zamanda dikilen genç asmalarda zayıflama ve ölümler görülmüş ve genellikle bitki stresine ilişkilendirilmiştir. Esca enfekteli asmaların salkımları pazarlanamaz hale geldiğinden, sofralık üzümlerde de bir problemdir (Rolshausen ve ark., 2008). Esca dış ve iç semptomlarının en azından bir kısmı, büyük olasılıkla rengi bozulmuş veya çürümüş odunsu dokuda üretilen fitotoksik mantar metabolitlerinden veya oksidasyonundan kaynaklanır. Mantar enfeksiyonu sonucu

üretilen bazı kimyasallar asmalar için toksiktir. Özellikle  $\alpha$ -glukanlar ve scytalon ve isosclerone olarak adlandırılan iki naphthalenone pentaketides, çeşitli mantarların ikincil metabolitleridir ve bu mantarlar tarafından *in vitro* olarak da üretilmiştir (Bruno ve Sparapano, 2007). Esca, semptomları birkaç faktörün eşzamanlı etkisine bağlı olabilen karmaşık bir hastalıktır (Andolfi ve ark., 2011; Bénard-Gellon ve ark., 2015).

### **Phomopsis Dieback**

*Phomopsis viticola* ve diğer *Phomopsis* spp.'nin neden olduğu *Phomopsis* sürgün ve yaprak lekesi global ölçekte önemli bir asma hastalığıdır (Lal ve Arya, 1982; Phillips, 1998; Mostert ve Crous, 2000; Aroca ve ark., 2006). *Phomopsis* sürgün ve yaprak lekесinin %30'a varan ürün kayıplarına neden olduğu bildirilmiştir (Úrbez-Torres ve ark., 2013).

*Phomopsis viticola*, asmanın tüm yeşil kısımlarını enfekte edebilir ve tüm otsu organlarda (sürgünler, yapraklar, gövdeler veya taneler) semptomlar oluşturur (Muntean ve ark., 2022). Genç sürgünlerde ilk boğum aralarında küçük siyah lekeler oluşturur ve bunlar daha sonra siyahımsı-kahverengi veya 'çikolata' renkli kabuk şeritleri haline dönüşür. Sürgün tabanındaki zayıflama, belirli koşullarda (rüzgâr, ürün yükü) kırılmaya neden olabilir. Uyku döneminde sürgünlerin boğum araları siyah noktalı beyaz bir görünüme bürünür. Ana ve yan damarlar ile yaprak sapında siyah nekrotik lekeler oluşabilir. Bazı yaprak kısımları da sarı, uçuk yeşil veya kahverengine dönebilir. Nadiren ağır şekilde enfekteli yapraklar veya yaprak sapları düşebilir, taneler kahverengine döner, hasada yakın dönemde buruşur ve kurur (Larignon, 2012; Úrbez-Torres ve ark., 2013).

*Phomopsis theicola* gibi diğer ilişkili mantarlar ve semptomları, genç bir asmanın büyük bir bölümünün ölümüne neden olur. Odunsu dokuda genellikle belirli sektörel nekroz ve kahve renkli bazı beneklenmeler gözlenir (Larignon, 2012). Patojen, sürgünlerin ve tomurcuk pullarının dış kabuğunda kışı geçirir. Erken ilkbaharda sıcaklıklar artınca, kabuk dokusunda gelişen piknidialarda konidyumlar oluşur. İlkbahar yağmurları sırasında, bu konidyumlar, piknidiyumdan sızıntı halinde dışarı atılır ve daha sonra enfeksiyonun meydana geldiği genç sürgünlerin üzerine yağmur damlacıklarıyla yayılır (Chakraborty ve ark., 1998; Fontaine ve ark., 2016).

### **Eutypa Dieback**

*Eutypa lata*'nın en yaygın olduğu Diatrypaceous türlerin neden olduğu *Eutypa* geriye doğru ölüm, olgun asmaların gövde ve kollarını enfekte eder (John ve ark., 2005; Octave ve ark., 2006). Uzun yıllar boyunca *Eutypa* geriye doğru çöküş, *Eutypa armeniaceae/Eutypa lata*'ya atfedilmiş, ancak daha yakın zamanlarda *Eutypa leptoplaca*, *Cryptosphaeria pullmanensis*, *Cryptovalsa ampelina*, *Cryptovalsa rabenhortsii*, *Diatrype sp.*, *Diatrype oregonensis*, *Diatrype stigma*, *Diatrype whitmanensis*, *Diatrype vulgaris*, *Diatrypella verrucaeformis*, *Eutypella vitis*, *Eutypella leprosa*, *Eutypella citricola*, *Eutypella microtheca* ve *Eutypella scoparia* gibi diğer Diatrypaceous türlerinin de dünya çapında asmalarda *Eutypa dieback* semptomlarına neden oldukları bildirilmektedir (Trouillas ve Gubler, 2010;

Trouillas ve ark., 2010; Rolshausen ve ark., 2014; Moyo ve ark., 2018; Kenfaoui ve ark., 2022; Muntean ve ark., 2022).

Hastalığın ilk dış semptomları genellikle enfeksiyondan 3-10 yıl sonra ortaya çıkar ve tipik olarak büyük budama yaraları gibi enfeksiyon bölgelerinin çevresinde kanser oluşturur. Gövdelerin enine kesitleri, asma gövde ve kol odun dokularında koyu kahverengi, genellikle kama şeklinde nekrozlar ortaya çıkarır (Highet ve Wicks, 1998; Rudelle ve ark., 2005). Patojen ayrıca ilkbaharda yeni sürgünlerin boğum aralarının kısılmasına, deformasyona ve yapraklarda kloroza neden olan toksik metabolitler üretir (Rudelle ve ark., 2005; Amponsah, 2010).

### **Botryosphaeria Dieback**

Botryosphaeriaceae türleri son zamanlarda global ölçekte asma patojenleri olarak önem kazanmıştır (Taylor ve ark., 2005). Geçmişte asmalar da dahil olmak üzere ağaçlar ve çalılardaki varlıkları, saprofitik veya endofitik organizmalar olmaları nedeniyle genellikle göz ardı edilmiştir (Castillo-Pando ve ark., 2001; Phillips, 2002). Bildirilen türlerin yaygınlığı ülkeler arasında farklılık gösterse de dünya çapında bugüne kadar *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum*, *Neoscytalidium*, *Phaeobotryosphaeria* ve *Spencermartinsia* cinslerinden 26 Botryosphaeriaceae taksonu asmalarda *Botryosphaeria* geriye doğru ölümü ile ilişkilendirilmiştir (Urbez-Torres, 2011; Pitt ve ark., 2013; Rolshausen ve ark., 2013; Pitt ve ark., 2015; Yang ve ark., 2017). Yaprak belirtileri, kırmızı çeşitlerde belirtilerin ortaya çıkışının ilk aşamalarında sarı kenarlı olmayan, ancak sonunda Esca'ya benzer şekilde sarı kenarlı olan damar arası alanları ile karakterize edilir (Lecomte ve ark., 2006; Reis ve ark., 2016). Bazı üzüm çeşitleri bu hastalığa (Cabernet, Sauvignon, Ugni-Blanc, vb.) diğerlerine (Merlot) göre daha duyarlıdır. Etkilenen asmalar, zayıflamış vegetatif gelişme gösteren ölü dallarla karakterize edilir, bazen hala canlı ancak düşük tomurcuk patlama yüzdesi vardır. Karakteristik yaprak belirtilerini tespit etmek olağan değildir, ancak bazen yapraklarda kloroz zayıflıkları veya bazı deformasyonlar görülebilir (Larignon, 2012).

### **SSU ile Kontrol Edilebilen Diğer Patojenler**

#### ***Agrobacterium vitis*, *Allorhizobium vitis***

Asmadaki taç gal hastalığına, *Allorhizobium vitis*'in patojenik suşları neden olur. *Agrobacterium vitis*'in neden olduğu asma tacı artık *Allorhizobium* cinsinde yeniden sınıflandırılmıştır (Mousavi ve ark., 2014; Kuzmanović ve ark., 2018). Bu patojen asmada üretim azalması ve erken asma ölümüne neden olabilen ciddi bir kronik hastalıktır (Gillings ve Ophel-Keller, 1995). *A. vitis* asmada tümör indüksiyonu için elverişli koşullar oluşana kadar asemptomatik kalarak, fidan çoğaltma materyallerini sistemik olarak istila edebilir (Diana ve Dejeu, 2011). Bu hastalık, global ölçekte özellikle soğuk iklim bölgelerinde bağcılığı sınırlandıran bir etmendir (Kerr ve Panagopoulos, 1977; Ophel ve Kerr, 1990). Diğer bakteri türleri gibi *A. vitis*, T-DNA'nın bitki hücre çekirdeğine aktarıldığı, bitki genomuna entegre edildiği ve ifade edildiği bir Ti plazmidi üzerinde tümör genetik belirleyicilerini taşır (Gelvin, 2003).

Bakteri asmaların gövde ve sürgünlerinde ur oluşumuna ve köklerde nekroza neden olur (Burr ve ark., 1987). Asmalarda, özellikle dondurucu, düşük sıcaklıklar veya aşılardan kaynaklanan yaralanmalar, kambiyal hücre bölünmesini uyarır ve bu alanlarda daha sonra taç gal enfeksiyonları başlar ve büyüme mevsimi boyunca belirgin hale gelen ur oluşumuna neden olur (Diana ve Dejeu, 2011). *A. vitis*, enfekteli bir asmanın ölümü veya bağdan çıkarılmasından sonra topraktaki kalıntılar üzerinde en az 2 yıl hayatta kalabilir, hatta 5 yıl sonra sağlıklı bir asma aynı toprağa dikildiğinde enfekte olabilir (Burr ve ark., 1989; Burr ve ark., 1995).

Patojen, asmalarda sistemik olarak hayatta kalır, ksilem sıvısında tespit edilebilir ve çoğaltma materyalleri ile yayılabilir (McCartney ve ark., 2003). Kalem ve anaçlık çoğaltma materyalleri görünür olmayan hastalıkları taşıyabilir. Patojen içermeyen çoğaltma materyalinin kullanımı, bağlarda bu hastalığın yayılmasını azaltabilir (Diana ve Dejeu, 2011).

Hastalık, köklerde ve gövdelerde, başta bezelye gibi küçük, yumuşak, süngerimsi, ince dokulu, beyaz veya sarı-yeşil, parankimal dokular oluşturur, bunlar daha sonra koyu, sert, odunsu hale gelir. Bu tümörler otsu veya odunsu bitkilerin tüm organlarında ortaya çıkabilir, ancak büyüme mevsiminin sonunda düşerler. Bazen tümörler kalıcıdır ve her yıl giderek daha iri hale gelirler (Pârvu ve Vedinaş, 2000; Docea ve Stelica, 2003; Stewart ve Wenner, 2004).

*A. vitis*, çeşit seçimleri ve kültürel uygulamalarla kısmen yönetilebilir, sera deneylerinde biyokontrol ajanları kullanılarak değişen oranlarda baskılanabilmiştir (Zheng ve Burr, 2016). Biyokontrol ajanları arasında *A. vitis* suşları ARK1, E26, VAR03-1 ve F2/5 bulunur (Staphorst ve ark., 1985; Liang, 1990; Kawaguchi ve ark., 2008; Kawaguchi, 2013). *A. vitis* suşu F2/5, daha önce taç safra hastalığına karşı bir biyolojik kontrol ajanı olarak hareket ettiği belirlenen ve ilk defa Güney Afrika'dan izole edilen, patojenik olmayan bir biyokontrol ajanıdır. Genom sekansında saptanabilir T-DNA sınır sekansları yoktur ve bakterilerin patojenik olmamasıyla tutarlı olan anahtar virülans genleri eksiktir (Xi ve ark., 2022). F2/5 suşu tümörojenik değildir. Patojenik *A. vitis* suşlarına karşı verilen antibiyozun, patojenin yaralı hücrelere bağlanmasını veya patojenin hücrelerde büyümesini engellemek yerine, yaralı asma dokusunun patojen tarafından transforme edilmesini engellediği düşünülmektedir (Kaewnum ve ark., 2013). F2/5 tarafından sağlanan antibiyoz veya asma tümörü inhibisyonu (GTI) canlı hücreler gerektirirken, ısıyla öldürülen veya sonikasyona tabi tutulan hücre preparasyonlarında GTI kabiliyetini kaybeder (Burr ve ark., 1997). clpA (F-avi2537), alpP1 (F-avi1696), aviR (F-avi4374) dahil olmak üzere GTI için gerekli olan bir dizi gen tanımlanmıştır (Zheng ve Burr, 2016). Bugüne kadar, potansiyel biyokontrol suşları ARK1, E26 ve F2/5'in hiçbir genom dizisi yayınlanmamış, ancak VAR03-1 için genom dizisi yakın zamanda yayınlanmıştır (Noutoshi ve ark., 2020).

### **Flavescence dorée**

Flavescence dorée (FD), Avrupa'da asmanın ekonomik olarak önemli bir karantina hastalığıdır (Morone ve ark., 2007). Bu hastalık, fitoplazma kaynaklıdır. Fitoplazmalar, dünya çapında yüzlerce bitki hastalığından sorumlu,

sürekli çoğalma yoluyla bulaşan, duvarsız bakteriyel patojenlerdir (Lee ve ark., 2000; Duduk ve ark., 2004; Bertaccini ve Duduk, 2009; Strauss, 2009). Günümüzde, Avrupa bağcılığı iki ciddi fitoplazma kaynaklı hastalık FD (Angelini ve ark., 2003) ve "*Candidatus* Phytoplasma solani'nin neden olduğu Bois noir ile karşı karşıyadır (Quaglino ve ark., 2013). FD 16S ribozomal grup V'ye ait farklı fitoplazma suşlarından kaynaklanır (Angelini ve ark., 2001). İtalya'da FD ilk defa 1973'te rapor edilmiş (Belli ve ark., 1973) olup esas olarak ribozomal alt gruplar C ve D'ye (Martini ve ark., 2002) ait fitoplazmalardan (FDP'ler) kaynaklanır. Her iki FDP türünün doğadaki vektörü yaprak biti *Scaphoideus titanus* Ball'dır (Schvester ve ark., 1961; Caudwell, 1983; Mori ve ark., 2002).

Enfekte asmalar genellikle dikimden sonraki yıl semptom gösterir, ancak çeşide ve muhtemelen yaşa bağlı gecikmeler bildirilmiştir (Caudwell ve ark., 1987). Yapraklarda sararma, aşağı doğru kıvrılma, tane dökümü, bodurluk ve yeni sürgünlerin odunlaşmaması en önemli belirtiler arasındadır (Caudwell, 1983; 1990). Semptomların ortaya çıktığı ilk yıldan sonra hastalığın gelişimi üzüm çeşidine göre değişiklik gösterebilir. Semptomların kendiliğinden gerilemesi (iyileşme) meydana gelebilir ve geri kazanılan bitki, enfektif vektörlere maruz kalmazsa asemptomatik kalabilir (Caudwell ve ark., 1987). Konaktan fitoplazmanın yokluğuna bağlı olsun ya da olmasın geri kazanım, farklı fitoplazmalardan etkilenen meyve ağaçlarında da rapor edilmiş (Musetti ve ark., 2004; Musetti ve ark., 2005) ve bu durum, patojen ve konukçu genotiplerinin yanı sıra çevresel koşullardan da etkilenmiştir (Kunze, 1976).

### **Filoksera**

Bağ filokserası, *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae), dünya çapında ciddi bir asma (*Vitis* spp.) zararlısıdır (Granett ve ark., 2001). Kuzey Doğu Amerika'nın bir yerlisi olan bağ filokserası, genetik türüne bağlı olarak *Vitis* türlerinin kökleri ve yaprakları ile beslenerek gal oluşumunu uyarır (Wapshere ve Helm, 1987; Benheim ve ark., 2012; Powell ve ark., 2013). Avrupa'daki büyük bağ bölgelerine, başlangıçta asma küllemesini yönetmek için Amerika'dan getirilen asma anaçları üzerinde taşınmıştır (Gale, 2002). 1868'de Fransa'da asma filokserasının keşfi ve müteakip on yılda yayılması iyi belgelenmiştir. Zararlı, yüzyılın başında 1 milyon hektardan fazla aşısız *V. vinifera* bağını yok ederek Fransız şarap endüstrisini olumsuz etkilemiş (Ordish, 1972; Campbell, 2004) ve kırsalda yaşayan topluluklara sosyoekonomik etkileri olmuştur (Banerjee ve ark., 2007; Bignon ve ark., 2011). Geçtiğimiz 150 yılda, bağ filokserası Kuzey ve Güney Amerika, Asya, Avrupa, Orta Doğu, Afrika ve Avustralya dahil olmak üzere dünyadaki hemen tüm büyük bağ bölgelerine yayılmıştır (EPPO, 1990). Asma filokserasının genetik soylarına bağlı olarak istila sıklığı, şiddeti ve dağılımı, konukçu bitkilerin doğal direnç mekanizmalarının bir fonksiyonu olarak önemli ölçüde değişir (Corrie ve ark., 2002; Corrie ve ark., 2003; Forneck ve Huber, 2009; Benheim ve ark., 2012).

Radicolae (kökte beslenen) filoksera, Avrupa asma *V. vinifera* kökleriyle beslenirken galleri indükler ve sonuç olarak konakçı kökleri ikincil mantar enfeksiyonlarına maruz bırakır (Omer ve ark., 1999) ve bu formu en zararlı olanıdır (Powell ve ark., 2013). İlk dönem nimfleri, asma

gövdelerinde kabuk altında veya kökler üzerinde kışı geçirir (Coombe, 1963; Granett ve ark., 2001). İlkbaharda ilk evreler aktif hale gelir; beslenme yerleri kurar, olgunlaşmamış, odunsu olmayan ve daha yaşlı, odunsu köklerde gelişir (Omer ve ark., 1997; Powell, 2002). Yaz aylarında, ilk evreler aktif olarak toprak üstünde sürünür ve toprak yüzeyinde asma gövdeleri, sürgünler, yapraklar ve üzüm salkımlarında bulunabilir (King ve Buchanan, 1986; Deretic ve ark., 2001). Bu nedenle ilk evre en hareketli olanıdır ve hem alet-makineleri ile hem de üzüm silolarında kolayca taşınabilir (Powell ve ark., 2001; Powell, 2002).

*V. vinifera* kökleri bağ filokserasıyla enfekte olduğunda, büyüme gücü zayıflar, yapraklar erken sararır ve asma tacı ve üzüm salkımları küçülür (Powell ve ark., 2013). Filoksera, on dokuzuncu yüzyılın sonlarından itibaren dayanıklı anaçların kullanılmasıyla etkin bir şekilde yönetilmektedir. Bugüne kadar, bağ filokserası yönetimi ağırlıklı olarak filokseraya dirençli anaçlara aşılı fidanların kullanımına ve bazı ülkelerde belirli filoksera karantina protokollerine veya iki yaklaşımın kombinasyonuna bağlı kalmaya odaklanmıştır. (Powell ve ark., 2013). Bağ filokserası için geliştirilmiş bir yönetim sistemine entegre edilebilecek bir dizi kontrol seçeneği mevcuttur. Gelecekteki değerlendirme ve daha fazla geliştirme için öncelikli alanlar arasında erken tespit teknikleri, biyolojik kontrol ajanlarının kullanımının araştırılması ve filoksera yönetimine entegre bir yaklaşımın geliştirilmesi yer almaktadır (Benheim ve ark., 2012).

Bağ filokserasına dayanıklı Amerikan *Vitis* türlerine ait anaçlar, farklı filoksera genetik suşlarına karşı test edilmiştir (Korosi ve ark., 2010; Powell ve Krstic, 2015). Karantina düzenlemeleri, filoksera transferi riskini azaltmak için özel olarak belirlenmiş karantina bölgeleri ve hareket protokollerinin kullanımını içerir ve bunlar Avustralya, Çin ve Avrupa'da kullanılmaktadır (Galet ve ark., 1980; Litvinov, 1984; EPPO, 1990; Powell, 2008). Karantina bölgesi, filoksera hareketini istila edilmiş bölgelerden kısıtlamak için kurulmuş yasal bir coğrafi alandır. Karantina kısıtlamaları hayati önem taşır ve filoksera yönetimi için birinci önceliktir, çünkü dirençli anaçlarla yeniden dikim yetiştiriciler için nispeten maliyetlidir. Ayrıca, böceğin küçük boyutu, esas olarak toprak altı habitatu (Benheim ve ark., 2012) ve standart bağ operasyonları sırasında ilkbahar ve yaz aylarında toprak üstünde mevsimsel hareket meydana gelmesi nedeniyle erken teşhisi karmaşıktır (Powell, 2002).

Bağlar arası filoksera bulaşma riskini azaltmak için geliştirilen karantina protokolleri, çeşitli ilaçlama işlemlerini kapsamaktadır. Dezenfeksiyon protokolü, bağlarda kullanılan ekipmana ve filoksera aktarma potansiyeline sahip olan asma konukçu materyallerinin kaldırılmasına bağlıdır. Bağ makinelerinin, ekipmanlarının, asma materyallerinin, toprağın ve ayakkabıların dezenfeksiyonu için ısı ve kimyasal işlemler önerilmektedir (Committee, 2009). Örneğin, ev tipi ağartıcı (yani sodyum hipoklorit) ayakkabıları ve küçük aletleri dezenfekte etmek için kullanılırken (Dunstone ve ark., 2003; Clarke ve ark., 2017), bağ makine ve araçları, dikim materyali, teşhis numuneleri ve üzüm hasat kapları için ısı bazlı işlemler kullanılmaktadır. (Committee, 2009).

Sıcaklık genellikle zararlılar ve hastalıklar için bir dezenfeksiyon tedavisi olarak kullanılır. Çoğu böcek,

yüksek bir sıcaklığı yalnızca kısa bir süre için tolere edebilir. Dezenfeksiyon için 24 ila 36 saniye arasında 50-60°C arasındaki bir sıcaklık yaygın olarak kullanılır, çünkü bu, ısı en dayanıklı böcek yaşam evrelerini bile öldürmek için en uygundur (Beckett ve ark., 2007).

Bağ alet-makineleri ve araçlarında bağ filokserasına karşı mevcut ilaçlama protokolü, belirtildiği gibi, görünür bir buhar jeti ile 100°C'nin üzerinde buhar uygulamaktır (Committee, 2009). Buna karşılık, sıcak suya daldırma, bağ filokserası da dahil olmak üzere karantina açısından önemli birçok eklem bacaklıyı (Graham, 2007b; European ve Organization, 2009; Gramaje ve ark., 2014) dezenfekte etmek için etkili, pratik ve nispeten ucuzdur (Sakai ve ark., 1985). Avustralya'da, üzüm hasat kaplarını dezenfekte etmek için kullanılan protokol, 70°C'ye ayarlanmış sıcak suya 120 saniye daldırmayı içerir (Committee, 2009). Filokseraya karşı sıcak suya daldırma ve buhar uygulamalarının etkinliği, Avustralya'daki endemik suşlar için bilimsel olarak doğrulanmamıştır. Umina ve ark. (2007), kuzey ve orta Victoria'daki çeşitli coğrafi bölgelerde 83 farklı bağ filokserası genetik suşunu belirlemişlerdir. Çalışmalar, son zamanlarda farklı genetik suşlar arasında kuru ısı ve kimyasal dezenfeksiyon işlemlerine yanıt farklılıklarını vurgulamıştır (Korosi ve ark., 2012; Clarke ve ark., 2017).

Avustralya'da, altı filoksera genetik suşunun ilk evreleri, G1, G4, G7, G19, G20 ve G30, 10, 20 ve 30 saniye 8 ve 24 cm'den ve sadece G1 için 92 cm'den verilen buhara maruz bırakıldığında tüm buhar uygulamaları, altı genetik suşta %100 ölüm sağlamıştır. SSU'nun filokseraya karşı etkinliği, ilk evrelerin 22, 40, 45, 50, 60 ve 70°C'ye ayarlanmış bir su banyosuna 60 ve 120 saniye süreyle daldırılmasıyla incelenmiştir. En az 60 saniye 50°C ve üzeri uygulamalar, altı genetik suşta %100 ölümlerle sonuçlanırken 40 ve 45°C'de hayatta kalma gözlenmiş, ilk evreler daha sonra beslenme alanları oluşturan yetişkinlere dönüşmüş ve asma kök çeliklerinde çoğalmıştır (Clarke ve ark., 2018).

Altı endemik filoksera genetik suşunun, G1, G4, G7, G19, G20 ve G30'un hayatta kalması, ilk evrelerin 30, 40 ve 60 saniye %0, 2, 3 ve %4'lük sodyum hipoklorite daldırılması ve ardından 30 saniye su ile durulanmasıyla test edilmiş, sodyum hipoklorit, konsantrasyonu ve uygulama süresinin artmasıyla filokseranın sağkalımı önemli ölçüde azalırken, uygulama kombinasyonlarının hiçbirisi %100 etkin olmamıştır. Suyla durulama yapılmadan, 60 saniye %2 sodyum hipoklorite daldırma uygulanan altı genetik suşun tamamında %100 mortalite sağlanmıştır (Clarke ve ark., 2017).

### **Nematodlar**

Nematodlar, hayvanlar, bitkiler ve diğer nematodlarla da beslenen parazitik pseudoceolomic yuvarlak kurtlardır (Malik ve ark., 2022). Dünya genelinde, bitki paraziti nematodları neredeyse tüm tarımsal ürünler için büyük bir tehdit oluşturmakta ve bu parazitik nematodların küresel olarak yıllık 358 milyar ABD Doları'nın üzerinde bir kayba neden olduğu tahmin edilmektedir (Abd-Elgawad ve Askary, 2015). Dünya çapında en önemli tarımsal ürünlere zarar veren başlıca bitki paraziti nematod türleri arasında *Meloidogyne*, *Tylenchulus*, *Helicotylenchus*, *Heterodera*, *Xiphinema*, *Longidorus* vb. bulunur (Askary ve ark., 2018). Asmalarda neden olduğu verim kaybının yıllık

bazda yaklaşık %12,5 olduğu tahmin edilmektedir (Sasser, 1987). Asmalarla ilişkili parazitik nematodlar, genellikle asmanın toprak altı kısımlarına yani köklere zarar verir ve bu organları fonksiyonlarını yapamaz hale getirir (McKenry ve Bettiga, 2013). Ayrıca bitki paraziti nematodların köklerde neden olduğu yaralar, bakteri, mantar vb. diğer patojenlerin enfeksiyonlarına yatkın hale gelerek ikincil enfeksiyonlara neden olur (Walker, 1995). Birkaç bitki paraziti nematod (%1'den az) virüslere vektör olarak hareket eder ve bu nedenle bitkilerde viral hastalıkların bulaşmasına yol açar (Brown ve ark., 2004). Bitki paraziti nematodların üç ana cinsin, yani *Xiphinema*, *Longidorus* ve *Trichodorus*'un farklı bitkilere virüs bulaştırdığı bilinmektedir (Malik ve ark., 2022).

### Sıcak Su Uygulamaları

SSU, tohumlar ve depolama ürünleri de dahil olmak üzere bitki materyalini dezenfekte etmek için 19. yüzyıldan beri başarıyla kullanılmaktadır. Asma çoğaltma ve dikim materyalinde bir dizi önemli haşere ve patojeni kontrol etmenin tek etkili yolu olmaya devam etmektedir (Morton ve Waite, 2007). Filoksera ve daha sonra kök ur nematodlarının kontrolü için 1 yaşlı köklü asmalara SSU 20. yüzyılın başlarında geliştirilmiş (Lear ve Lider, 1959; Suatmadji, 1982; Stonerod ve Strik, 1996), o zamandan beri, dikim materyalinde Pierce hastalığı (Goheen ve ark., 1973), *Phytophthora cinnamomi* (Von Broembsen ve Marais, 1978), asma kanseri (Burr ve ark., 1989; Ophel ve ark., 1990; Bazzi ve ark., 1991), fitoplazmalar (Haviland ve ark., 2005), unlu bitler ve *Phaeoconiella chlamydospora* (Crous ve ark., 2001; Laukart ve ark., 2001; Fourie ve Halleen, 2004b) dahil endojen mantar patojenlerinin yayılmasını önlemek için 1 yaşlı köklü asmalara ve çeliklere uygulanmaktadır. SSU, 1990'ların ortalarından itibaren daha yaygın kullanılmakla birlikte önemli bir streşir ve doğru uygulanmadığında materyalin kaybına neden olabilir (Morton ve Waite, 2007). *V. vinifera* çeşitleri, SSU'na karşı farklı düzeylerde hassasiyete sahiptir. Çeliklerin büyütüldüğü büyüme mevsimi sıcaklıklarından etkilenebilir. Ayrıca, kullanılan sıcaklık aralığı, kontrol edilmesi gereken patojenlere bağlıdır.

SSU, genç asmaların (çelikler, köklü veya aşılı köklü fidanlar) belirli zararlıların ve patojenlerin yayılmasını yavaşlatmaya yetecek kadar yüksek sıcaklıklarda 50–53°C arasında 30 – 45 dakika süreyle uygulama gerektiren bir yöntemdir (Waite ve Morton, 2007; Eichmeier ve ark., 2018; Lade ve ark., 2022). Örneğin, 53°C'de 30 dakika veya 50°C'de 45 dakika standart SSU protokolleri, asma çoğaltma materyalinde Petri veya Siyah ayak patojenlerini kontrol edebilir (Gramaje ve Armengol, 2011; Agusti-Brisach ve Armengol, 2013).

SSU yapılan çelikler, özellikle hassas çeşitler, genellikle uygulama yapılmayan çeliklerden daha yavaş gelişir. SSU çeliklerde tomurcuk ve kök gelişimini geciktirmesi, büyüme mevsimi başında çeliklerin iyileşme süreci yaşadığını düşündürmektedir. Bununla birlikte ilk büyüme mevsimi sonunda, SSU yapılmış çelikler büyümedeki farkı kapatır ve SSU yapılmamış çeliklerden ayırt edilemez hale gelir (Waite, 1998; Waite, 2002).

Ayrıca, SSU'na toleransın, çeliklerin yetiştirildiği iklimden etkilendiğini gösteren çok sayıda kanıt vardır.

Serin iklimlerde yetiştirilen çelikler, sıcak iklimlerde yetiştirilen çeliklere göre SSU'nda zararlanmaya daha duyarlıdır (Fletcher ve ark., 2002; Graham, 2007a), Hem çeliklerin hem de sıcak iklimlerde beraberinde gelişen patojenlerin SSU'na daha az duyarlı olduğu ve yeterli patojen kontrolü için uygulama sıcaklığının 51-53°C kadar yüksek olması gerekebileceğini gösteren kanıtlar vardır (Armengol ve ark., 2006).

Vigues ve ark. (2009), Fransız fidanlıklarında *Botryosphaeria dothidea*, *Diplodia seriata* ve *Phaeoconiella chlamydospora* enfeksiyonlarını azaltarak umut verici sonuçlar gösteren test edilen farklı kontrol yöntemleri (kimyasal, biyolojik ve teknolojik yöntemler) arasında SSU çalışmalarının tek etkin uygulama olduğu sonucuna varmıştır. Yakın zamanda Elena ve ark. (2015), 30 dakika 51-53°C'de sıcak su uygulamasının fidanlıkta asma çoğaltma sürecinde asma için patojenik sekiz *Botryosphaeriaceae* türünü kontrol edebildiğini belirlemişlerdir. AGH baskılamaya yetecek kadar yüksek sıcaklıklara (50-53°C) maruz bırakılmasını gerektiren bir kontrol yöntemi olarak tanımlanırken, daha yüksek sıcaklıklar ( $\geq 54^\circ\text{C}$ ) AGH etmenlerini tamamen yok etme potansiyeline sahiptir (Gramaje ve ark., 2009).

SSU bitki ve mantar gelişimi üzerindeki kısa ve uzun vadeli etkileri değişkenlik göstermektedir. Daha önce yapılan çalışmada, SSU yapılarak üretilmiş asma fidanlarının, SSU yapılmadan üretilenlere kıyasla, muameleden hemen sonra daha düşük seviyelerde AGH-mantar çeşitliliği sergilediği, ancak dikildikten sonra bağda yeniden enfekte olabileceğini ortaya konmuştur (Eichmeier ve ark., 2018). Bitki sağlığını değerlendiren başka bir çalışmada, dinlenme halindeki asma anaç çelikleri ve aşılı fidanlara yapılan SSU'nun, bir büyüme mevsiminin ardından sağlıklı ve yaşayabilir bitkilerle sonuçlandığı belirlenmiştir (Gramaje ve ark., 2009; Gramaje ve Armengol, 2012; Lade ve ark., 2022). Dört yıllık bir SSU saha denemesini içeren başka bir çalışmada benzer bulgular bildirilmiş, SSU'nun bitki büyümesi üzerinde hafif bir uzun vadeli etki gösterdiğini, ancak bu etkinin istatistiksel olarak anlamlı kabul edilecek düzeyde olmadığı belirlenmiştir (Gramaje ve ark., 2014; Lade ve ark., 2022).

### Tedavi Koşulları

SSU stresinin olumsuz etkisinden kaçınmak için uygulama öncesi, sırası ve sonrasında aşağıda sıralanan bazı önlemler alınmalıdır (Europe, 2012).

### Ön Uygulama

SSU yapılacak bitki materyali tamamen odunlaşmış olmalıdır. Bitkiler, budama veya fidanların sökülmesi sırasında vegetatif döngülerini tamamlamış ve tam dinlenmeye girmiş olmalıdır (Europe, 2012).

Çelik hazırlama veya fidanların sökülmesi işleminden sonra materyal optimum sıcaklık ve nemde muhafaza edilmelidir. Tamamen dinlenmeye girmeyen çelikler veya köklü asma fidanları, SSU işlemlerine karşı çok hassastır ve uygulamadan sağ çıkamayabilir (Europe, 2012).

Asma çelikleri, aşı kalemleri ve anaç çelikleri aşılama öncesi dinlenme halini korumak ve kaliteyi artırmak için soğuk depoda (1–5°C ve yüksek bağıl nemde (%85 ve üzeri) tutulmalıdır. Ancak asma çoğaltma materyali, uygulamadan 12-24 saat önce soğuk hava



deposundan çıkarılmalı ve oda sıcaklığında nemli ve havalandırılmış bir odada bekletilmelidir (Waite ve Morton, 2007; Europe, 2012).

### Sıcak Su Uygulaması

SSU, aşılama öncesi, depolama sürecinin sonunda yapılmalıdır. Daldırma sonrası sıcaklık (50-55°C) ve uygulama süresine (45 dakika) uyulmalıdır. Çıplak

köklü fidanlar SSU tankına daldırıldıktan hemen sonra su sıcaklığının 50°C'nin altına düşebileceği ihtimalini göz önünde bulundurarak su sıcaklığı sirkülasyon halinde düzenli kontrol edilmelidir. Tanktaki su, uygulama sıklığına göre düzenli olarak, ancak günde en az bir kez değiştirilmelidir. Farklı SSU çalışmalarının bir özeti Çizelge 1a ve 1b'de sunulmuştur (Europe, 2012).

Çizelge 1a. Sıcak su uygulaması ile yapılan çalışmalar

Table 1a. Studies on Hot Water Treatment

Kaynaklar	Hastalık türü	SSU protokolü	Sonuç
Von Broembsen ve Marais (1978)	Dinlenme halindeki ve aktif büyüyen asmalarda <i>Phitophthora cinnamomi</i>	50°C'de 5 ila 30 dakika	<i>P. cinnamomi</i> 'nin başarılı bir şekilde arındırılması için tüm materyallere 15 dakika 50°C SSU önerilmiştir
Ophel ve ark. (1990)	<i>Agrobacterium vitis</i> ile bulaşık asma çubukları	50°C 30 dakika	Bulaşık materyaller <i>A. vitis</i> 'ten arındırılmıştır
Stonerod ve Strik (1996)	Filokseradan [Daktulosphaera vitifoliae (Fitch)]	Tüm yaşam evreleri için 43°C'de 5 dakika + 52°C 5 dakika	Aşılı ve aşısız asma fidanları 52°C 5 dakika SSU ile filokseradan arındırılmıştır
Clarke ve ark. (2004)	Asma çeliklerinde <i>Phomopsis viticola</i>	50°C 30 dakika	<i>Phomopsis viticola</i> etmeninin hayatta kalma oranı %75,6'dan %0,7'ye azaltılmıştır
İlgin ve Gürsoy (2005)	Asma anacı ve üzüm çeşidi kalemlerinde, SSU'nun göz canlılığı ve fidan randımanına etkileri incelenmiştir	50°C'de 30, 45 ve 60 dakika	50°C 30 dakika, zarar oluşturmazken, 50°C'de 45 dakika, büyük oranda canlılık kaybına neden olmuştur
Gramaje ve ark. (2008)	Asma çeliklerinde <i>Esca</i> sendromuyla ilişkili <i>Phaeoconiella chlamyospora</i> ve <i>Phaeoacremonium</i> spp.	30 dakika 51°C ve üzeri	Asma çubuklarını fungal patojenlerden arındırmak için 51°C'nin üzerindeki SSU önerilmiştir
Vignes ve ark. (2009)	Fransa'da asma fidanlıklarında <i>B. dothidea</i> , <i>D. seriata</i> ve <i>Pa. chlamyospora</i> 'dan	Enfeksiyonları azaltan kimyasal, biyolojik ve teknolojik yöntemler + sıcak su uygulaması karşılaştırılmıştır	Denenen yöntemler arasında sadece SSU tam temizleme sağlamıştır
Gramaje ve ark. (2010)	Asmalarda <i>Cadophora luteo-olivacea</i> , <i>Cylindrocarpon lirioidendri</i> ve <i>Phaeoacremonium</i>	<i>Cylindrocarpon</i> için 41-49°C'de ve <i>Phaeoacremonium</i> spp. ve <i>Ca. luteo-olivacea</i> içinde 49-55°C' de 30, 45 ve 60 dakika etkinlikleri test edilmiştir	<i>Ca. luteo-olivacea</i> konidyal çimlenmesi, 51°C 30 dakika, misel büyümesi 54°C-60 dakika SSU ile engellenmiştir. <i>Cylindrocarpon</i> spp. konidyal çimlenmesi 45°C 45 dakika, misel büyümesi 48°C 45 dakika üzeri uygulamasıyla engellenmiştir
Serra ve ark. (2011)	Fidanlıkta <i>Phaeoconiella chlamyospora</i>	SSU ve siprokonazol kombine uygulamaları incelenmiştir	Yapay enfeksiyon yapılan çeliklerde SSU ve siprokonazol tek başına enfeksiyon yüzdesini azaltmamış, Siprokonazolün hemen ardından SSU, enfekteli çelik sayısını önemli ölçüde azaltmış, ancak patojeni yok edememiştir
Poyraz ve Onoğur (2011)	Aşılı köklü asma fidanlarında Petri Hastalığı ( <i>Phaeoconiella chlamyospora</i> , <i>Phaeoacremonium aleophilum</i> )	45-55°C' de 15, 30, 45 ve 60 dakika	50-51°C-30 dakika uygulamasının çeliklerin dekontaminasyonu için en iyi sonucu sağladığı, ancak çeliklerin gelişimini olumsuz yönde etkilediği bulunmuştur
Bleach ve ark. (2013)	Dinlenme halindeki asma fidanlarında Siyah ayak patojenleri <i>Ilyonectria</i> ve <i>Dactylonectria</i>	48,5°C-30 dakika	SSU dinlenme halindeki asma fidanlarında enfeksiyonu azaltırken dikim sonrasında bitki büyümesini olumsuz etkilememiştir
Gramaje ve ark. (2014)	Dinlenme dönemindeki aşılı asma fidanlarına SSU uygulamasının etkileri incelenmiştir	53°C-30 dakika	Dört büyüme mevsiminde SSU uygulaması fidanların büyüme gücü ve verim parametrelerini olumsuz etkilememiştir
Elena ve ark. (2015) (in vitro)	Asma fidan üretimi sürecinde sekiz <i>Botryosphaeriaceae</i> türüne karşı SSU etkileri incelenmiştir	50-54°C sıcaklık ve 15, 30 ve 45 dakika uygulama sürelerinin etkinliği test edilmiştir	<i>Diplodia seriata</i> , <i>Neofusicoccum luteum</i> , <i>Neofusicoccum parvum</i> ve <i>Spencermartinsia viticola</i> sıcaklığa en duyarlı türler olurken, <i>Lasiodiplodia theobromae</i> ve <i>N. vitifusiforme</i> en toleranslı türler olarak belirlenmiştir

## Çizelge 1b. Sıcak su uygulaması ile yapılan çalışmalar

Table 1b. Studies on Hot Water Treatment

Kaynaklar	Hastalık türü	SSU protokolü	Sonuç
Elena ve ark. (2015) (bitkide)	110R anacına yapay olarak bulaştırılan <i>Botryosphaeriaceae</i> mantarının arındırılması çalışılmıştır	50-53°C-30 dakika	51°C ve daha yüksek sıcaklıklarda 30 dakikalık SSU uygulanması enfeksiyonda önemli bir azalma sağlamıştır
Billones-Baaijens ve ark. (2015)	<i>Neofusicoccum luteum</i> ve <i>N. parvum</i> enfekteli Dormant 5C anaç çeliklerde SSU ile arındırma çalışılmıştır	50-53°C-30	<i>N. luteum</i> ve <i>N. parvum</i> ile enfekte dormant çeliklere 50°C-30 dakika SSU uygulanması sırasıyla %45 ve %0 azaltırken 53°C-30 dakika SSU uygulanması enfeksiyon insidansını %100 ve %91,5 oranında azaltmıştır
Bruez ve ark. (2017)	Pinot noir fidanlarında <i>Botryosphaeria</i> sp., <i>Diplodia seriata</i> ve <i>N. Parvum</i>	50°C-45 dakika	Bağda 15 yıllık büyümeden sonra yapılan testlerde, SSU uygulaması uzun süreli kontrol sağlamamış, araziye dikilen fidanlar yeniden bulaşmalara açık bulunmuştur
Akgül ve ark. (2016) (in vitro)	Fungusların miselyal agar disklerini içeren santrifüj tüplerinde <i>Botryosphaeria dothidea</i> , <i>Diplodia seriata</i> , <i>Lasioidiplodia theobromae</i> ve <i>Neofusicoccum parvum</i>	47- 52°C - 30, 45 ve 60 dakika	SSU'na en dayanıklı tür <i>L. theobromae</i> olurken, en duyarlı tür <i>D. seriata</i> olmuştur. <i>D. seriata</i> ve <i>L. theobromae</i> için öldürücü sıcaklık ve zaman kombinasyonu sırasıyla 47°C-30 dk ve 51°C-45 dk olarak bulunmuştur
Akgül ve ark. (2016) (bitkide)	30 cm boyundaki asma çubuklarında <i>Botryosphaeria dothidea</i> , <i>Diplodia seriata</i> , <i>Lasioidiplodia theobromae</i> ve <i>Neofusicoccum parvum</i>	51-53°C – 30 ve 45 dakika	İtalya ve Kober-5BB asma anaç ve çeşitlerinde SSU'na en tolerant olarak gözlenirken, 53°C'de 45 dakikalık uygulamalar bu çeşitlerin göz canlılığında sırasıyla %37.3 ve %46.7'lik azalmaya yol açmıştır
Soltekin ve ark. (2017)	SSU'nun tüplü aşılı asma fidanı üretiminde fidan randımanı ve kalitesi üzerine etkileri	53°C-30 dakika	41B anacına aşılı Sultan 1, Altın sultani ve Saruhan bey çeşitlerine ait fidan randımanı n kontrol grubuna göre, sırasıyla %9,53, %14,99, %5,28 artış sağladığı gözlenmiştir
Yağcı ve Yıldırım (2019)	Narince üzüm çeşidi aşı kalemlerinde <i>Agrobacterium vitis</i>	45-56°C-30 dakika	Kanserli kalemlerde uyanma oranı (%65.8) düşük olmuştur, sonuçta sıcak su uygulamaları gözlerde sürmede/uyanmada azalma meydana getirmektedir
Knoetze (2020)	Köklendirilmiş asma anaç fidanlarında <i>Meloidogyne javanica</i>	53°C-20 dakika	Köklü anaçlardaki nematod popülasyonları önemli ölçüde azaltılmıştır
Lade ve ark. (2022)	Aşılı asma fidanlarının AGH arındırılması	53°C-30 dakika	Aşı dokularında <i>Cadophora luteo-olivacea</i> ve kök boğazında <i>Phaeoconiella chlamydospora</i> ve <i>Neofusicoccum parvum</i> gibi bazı AGH ile ilişkili patojenlerin ciddi bir şekilde azaltılmıştır SSU Chardonnay ve Xarello'nun sırasıyla toplam ve AGH ile ilişkili mantar türleri üzerinde en büyük etkiye sahip bulunmuştur
Poyraz ve Uysal-morca (2022)	Asma fidanlarında Petri Hastalığı ( <i>Phaeoconiella chlamydospora</i> (Pcl), <i>Phaeoacremonium aleophilum</i> (Pa))	53°C-30 dakika	Sonuçta bazı fungusitler ile sıcak su uygulaması (50°C 30 dk) ve biyopreparat kombinasyonlarının asma çeliklerindeki Pcl ve Pa etmenleri üzerinde büyük etkisi bulunmuştur

**Sıcak Su Uygulama Sonrası**

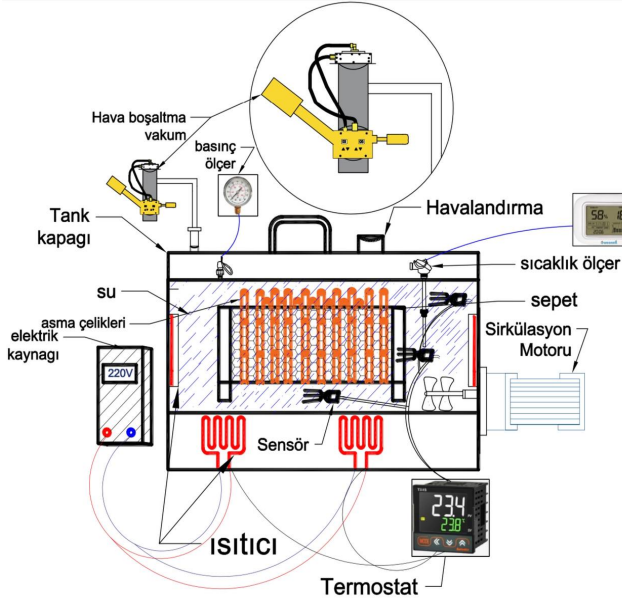
Yüzeysel küflenme ve vegetatif gelişmede geçici bir gecikmeye neden olabileceğinden SSU'ndan sonra uzun süre depolamadan kaçınılmalıdır (Boudon-Padieu ve Grenan, 2002; Metlitskiy, 2002). SSU yapıldıktan sonra, bitki materyali kısa bir süre soğuk depoda saklanmadan veya aşılardan önce nemli ve havalandırılmış bir ortamda 12–24 saat oda sıcaklığına erişmesi için bırakılmalıdır. Artan mortalite veya patojen enfeksiyonlarına neden olabileceğinden doğrudan soğuk su ile temastan kaçınılmalıdır (Boudon-Padieu ve Grenan, 2002; Waite ve Morton, 2007; Europe, 2012).

**Teçhizat**

SSU yapılacak sıcak su tankları amaca yönelik olarak paslanmaz veya galvanizli çelikten yapılmalıdır. Sürekli ve üniform sıcaklık sağlamak için sirkülasyon ünitesi ve su ısıtma araçları içermeli ve ısı kaybını sınırlamak için bir kapaklı, uygun ısı yalıtımına sahip, uygun ölçüm ve kayıt ekipmanı içermelidir (Boudon-Padieu ve Grenan, 2002; Metlitskiy, 2002; Europe, 2012).

Asma materyalinin tanka daldırılması için açık ağ kafes veya benzeri bir kısmı şunları sağlamalıdır: Asma materyali etrafında yeterli sıcak su sirkülasyonu sağlanmalıdır. Su sirkülasyonunu kolaylaştırmak için

tankın her tarafında (yaklaşık 150 mm) bir boşluk olmalıdır; Uygulama sırasında tüm çoğaltma materyalinin SSU tankına tamamen daldırılmış durumda kalmasını sağlamak için bir ağ kapak veya benzeri bir daldırma aparatı kullanılmalıdır. İdeal olarak, her tank için en az üç sıcaklık sensörü biri tankın tabanından 100 mm yükseklikte, diğeri yüzeyden 100 mm derinlikte ve üçüncüsü yüklem kütlesinin merkezine yer almalıdır (Şekil 1) (Boudon-Padieu ve Grenan, 2002; Metlitskiy, 2002; ICA-37, 2007; Europe, 2012).



Şekil 1. Sıcak su uygulama ünitesi tasarımı  
Figure 1. Hot water application unit design

## Sonuç

AHG bağlarda asmaların en yıkıcı hatalığı olarak bilinmektedir. SSU bu hastalıklarla ve aynı zamanda filoksera, nematodlar ve asma kanseri ile mücadelede en etkili yöntem olarak tanımlanmaktadır. Yapılan önceki çalışmalara göre 50-52°C arasındaki sıcaklıkların *Phaeomoniella chlamydospora*'yı ortadan kaldırdığı bildirilirken, Petri hastalığı ve Esca sendromuna karşı daha yüksek (51-53°C) sıcaklıklar gerekmektedir. *Botryosphaeriaceae* hastalığının temizlenmesi için 53°C ve üzeri SSU yapılması gerekmektedir. Diğer sonuçlar, 53°C'de 30 dakika veya 50°C'de 45 dakika standart SSU protokollerinin, asma çoğaltma materyalinde Petri veya Siyah ayak patojenlerini kontrol etmek için yeterli olabilecektir. SSU, genç asmalarda (dinlenme halindeki aşı gözü veya anaç çelikleri, köklü veya aşıllı köklü fidanlar) AGH ve filoksera, nematodlar ve asma kanserini baskılamaya yetecek kadar yüksek sıcaklıklara (50-53°C) maruz bırakılmasını gerektiren bir kontrol yöntemi olarak tanımlanırken, daha yüksek sıcaklıklar ( $\geq 54^\circ\text{C}$ ) AGH etmenlerini tamamen yok etme potansiyeline sahiptir. Sağlıklı bir asma fidanı üretebilmek için patojenlerin türü, asma anacı veya üzüm çeşitlerine ve hatta bunların üretildiği lokasyonlar be bu lokasyonlardaki mevsimlik sıcaklıklar da dikkate alınarak önerilen SSU protokollerinin modifiye edilmesi gerekmektedir.

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