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RESEARCH PAPER

Effect of Different Nutritional Conditions on The Activity of Citrus Leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on Citrus Trees in Northern Iran Orchards

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Abstract

Citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the most important pests of citrus in nurseries and young orchards of the world and Iran. In this study, two separate experiments, the effects of nutrients-nitrogen, phosphorus, potassium, and calcium compounds-on the activity of citrus leaf miner moths were investigated during the 2018–2019 and 2019–2020 growing seasons in Mazandaran province, Iran. The experimental design was randomized complete block design with 4 and 6 treatments, and 4 replications that was carried out in Bessat citrus orchard in the city of Sari. The results of the first experiment showed that in the first year, effect of different treatments was signified in the number of leaf buds, total leaves, and uninfested leaves and the highest numbers were related to soil application and foliar spraying with averages of 6.24, 5.79 and 5.77, respectively. The results obtained in the second year also showed that effect of different treatments on the number of branches was not significant. However, the highest number of leaf buds, total leaves and uninfested leaves were obtained by soil application + foliar spraying with averages of 6.12, 6.01, and 5.85, respectively. In addition, the results of the second experiment showed that in the first year, the highest number of leaf buds, the total leaves and uninfested leaves with averages of 2.52, 12.59, and 12.34, respectively, were observed in calcium nitrate treatments. In the second year, the results showed that the highest values of the growth traits were obtained in calcium nitrate treatment with averages of 19.51, 96.14, and 94.29, respectively.

1. Introduction

Citrus is one of the most important horticultural crops in the world, belonging to the genus *Citrus*, family Rutaceae, and subfamily Aurantioidea, and is one of the most important subtropical fruits (Fifaei and Ebadi, 2019). Iran ranks tenth in the world with a production of nearly 4.2 million tons of citrus fruits in 2023 (FAO, 2024). In 2024, the production of citrus fruits in Iran was more than 5.97 million tons, and among citrus producing provinces, Mazandaran province (northern Iran) was the largest producer of these products in Iran with a production of more than 3.11 million tons of citrus fruits (Anonymous, 2024). Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the major pests of citrus trees in most parts of the world including Africa,

Australia, Middle East, Caribbean Islands, Central, North and South America (Damavandian and Kiaeian Moosavi, 2014; Diez et al., 2006; Legaspi et al., 2001). Since 1994, this pest appeared in the citrus orchards of Mazandaran province (northern Iran), and in less than a year, it spread to all citrus growing regions in the north of Iran. This situation has occurred not only in Iran but also in the countries of the Near East and most citrus orchards in the world. The larvae of CLM mine immature foliage and leaf mining causes severe curling of the leaves and leaf chlorosis, necrosis and leaf drop, which ultimately results in a reduction in photosynthetic capacity of citrus trees (Amiri-Besheli, 2008; Heppner and Fasulo, 2016; Sarada et al., 2014). These serpentine mines will also increase the sensitivity of the leaves to plant pathogens such as *Xanthomonas axopodis* pv. *citri*, causing citrus bacterial canker (Das, 2003; Gottwald et al., 1997; Jesus Junior et al., 2006). Severe infestations can result in a significant reduction in fruit production (Pena et al., 2000). This pest mostly damages the young citrus foliage on the nursery trees and prevents the development of young leaves and sometimes results in their fall (Diez et al., 2006). Trees five years of age or less are especially to CLM damage. The presence of 2 or more mines per leaf can severely damage the young trees, and may result in delayed maturity of 1 to 2 years (Lapointe et al., 2014; Sarada et al., 2014). A wide range of synthetic pesticides have been used to control CLM (Beattie et al., 1995). On the one hand, hiding CLM larvae in the mines and not exposing them to the pesticides has led to the continuous and long-term use of these chemicals, unsuccessful control of CLM and the outbreak of other pests (Damavandian and Kiaeian Moosavi, 2014). On the other hand, environmental pollution caused by the use of pesticides has resulted in the interference in biological control by the natural enemies of citrus pests, which play an important role to control of this pest (Damavandian, 2007). Therefore, the use of these synthetic insecticides should be replaced by safe and low-risk control strategies in order to protect active natural enemies and provide effective control against CLM. Patsias (1996) mentioned cultural practices as a complementary method to control CLM. Based on the principles of pest control, the use of suitable cultural practices is one of the effective strategies to prevent or reduce the damage caused by pests (Abbas and Fares, 2009; Belasque et al., 2005). Proper maintenance of soil fertility and attention to plant nutritional requirements is at the heart of an effective IPM or Plant Health Care program. Fertilizing trees with appropriate fertilizers at the proper time can play a valuable role in reducing or avoiding damage caused by pests (Boman and Obreza, 2002; Futch et al., 2009). Mahmoodi and Alavi (2005) stated that foliar application of potassium nitrate, potassium sulphate, and nitrogen reduced the damage percentage of CLM larvae on the trees treated.

In Mazandaran province (northern Iran), the CLM strats acting since mid June with the increase in temperature, and damages the young summer flushes on citrus trees, while in early spring, despite the flushing of young foliages, it is practically inactive due to unsuitable temperature (Jafarzadeh, 2000). Considering the importance of citrus trees and the impact of the CLM damage on its growth and fruit yield, and considering moderate efficacy of only a few common insecticides in the region (imidacloprid and abamectin) and the repeated sprayings and the emergence possibility of resistance in this pest. This study aimed to investigate the effect of proper nutritional management on activity of CLM on the citrus trees, as a result of a proper feeding plan, maximum flushing of young leaves occurs in the spring when CLM are not able to damage them.

2. Materials and Methods

This study was designed and conducted in two separate experiments:

2.1. Effect of nitrogen, phosphorus, and potassium fertilizers on CLM activity

This experiment was carried out in the Besat 1 citrus orchard (affiliated with Fajr Agriculture and Horticulture Company) located in Sari City, Mazandaran province, Iran, for 2 consecutive years, 2018 and 2019. The study was performed as a randomized complete block design with four treatments in four replications. Each replication included eight trees in a row. Replications were spaced two trees apart. The trees in this orchard were 3-year-old pre-ripe Japanese mandarin trees (*Citrus unshiu* cv. *miyagawa*) and 1.5 m in height with 5 m spacing between and within planting lines. The studied treatments were explained below:

1- The control (without fertilization),
2- Optimal use of nitrogen, phosphorus, and potassium nutrients by soil application at a rate of 140, 100, and 200 g tree^{-1}, respectively,
3- The use of 20:20:20 (N: P: K) fertilizer by foliar spraying at a concentration of 1.5 L 1000 L^{-1},
4- The application of 20:20:20 (N: P: K) fertilizer by soil application (150 g tree^{-1}) and foliar spraying at a concentration of 1.5 L 1000 L^{-1}.

Before applications soil samples were taken from 0-30 and 30-60 cm depths of the soil of the desired orchard to analyze its physical and chemical attributes. Based on the results of the soil analysis, the required fertilizers (NPK) were used at the appropriate and recommended time.

In fertilizing by soil application for the first time, after weighing and mixing, the fertilizers were poured inside the furrow with a 10 cm depth created in surrounding the trunk till the external end of canopy shade at the second week of March 2018 and 2019. Next time, only nitrogen fertilizer (ammonium sulphate) was used in the

recommended amount in the same method at an interval of one and two months after the first time. In foliar spraying method, 20:20:20 fertilizer (Omex Bio 20; produced by Omex Co., UK) was selected and sprayed at a concentration of 1.5 L 1000 L^{-1} of water using a 20-liter back pump sprayer three times at a 10-day interval from May 5 to 25 after the beginning of leaf flushing in spring. In soil application + foliar spraying treatment, 20:20:20 fertilizer was used in a combination of soil application and foliar spraying methods three times. For sampling, in the first year, two branches in length of 20 cm were randomly selected on the trees, and in the second year, four branches in length of 20 cm were selected from the main directions (north, east, south, and west). The number of new branches, the number of leaf buds and leaves that emerged on marked branches were counted and recorded every two weeks. In total, 96 branches on treated trees were counted and examined over two years. Sampling started in the middle of May and continued until the end of October.

2.2. Effect of calcium and potassium compounds on CLM activity

This survey was carried out in the Besat 1 Citrus orchard (affiliated with Fajr Agriculture and Horticulture Company) in Sari city, Mazandaran province, Iran, for two consecutive years, 2019 and 2020. This orchard consisted of 3-year-old pre-ripe Japanese mandarin trees (*Citrus unshiu* cv. *miyagawa*). First, samples were taken from the soil, and then the amount of micronutrients required for treatments was determined based on the soil analysis results. This experiment was conducted as a randomized complete block design with six treatments in eight replications. Each tree presented as a replication, and replications for each treatment (i.e., four trees in total) were located in two adjacent rows. The treatments included:
1- Foliar spraying of calcium chloride at a concentration of 5 L 1000 L^{-1} of water three times,
2- Foliar spraying of calcium nitrate at a concentration of 6.6 L 1000 L^{-1} of water three times,
3- Foliar spraying of potassium nitrate at a concentration of 6 L 1000 L^{-1} of water three times,
4- Foliar spraying of potassium chloride at a concentration of 3.5 L 1000 L^{-1} of water three times,
5- Foliar spraying of potassium sulphate at a concentration of 2.4 L 1000 L^{-1} of water three times,
6) Control.

No application of fertilizer was done for the control treatment. The first application of fertilizers was done at the beginning of leaf flushing in spring. The second and third application was done at a 7-day interval with the first and second applications, respectively.

With the beginning of the spring, four branches on each tree in different directions were randomly selected and numbered. Sampling started when new buds appeared and continued until early October at an interval of every two weeks. The number of buds, the total number of leaves, and the number of healthy and infested leaves on four branches (A total of 96 branches) were counted and recorded on each time. In addition, to prevent CLM damage, all trees studied were treated with imidacloprid (Confidor® 35%SC, Ariashimi Co., Iran) by pouring the solution into the soil surrounding of the tree trunk.

2.3. Statistical analyses

Before data analysis, Shapiro–Wilk and Levene's tests were conducted to determine normality and homogeneity of variance, respectively. If data did not meet these assumptions, square-root transformation was applied to normalize and standardize, then was analyzed through one-way analysis of variance (ANOVA) using SAS software (SAS Institute, 2017). Means were separated by using Duncan's Multiple range test at $p = 0.05$.

3. Results and Discussion

3.1. Effect of nitrogen, phosphorus, and potassium fertilizers on CLM activity in 2018

The results of the analysis of variance showed that there was a significant difference among the treatments in the number of leaf buds, the total number of leaves appearing on each seedling, and the number of uninfested leaves, while the difference in the number of branches was not significant (Table 1). As it is clear from Table 1, although the highest number of leaf buds was related to the soil application of fertilizers, there was no significant difference between this method and soil application+foliar spraying. On the other hand, the lowest number of leaf buds was counted for the control, which was significantly different from other treatments (Table 1). According to the results of the mean comparison, foliar spraying treatment followed by soil application + foliar spraying produced the highest total number of leaves and the number of uninfested leaves, while the control yielded the lowest values in these characteristics (Table 1). Data in Table 2 clearly indicated that the highest number of leaf buds, total number of leaves, and uninfested leaves were significantly produced in the first three months of sampling that is when the pest was not present.

3.2. Effect of nitrogen, phosphorus, and potassium fertilizers on CLM activity in 2019

The results in the second year indicated that the seedlings treated by soil application + foliar spraying of fertilizer significantly produced the highest number of leaf buds, uninfested leaves, and

Table 1. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on trees treated by macronutrients and control in the first year (2018).

Treatments	Mean ± SE†			
	No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
Soil application	2.49±0.01 a	6.24±0.01 a	4.02±0.01 c	4.00±0.02 c
Foliar spraying	2.59±0.01 a	5.54±0.01 b	5.79±0.02 a	5.77±0.02 a
Soil appl.+foliar spray.	2.54±0.03 a	5.99±0.02 a	5.31±0.04 b	5.30±0.04 b
Control	2.52±0.02 a	5.01±0.02 c	3.48±0.05 d	3.41±0.06 d
F value	0.11^{ns}	8.60**	14.17**	15.27**

†Different letters in each column indicate statistically significant differences between treatments.
(ns)= non-significant difference between the treatments at p>0.05; (**)= significant difference between the treatments at p<0.01.

Table 2. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on the trees treated by macronutrients before and after emerging of the pest in the first year (2018).

Period*	Treatments	Mean ± SE†			
		No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
BE	Soil application	4.65±0.05 a	9.72±0.07 a	6.03±0.10 c	6.02±0.05 c
	Foliar spraying	4.87±0.07 a	8.48±0.06 b	8.67±0.05 a	8.66±0.07 a
	Soil appl.+foliar spray.	4.73±0.05 a	9.22±0.07 a	7.94±0.05 b	7.94±0.06 b
	Control	3.55±0.04 b	5.42±0.08 c	4.23±0.09 d	4.21±0.06 d
	Total mean (all treatments)	4.45±0.05 A	8.21±0.06 A	6.72±0.07 A	6.71±0.06 A
AE	Soil application	0.33±0.02 d	2.76±0.05 d	2.01±0.04 e	1.97±0.04 f
	Foliar spraying	0.31±0.03 d	2.60±0.05 d	2.90±0.06 e	2.87±0.06 e
	Soil appl.+foliar spray.	0.35±0.03 d	2.75±0.04 d	2.68±0.05 e	2.66±0.05 e
	Control	1.48±0.05 c	4.60±0.07 c	2.72±0.05 e	2.60±0.05 e
	Total mean (all treatments)	0.62±0.04 B	3.18±0.06 B	2.58±0.05 B	2.53±0.05 B
F value		21.18**	7.23**	8.31**	6.81**

* BE: Before emerging; AE: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at p<0.01.

Table 3. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on trees treated by macronutrients and control in the second year (2019).

Treatments	Mean ± SE†			
	No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
Soil application	2.53±0.04 a	5.56±0.04 b	4.54±0.03 c	4.18±0.04 c
Foliar spraying	2.62±0.02 a	5.63±0.04 b	5.23±0.04 b	5.22±0.04 b
Soil appl.+foliar spray.	2.51±0.02 a	6.12±0.03 a	6.01±0.04 a	5.85±0.05 a
Control	2.46±0.03 a	5.32±0.05 c	3.18±0.03 d	2.41±0.03 d
F value	1.61^{ns}	5.14**	6.86**	4.41**

†Different letters in each column indicate statistically significant differences between treatments.
(ns)= non-significant difference between the treatments at p>0.05; (**)= significant difference between the treatments at p<0.01.

the total number of leaves, with statistically significant differences compared to the control treatment (Table 3). The mean comparison of the traits measured in two periods (before and after the emergence of the pest) also showed that the highest number of leaf buds, uninfested leaves, and the total number of leaves were produced before the emergence of the pest. It is clearly noticed in Table 4 that the number of uninfested leaves on the trees in these two periods did not differ significantly (Table 4).

3.3. Effect of calcium and potassium compounds on CLM activity in 2019

The results in year 2019 showed that there was a statistically significant difference between the studied treatments in all the examined characteristics (p<0.05) (Table 5). Based on the mean comparison results, the highest number of leaf buds were related to calcium nitrate with 2.52 buds per four branches, which was not significantly different from potassium nitrate, potassium chloride, and potassium sulphate. On the other hand, the lowest number of leaf buds was counted for the control with 1.61 buds per four branches, which was not significantly different from other treatments except for calcium nitrate. Also, the highest total number of leaves counted belonged to calcium nitrate with 12.59 leaves per four branches, which was no significantly different from potassium nitrate and potassium chloride. The lowest total number of leaves was recorded for control with 6.18 leaves per four branches, which was not significantly different from the calcium chloride and potassium sulphate. The results showed that the highest infested leaves were obtained from the control with 1.01 leaves per four branches, and the difference between control and other treatments was significant. Also, no infested leaves were observed on the trees treated

Table 4. Mean comparison of number of branch, leaf buds and uninfested leaves and total number of leaves on the trees treated by macronutrients before and after emerging of the pest in the second year (2019).

Period*	Treatments	Mean ± SE†			
		No. branch	No. leaf buds	Total No. leaves	No. uninfested leaves
BE	Soil application	4.59±0.05 b	9.03±0.06 b	6.68±0.07 c	6.54±0.07 c
	Foliar spraying	4.81±0.07 a	9.35±0.09 b	7.85±0.07 b	7.85±0.06 b
	Soil appl.+foliar spray.	4.55±0.06 b	10.04±0.07 a	9.29±0.08 a	9.20±0.08 a
	Control	3.74±0.04 c	5.85±0.07 c	3.52±0.05 d	3.43±0.06 d
	Total mean (all treatments)	4.42±0.05 A	8.57±0.07 A	6.84±0.07 A	6.76±0.07 A
AE	Soil application	0.47±0.03 e	2.08±0.05 e	2.39±0.06 e	1.82±0.05 f
	Foliar spraying	0.43±0.04 e	1.90±0.05 e	2.60±0.05 e	2.59±0.03 e
	Soil appl.+foliar spray.	0.47±0.05 e	2.20±0.04 e	2.73±0.06 e	2.49±0.05 e
	Control	1.18±0.04 d	4.78±0.07 d	2.84±0.05 e	1.38±0.04 g
	Total mean (all treatments)	0.64±0.04 B	2.74±0.05 B	2.64±0.05 B	2.07±0.04 B
F value		7.14**	5.64**	6.63**	7.69**

* BE: Before emerging; AE: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at p<0.01.

Table 5. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves on trees treated by different fertilizers and control in the first year (2019).

Treatments	Mean ± SE†			
	No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
Calcium chloride	1.71±0.01 b	6.67±0.04 c	0.14±0.003 d	6.53±0.03 cd
Calcium nitrate	2.52±0.02 a	12.59±0.07 a	0.25±0.00 c	12.34±0.06 a
Potassium nitrate	2.08±0.05 ab	10.88±0.06 ab	0.45±0.002 b	10.43±0.06 ab
Potassium chloride	2.41±0.02 ab	10.83±0.07 ab	0.19±0.01 cd	10.64±0.05 ab
Potassium sulphate	2.18±0.02 ab	7.98±0.05 bc	0.00±0.003 e	7.98±0.04 c
Control	1.61±0.02 b	6.18±0.03 c	1.01±0.001 a	5.17±0.04 d
F value	2.04*	2.83*	1.49*	2.63*

†Different letters in each row indicate statistically significant differences between treatments
(*)= significant difference between the treatments at p<0.05

Table 6. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves before and after emerging of the pest in the first year (2019).

Period*	Treatments	Mean ± SE†			
		No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
BE	Calcium chloride	2.94±0.05 b	9.84±0.07 d	0.00±0.00 b	9.84±0.07 d
	Calcium nitrate	4.03±0.06 a	17.44±0.08 a	0.00±0.00 b	17.44±0.08 a
	Potassium nitrate	3.34±0.05 b	15.19±0.09 b	0.00±0.00 b	15.19±0.09b
	Potassium chloride	4.03±0.04 a	14.25±0.07 b	0.00±0.00 b	14.25±0.07 b
	Potassium sulphate	3.81±0.07 a	11.00±0.08 c	0.00±0.00 b	11.00±0.08 c
	Control	3.28±0.06 b	7.06±0.06 e	0.00±0.00 b	7.06±0.06 e
	Total mean (all treatments)	3.57±0.06 A	13.13±0.08 A	0.00±0.00 B	13.13±0.08 A
AE	Calcium chloride	0.48±0.03 d	3.50±0.06 g	0.27±0.03 b	3.23±0.05 g
	Calcium nitrate	1.00±0.02 c	7.73±0.05 e	0.50±0.03 b	7.23±0.07 e
	Potassium nitrate	0.82±0.04 c	6.57±0.06 ef	0.89±0.04 b	5.68±0.05 f
	Potassium chloride	0.79±0.02 c	7.41±0.04 e	0.38±0.02 b	7.03±0.06e
	Potassium sulphate	0.55±0.04cd	4.96±0.07 f	0.00±0.00 b	4.96±0.07 f
	Control	0.14±0.03 d	5.29±0.06 f	2.02±0.05 a	3.27±0.06 g
	Total mean (all treatments)	0.63±0.03 B	5.24±0.05 B	0.34±0.04 A	4.90±0.06 B
F value		11.58**	23.03**	5.40**	25.69**

* BE: Before emerging; AE: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at p<0.01.

with potassium sulphate, and there was a significant difference between this treatment and other treatments. Based on the results, the highest number of uninfested leaves counted were related to calcium nitrate with 12.34 leaves per four branches, which was not significantly different from the potassium chloride and potassium nitrate. Also, control and calcium chloride yielded the lowest number of uninfested leaves with 5.17 and 6.54 leaves per four branches, respectively, and there was no significant difference between them (Table 5). Mean comparison of the traits before and after CLM emergence showed that the number of leaf buds and the total number of leaves appeared before CLM emergence were significantly higher compared to those after CLM emergence. Although the number of infested leaves counted after CLM emergence was higher than those before CLM emergence, but this difference was not significant (Table 6).

Table 7. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves on trees treated by different fertilizers and control in the second year (2020).

Treatments	Mean ± SE†			
	No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
Calcium chloride	12.89±0.06 bc	65.26±0.18 b	3.43±0.03 a	61.83±0.15 b
Calcium nitrate	19.51±0.10 a	96.14±0.36 a	1.85±0.01 b	94.29±0.22 a
Potassium nitrate	13.14±0.07 b	76.69±0.16 b	4.1±0.03 a	72.59±0.19 b
Potassium chloride	15.88±0.09 b	74.78±0.21 b	4.13±0.02 a	70.65±0.11 b
Potassium sulphate	13.67±0.08b	66.72±0.10 b	1.76±0.01 b	64.96±0.14 b
Control	9.72±0.07 c	45.92±0.12 c	4.51±0.03 a	41.41±0.10 c
F value	5.85**	3.98**	2.09*	3.75**

†Different letters in each row indicate statistically significant differences between treatments.
(**) and (*) indicate significant difference between the treatments at p<0.01 and p<0.05, respectively.

Table 8. Mean comparison of number of leaf bud, infested and uninfested leaves and total number of leaves before and after emerging of the pest in the second year (2020).

Period*	Treatments	Mean ± SE†			
		No. leaf bud	Total No. leaves	No. infested leaves	No. uninfested leaves
BE	Calcium chloride	21.20±0.32 d	92.91±0.52 c	0.68±0.07 d	92.23±0.51 c
	Calcium nitrate	30.97±0.27 a	148.09±0.66 a	0.57±0.08 d	147.52±0.53 a
	Potassium nitrate	21.70±0.25 d	118.75±0.61 b	0.82±0.07 d	117.93±0.61 b
	Potassium chloride	27.33±0.30 b	117.20±0.57 b	0.83±0.09 d	116.37±0.47 b
	Potassium sulphate	24.78±0.19 c	120.15±0.55 b	0.34±0.06 d	119.81±0.43 b
	Control	17.20±0.15 e	77.76±0.60 d	5.69±0.12 b	72.07±0.39 d
	Total mean (all treatments)	23.86±0.36 A	112.48±0.78 A	1.49±0.07 B	110.99±0.57 A
AE	Calcium chloride	4.58±0.06 g	37.60±0.47 ef	6.18±0.11 b	31.42±0.39f
	Calcium nitrate	8.05±0.08 f	44.18±0.38 e	3.13±0.08 c	41.05±0.35 e
	Potassium nitrate	4.58±0.05 g	34.63±0.43f	7.38±0.09 a	27.25±0.26 fg
	Potassium chloride	4.42±0.04 g	32.35±0.40 f	7.43±0.07 a	24.92±0.32 g
	Potassium sulphate	2.55±0.05 h	13.28±0.29 g	3.18±0.05 c	10.10±0.12 h
	Control	2.23±0.04 h	14.08±0.22 g	3.33±0.10 c	10.75±0.14 h
	Total mean (all treatments)	4.40±0.04 B	29.35±0.49 B	5.11±0.09 A	24.24±0.34 B
F value		12.23**	18.78**	15.22**	10.01**

* BE: Before emerging; A.: After emerging.
†Different lower and upper case letters in each column indicate statistically significant differences between treatments at two periods and between total means at two periods, respectively.
(**)= significant difference between the treatments at p<0.01.

3.4. Effect of calcium and potassium compounds on CLM activity in 2020

The results of variance analysis showed that the studied treatments had statistically significant differences with each other in all attributes (p<0.05). Mean comparison of number of leaf buds showed that the trees treated with the calcium nitrate produced the highest leaf buds with 19.51 buds per four branches, which was significantly different from other treatments (Table 7). Also, the highest total number of leaves was related to the calcium nitrate with 96.14 leaves per four branches, and this difference was significant from the other treatments. The lowest total number of leaves with 45.92 leaves per four branches was recorded for the control treatment that there was a significant difference between control and others. Based on the obtained results, the highest number of infested leaves was recorded for the control with 4.51 leaves per four branches that was not significantly different from potassium chloride, potassium nitrate, and calcium chloride treatments. On the other hand, the lowest infested leaves with 1.76 leaves per four branches belonged to the potassium sulphate treatment, which was not significantly different from calcium nitrate. The mean comparison of the number of uninfested leaves also showed that the highest and lowest number of uninfested leaves with 94.29 and 41.41 leaves per four branches related to calcium nitrate and control treatments, respectively, and their difference from other treatments was significant (Table 7). Based on the results presented in Table 8, the number of leaf buds and the total number of leaves on the trees treated with the fertilizers before CLM emergence were significantly higher than after CLM emergence, and also the low infestation level was observed on the leaves produced after CLM emergence due to the less production of young leaves in this period.

In the principles of control, one of the effective ways to reduce the damage of pests is to use suitable agricultural methods to avoid damage caused by harmful factors. Feeding trees with the suitable fertilizers at the appropriate time can play a valuable role in reducing or avoiding damage caused by pests (Abbas and Fares, 2009; Belasque et al., 2005). The investigations show that the use of macro fertilizers for citrus trees in Mazandaran province is usually recommended by experts and consumed by gardeners from the end of February, while the citrus trees need a lot of macro fertilizers, especially nitrogen during the flowering time in late April and later. In Mazandaran province, the use of fertilizers that have a high solubility in water such as Nitrogen before rainfalls in late winter or early spring

caused that these fertilizers was removed from the root zone, and not only does not have much effect on the vegetative and reproductive growth of the trees but also causes an increase in production costs and even environmental pollution (it is visual observation). Hence, special attention to this point can help in the optimal and balanced consumption of fertilizers and the appropriate vegetative and reproductive growth of the trees. In the first experiment, the optimum consumption of macronutrients by soil application+ foliar spraying showed a significant difference compared to the control and produced the highest leaf buds in the spring. Due to the inactivity of CLM at this time, all leaves and young shoots produced until late June 2018 and late July 2019 were not damaged by this pest. According to the results obtained in the first experiment, applying fertilizer by soil application+foliar spraying produced more leaf buds and leaves than other treatments. On the other hand, the mean comparisons showed that the macronutrients should be applied before the emergence of the pest in the spring season.

The results of the second experiment showed that the highest number of leaf buds and leaves produced from April 21 to June 21 was protected from the damage by CLM due to the absence of the pest during this period. According to the results obtained (unpublished data), the highest number of infested leaves in the first and second years was observed in the middle of August and from September 23 to October 22, when the ambient temperature increased and leaf buds and leaves were less produced on the trees. Mean comparison of the characteristics in the two periods, before and after the emergence of CLM showed that if the micronutrients are used before the emergence of the pest in the spring, it will produce the highest young leaves and leaf buds before the pest starts its activity.These results show the fact that applying macro- and micronutrients in the spring season and as a result, produce more leaf buds and leaves at this time when CLM is not active, and fewer leaf buds and leaves are exposed to damage by the pest when it is active. Therefore, it is possible to minimize CLM damage through proper nutrition management that will lead to the reduction in the use of chemical pesticides.

The results of the second experiment in 2020 showed that the highest number of uninfested leaves was counted on the trees treated with calcium and potassium nitrate (91.63 and 72.60, respectively). Calcium and potassium nitrate are easily soluble in water and absorbed by plants. Inherent nitrate content enables many nutrients including calcium and potassium to be taken by plants and increases resistance to pests (El-Enien et al., 2017). Accordingly, these two nutrients are multi-functional in physiological processes in the plant and effective in vegetative growth.

Patsias (1996) recommended control methods of CLM in Cyprus, which included increasing the amount of chemical fertilizers, especially nitrogen in early February, light irrigation during the two months (from January 20 to March 20), pruning citrus trees in early February, light irrigation during the summer and autumn and reducing the use of chemical fertilizers in these seasons. All of the mentioned cases aimed to reduce the production of leaf buds and leaves on trees during CLM activity, which is consistent with the findings of the present research. Mahmoodi and Alavi (2005) stated that spraying potassium nitrate and potassium sulphate caused an increase in the number of leaves and the length of new branches on citrus trees and the percentage of damage caused by the larvae of CLM was also reduced by the management of the micronutrients, which is in agreement with the results obtained in the present study.

Mustafa et al. (2014) evaluated the relationship between the level of CLM damage and the biochemical changes of citrus leaves (Ca^{2+}, K^{+}, and Mg^{2+}) caused by the use of chemical fertilizers during the growing season in Punjab, Pakistan. They reported a negative correlation between the CLM damage and potassium in the one-year orchard, while the results obtained in the two-year and three-year orchards showed a positive correlation between the CLM damage and potassium and calcium. These results clarify the effect of mineral nutrients on the level of damage caused by this pest. Concerning other types of fertilizers, research also showed that organic fertilizers such as different composts at 0.5 kg $plant^{-1}$ concentration reduced the CLM infestation by up to 55 and 39% during fall and summer, respectively (Ullah et al., 2019). El-Enien et al. (2017) reported that potassium and calcium compounds had a significant effect on the CLM infestation of Valencia orange seedlings so that potassium nitrate, potassium silicate, and calcium nitrate as soil application and foliar spraying caused up to 50% reduction in CLM infestation. Our results are in line with the findings obtained by El-Enien et al. (2017) and El-Sayed and Ennab (2008) found that spraying potassium sulphate at 2% decreased the CLM infestation on citrus trees. In addition, Dito and Lewis (2013) indicated that foliar spraying of potassium silicate on young citrus seedlings significantly reduced CLM damage.

In general, nutrients can directly or indirectly affect plants so that they become a susceptible host against pest or pathogen attacks. Nutrients can reduce or increase the severity of the damage, influence the environment to attract or deter the pest or pathogen, and induce resistance or tolerance in the host plant (Agrios, 2005; Zambolim et al., 2001). However, mineral nutrients also affect plant growth and reproduction by influencing plant resistance or sensitivity to pathogens and pests (Spann and Schumanu, 2010).

Although plant resistance to pests and diseases is controlled genetically, environmental factors also play a significant role in this process (Bairwa et al.,

2014). Some genes responsible for the pest resistance are activated only by environmental stimuli. Nutrients are one of the environmental factors that can significantly affect the management of agricultural systems (Ochola et al., 2014).

Several studies have reported a negative effect of fertilizer application on the population of various insect pests in fields and greenhouses. Chávez-Dulanto et al. (2018) stated that the foliar application of microelements such as calcium, magnesium, zinc, copper, iron, manganese and boron caused a significant reduction in the populations of *Panonychus citri* McGregor and *Phyllocoptruta oleivora* Ashmead, as key pests of citrus crop in Peru. Karim (2013) reported that the use of various manures and fertilizers, including cow-dung, triple superphosphate and micronutrients decreased the populations of aphid (28.02%), fruit borer (35.76%), whitefly (43.30%) and leafhopper (53.75%) in the tomato field in Bangladesh. Karungi et al. (2006) showed that the application of NPK fertilizer in the soil caused a reduction in *Aphis fabae* Scopoli population in *Phaseolus vulgaris* fields in Uganda. Moursy et al. (2021) reported that the sequential application of humic acid in foliar and soil methods resulted in the lowest population density of *Aphis gossypii* Glover, *Bemisia tabaci* Gennadius and *Tetranychus urticae* Koch, which are three main pests on eggplant in greenhouse conditions in Egypt. Oeller et al. (2025) revealed that the organic chicken manure applied to quinoa (*Chenopodium quinoa*) caused the largest reduction in the cowpea aphid (*Aphis craccivora* Koch) and *Lygus* sp. populations due to the lowest survival. In Iran, Olyaie Torshiz et al. (2017, 2020) stated that the pomegranate trees treated with both biofertilizers and humic acid showed the lowest fruit infestation (18.75-28.67%) with *Ectomyelois ceratoniae* Zeller in pomegranate orchards. Yardım and Ewards (2003) also reported that the population of aphids on tomatoes treated with organic fertilizer was lower than on those treated with the synthetic fertilizer as well as the control.

Mineral nutrients affect the primary resistance mechanism in two ways; First, the formation of mechanical barriers, which is mainly through forming a thicker cell wall, and second, making natural defence compounds, including phytoalexins, antioxidants, and flavonoids, which protect the plant against pests (Yadollahpour et al., 2015). Anyway, the role of nutrients in plant-pest interaction is well established. Identifying these interactions is useful for controlling and eradicating pests using the fertilizing program.

4. Conclusions

The CLM is one of the important citrus pests in the citrus growing regions of the world and Iran. The results of this research showed that the use of tested chemical fertilizers by foliar spraying in early spring to stimulate the trees to produce more leaves and branches and to accelerate the vegetative growth of new shoots to avoid pest damage is suitable and economical. The results of the present study showed that the use of macro fertilizers in the spring season by the method of soil application along with foliar spraying as well as the use of calcium chloride and calcium nitrate micronutrient fertilizers at the same time lead to more bud and leaf production before the appearance of CLM. Considering that the CLM damages the young leaves, therefore, when the pest starts to act, the young leaves are out of their sensitive stage and are not damaged by the pest. Considering the adverse environmental effects caused by the use of pesticides on natural enemies and even the outbreak of some pests such as mites due to the excessive use of these chemicals in citrus orchards. Therefore, the damage can be reduced by proper nutrition management using macro and micro fertilizers, and minimize the use of pesticides.

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RESEARCH PAPER

Changes in Morpho-Physiological Characteristics of Melon (*Cucumis melo* L.) Seedlings under Different Levels of Drought Stress

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Abstract

Drought stress is one of the most common abiotic stresses that negatively affects crop production. The purpose of this study was to determine the morpho-physiological characteristics of melon plants in response to increasing levels of drought stress induced by different polyethylene glycol (PEG-6000, molecular weight 6000) concentrations. Melon cultivar 'Kırkağaç 589' seedlings with a 2 true-leaf stage were grown in a growth medium containing peat: perlite: vermiculite (6:1:1, by volume) mixed with 5%, 10%, 15%, and 20% PEG-6000. Thirty days after drought treatment, plant height, stem diameter, fresh and dry weight, dry matter, leaf area, leaf temperature, chlorophyll content (SPAD), relative water content, turgor loss, and electrolyte leakage were measured. The findings indicated that as the severity of the drought increased, there was a notable decline in plant height, fresh weight, dry weight, and relative water content. In contrast, drought stress led to increased dry matter, leaf temperature, chlorophyll content, electrolyte leakage, and turgor loss. The plant height and fresh weight were particularly susceptible to drought stress, with significant inhibition observed even at a concentration of 5% PEG. Compared to the control, the relative water content decreased from 81.3% to 69.0%, while electrolyte leakage increased from 20.9% to 27.2%. It was concluded that electrolyte leakage serves as an indicator of drought stress and that the drought severity of 10% PEG should be regarded as the critical level for melon plants at the seedling growth stage.

1. Introduction

Melon (*Cucumis melo* L.), a member of the Cucurbitaceae family, is a vegetable plant with a variety of plant growth habits, fruit shape, size, and color. They can adapt to various soil and climatic conditions in open fields and greenhouses (Vural et al., 2000). In Türkiye, the total sowing area of melon was 55.000 ha, with a fruit production of 1.4 million tons in 2023 (Anonymous, 2024). It is an important plant for crop rotation in the Central Anatolian region because of its tolerance and high adaptation ability to drought under rainfed conditions.

Abiotic stresses, including drought, salinity, extreme temperature, and heavy rainfall have adversely affected plant growth and productivity (Yang et al., 2021). Drought stress is an ordinary situation in arid and semi-arid regions with insufficient rainfall. In drought-stress conditions, the life cycle of plants, from germination to harvest, is constrained (Adıgüzel et al., 2023; Kaya, 2024). This leads to delayed or inhibited germination, emergence, seedling growth, plant development, and reduced yield, which are affected by some physiological processes (Seleiman et al., 2021). Similarly, drought affects the morphological and

physiological characteristics of the melon plant, leading to a reduction in fruit yield (Akhoundnejad and Dasgan, 2020; Astaraki et al., 2022). For example, Kavas et al. (2013) demonstrated that increased drought stress resulted in a decrease in both the fresh and dry weights of roots. A similar reduction in plant height was reported by Seymen et al. (2024) in melon plants subjected to drought stress. Furthermore, drought stress reduced stem diameter (Rehman et al., 2024), fresh weight (Seymen et al., 2024), dry weight (Wang et al., 2024), and leaf area (Bagheri et al., 2019). The decline in morphological characteristics is closely associated with the changes in physiological processes and consequently fruit yield. In response to drought stress, the water content within leaf tissues decreased, leading to a loss in relative water content, and an increase in dry matter (Ansari et al., 2018; Rehman et al., 2023). Melon plants subjected to drought stress had higher electrolyte leakage (Kavas et al., 2013; Rehman et al., 2023). Leaf temperature under drought stress imposed by limited irrigation water increased with a lower water supply (Akhoundnejad and Dasgan, 2020).

The responses of melon seedlings to increasing drought severity and the drought tolerance threshold have not been thoroughly examined. Therefore, the purpose was to examine the changes in morphological and physiological characteristics of melon plants under different drought stresses and to identify the threshold level of drought stress.

2. Materials and Methods

The laboratory experiment was performed under controlled conditions at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University, Eskişehir, Türkiye in 2024. Seeds of the melon cultivar 'Kırkağaç 589' and polyethylene glycol (PEG) 6000 creating different drought severities were used as materials.

2.1. Experimental setup

The seeds were planted into plastic tray with 80 cells and incubated in a plant growth chamber under a temperature regime of 22°C day/16°C night, and a photoperiod of 18/6 hours with a relative humidity of 65-70%, until the plants reached a 2-true leaf stage. Following the incubation period, they were transplanted into plastic pots with a volume of 0.45 L containing a total of 130 g of the mixture of peat, perlite, and vermiculite (6/1/1, by volume).

The severity of the drought was arranged by the addition of varying concentrations of PEG 6000 (5%, 10%, 15%, and 20% and 6.5, 13.0, 19.5, and 26.0 g PEG 6000 per pot, respectively) to each pot before seedling transplantation. These concentrations roughly correspond to osmotic potentials of 1.5, 3.0, 5.0, and 6.0 bar, (Michel and Kaufmann, 1973). One hundred milliliters of ½ strength of Hoagland's solution were applied to each pot. The pots were then placed in a growth chamber that was set at 24°C/18°C and 18/6 hours with a relative humidity of 50-60%. Weighing the pots every other day to determine moisture loss and the deficit of water was completed by adding distilled water. The plants were harvested thirty days after the drought stress and all measurements were taken.

2.2. Measurement of characteristics

The plant height, stem diameter, fresh and dry weights, and dry matter of above ground parts of melon seedlings were measured after cutting them from the soil surface. The ImageJ Program was used to measure the leaf area on the scanned leaves. After the above-ground parts of the plants were dried in an oven at 80°C for 48 h, the dry matter was calculated as a percentage by dividing the dry weight by the fresh weight and multiplying the result by 100. The portable chlorophyll meter Konica Minolta SPAD-502 was used for determining the chlorophyll content as the SPAD index. The relative water content (RWC) of the leaf was determined with the following formula.

$$RWC(\%) = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100$$

Where,
FW= fresh weight of leaf
DW= dry weight of leaf
TW= turgid weight of leaf

$$Turgor\ loss\ (\%) = \left[\frac{(TW - FW)}{TW}\right] \times 100$$

Dry weight was determined after drying at 80°C for 24 h and turgid weight was weighed after the leaf samples were immersed in distilled water in a falcon tube for 24 h in the dark at 20°C (Kaya et al., 2003).

2.3. Statistical analysis

The data were statistically analyzed by a completely randomized design with five replicates, and differences between the means were compared by the Least Significant Differences (LSD) test at a 5% level. The JMP 13.0 software was used for all of the statistical analysis.

3. Results and Discussion

The analysis of variance revealed that there were significant differences in morphological characteristics including plant height, stem diameter, fresh and dry weight, leaf area, and dry matter due to the levels of drought severity (Figure 1). As drought severity increased, the height of melon plants exhibited a significantly reduced. However, no significant reduction in plant height

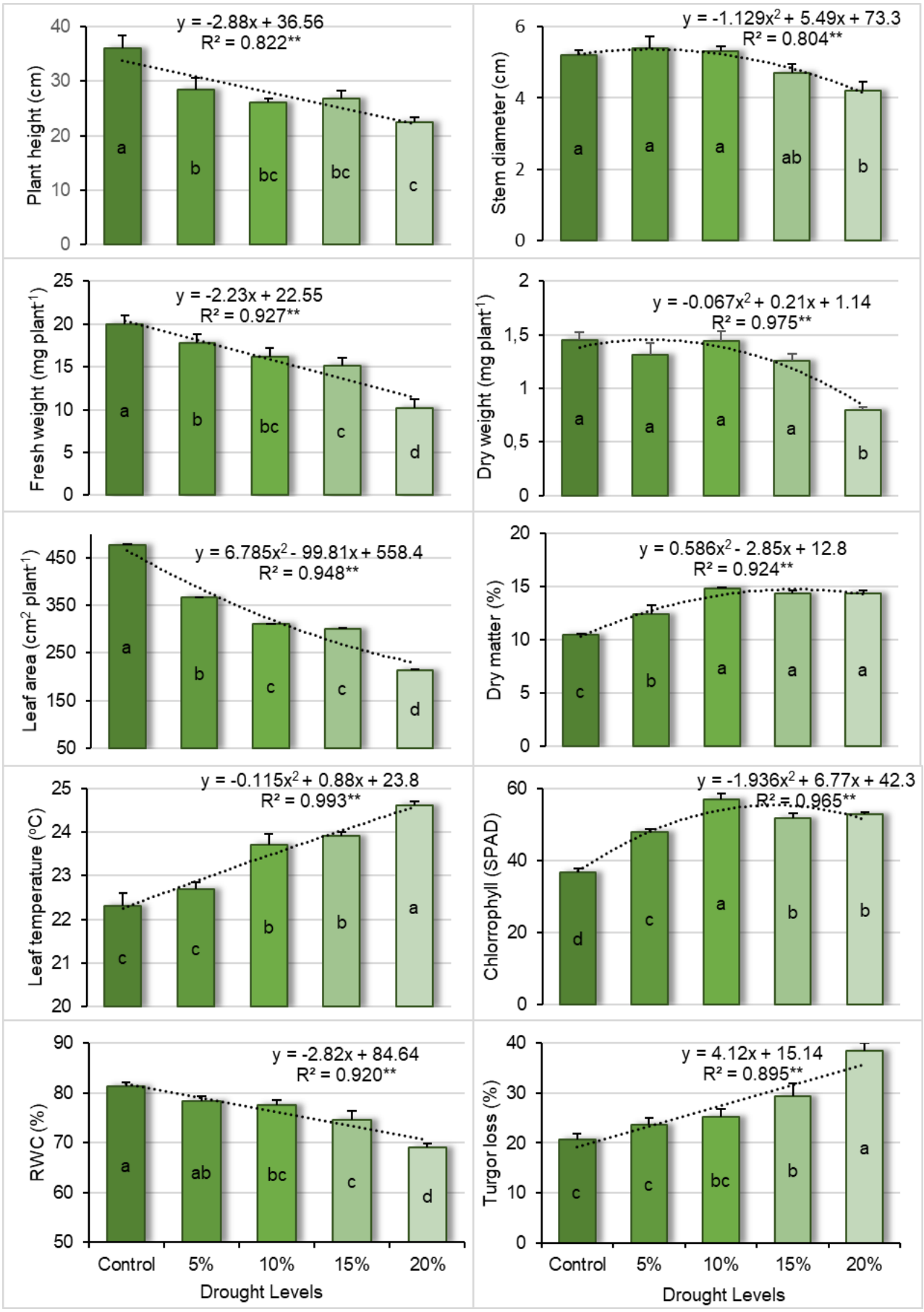

Figure 1. Changes in the investigated parameters of melon plants subjected to various drought stresses (Letters and bars on each column denote significance level at 5% and standard error, respectively. RWC: Relative water content).

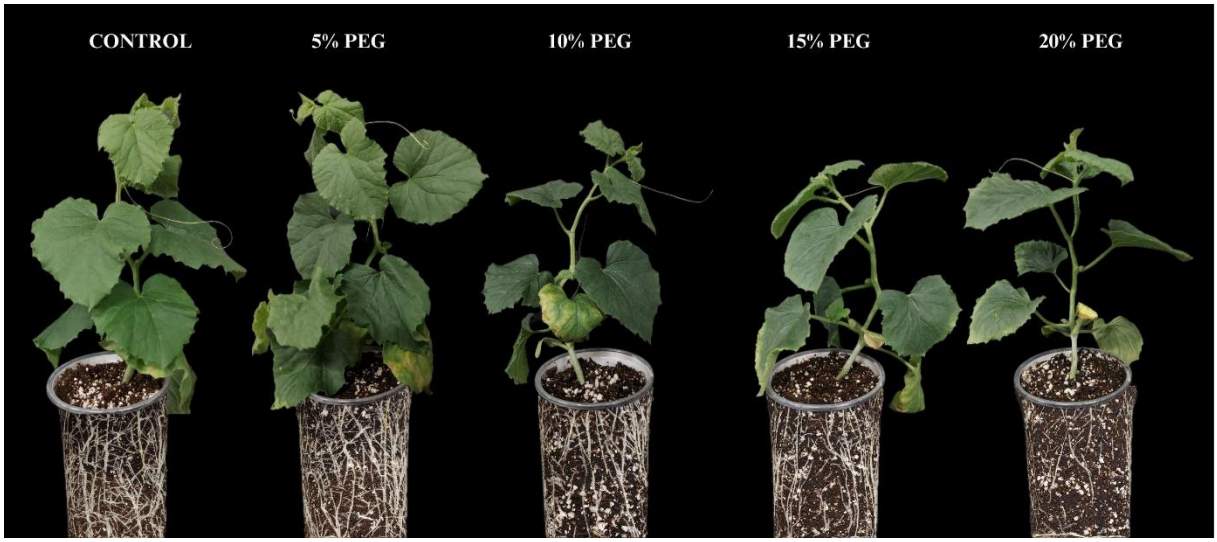

Figure 2. Melon plants subjected to various drought stresses.

was observed between 5% and 15% drought severity levels (Figure 2). The plant height decreased from 36.0 cm in control to 22.4 cm in 20% PEG. This result supports the findings of Seymen et al. (2024) in melon and Wang et al. (2024) in cucumber, who observed a reduction in plant height under water-deficit conditions.

The impact of increasing drought severity on stem diameter was not substantial up to a level of 15% PEG. Still, a notable decline was observed at the highest level of drought severity, which was 20% PEG. This result is confirmed by the finding of Rehman et al. (2024), who found a reduction in the stem diameter of melon. The application of increasing levels of drought stress reduced the fresh weight of melon plants. The fresh weight of the melon plants exhibited a 50% reduction due to the effects of drought stress, which indicates a high level of sensitivity to drought. Seymen et al. (2024) reported a 64.8% decrease in plant fresh weight due to drought stress. Although there was no change in dry weight with increasing drought severity up to 15% PEG, it was remarkably reduced under 20% PEG (Figure 1). Similarly, Wang et al. (2024) found a drastic decline in the dry weight of cucumber at 10% PEG and Seymen et al. (2024) recorded that the dry weight of melon declined by 52% under 50% water-deficit conditions. The enhancement of dry matter was observed in melon plants subjected to 5% and 10% PEG, as evidenced by changes in fresh and dry weight. Similar to this result, Kirnak and Dogan (2009) reported that dry matter production of watermelon plants was decreased by drought stress. However, the dry matter content was found to be similar at 10%, 15%, and 20% PEG. The total leaf area exhibited a decline in correlation with increasing drought severity. Bagheri et al. (2019) and Karimi and Zare (2023) reported similar results regarding decreased leaf area in melon with irrigation water supply. Furthermore, Ansari et al. (2019) observed that the prolonged duration of drought stress resulted in a reduction in the leaf area of melon.

The increase in drought severity significantly affected chlorophyll content, leaf temperature, relative water content, turgor loss, and electrolyte leakage of melon plants. The plants subjected to various drought stresses showed elevated chlorophyll content, while it declined at 15% and 20% PEG (Figure 1). No significant differences were observed between 10% and 20% PEG. Similarly, Astaraki et al. (2022) reported that chlorophyll content increased from 72 SPAD in control to 82 SPAD in drought-stressed plants of melon, and Seymen et al. (2024) found an increase in chlorophyll content (SPAD) in plants grown under a 50% water deficit. Leaf temperature gradually increased by elevating drought stress, reaching a maximum of 24.6°C in 20% PEG treatment. This result aligns with the findings of Akhoundnejad and Dasgan (2020) and Seymen et al. (2024), who determined that leaf temperature increased in melon plants grown under water-deficit conditions. The highest relative water content was identified in the control plants, and it was diminished by increasing drought severity. A significant decline was observed in 20% PEG. Similar findings were reported by Ansari et al. (2018) and Rehman et al. (2023), who demonstrated that the relative water content decreased under drought conditions, and this decrease was significantly greater when the duration of drought stress was extended. It is proposed that the reduction in the relative water content resulted in an elevation of leaf temperature, as indicated by their significant and negative correlation coefficient r=-0.724**. Furthermore, Kavas et al. (2013) found a sharp decline in the relative water content of melon at -0.4 MPa, which is similar to the result of the present study. Turgor loss was observed to be higher in melon plants subjected to drought, with an increase in the severity of drought resulting in a corresponding

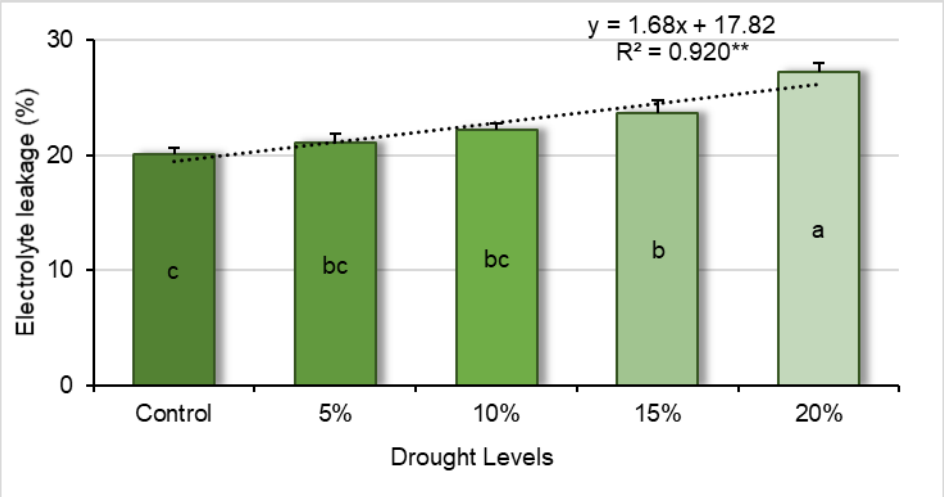

Figure 3. Changes in the electrolyte leakage of melon plants subjected to various drought stresses (Letters and bars on each column denote significance level at 5% and standard error, respectively).

Table 1. The correlation coefficient (r) between the investigated characteristics of melon plants exposed to various drought stresses.

	LT	Chl	PH	SD	FW	DW	RWC	DM	TL
Chl	0.661**	1.000							
PH	-0.637**	-0.711**	1.000						
SD	-0.391	-0.127	0.403*	1.000					
FW	-0.819**	-0.581**	0.774**	0.560**	1.000				
DW	-0.574**	-0.293	0.638**	0.638**	0.889**	1.000			
RWC	-0.724**	-0.470*	0.557**	0.409*	0.832**	0.725**	1.000		
DM	0.758**	0.849**	-0.567**	-0.073	-0.589**	-0.217	-0.583**	1.000	
TL	0.695**	0.413*	-0.546**	-0.429*	-0.829**	-0.752**	-0.995**	0.515**	1.000
EL	0.468*	0.279	-0.406*	-0.354	-0.629**	-0.671**	-0.591**	0.281	0.602**

LT: Leaf temperature, Chl: Chlorophyll content, PH: Plant height, SD: Stem diameter, FW: Fresh weight, DW: Dry weight, RWC: Relative water content, DM: Dry matter, TL: Turgor loss, and EL: Electrolyte leakage.
*, **: significant at $p<0.05$ and $p<0.01$, respectively.

increase in the observed loss. Electrolyte leakage increased markedly in melon plants subjected to drought stress (Figure 3). This result supports the findings of Kavas et al. (2013) and Rehman et al. (2023), who reported an increase in oxidative damage under drought stress, which was accompanied by a reduction in electrolyte leakage. The increase in electrolyte leakage was minimal at up to 15% PEG, while it increased considerably at 20% PEG, which should be considered as the critical threshold and used for the selection of drought-tolerant melon genotypes. Additionally, increased drought severity was associated with elevated electrolyte leakage; suggesting that it may serve as a valuable indicator for assessing drought tolerance levels in melon, as previously reported by Garty et al. (2000) and Bajji et al. (2002). Similarly, Jothimani and Arulbalachandran (2020) noticed that electrolyte leakage in black gram (*Vigna mungo* L.) linearly increased with increasing levels of PEG, reaching the maximum value at 20% PEG with a 60% increase.

The correlations between the characteristics affected by different drought severities were calculated with significance levels and are presented in Table 1. A significant and negative correlation coefficient (r= -0.819**) was observed between leaf temperature and fresh weight. Conversely, the correlation between chlorophyll content and dry matter was positively significant. Electrolyte leakage is negatively correlated with fresh weight, dry weight, relative water content, and leaf temperature, indicating that electrolyte leakage is relatively enhanced by a reduction in these characteristics. Plant height and fresh weight were negatively linked with dry matter, turgor loss, and electrolyte leakage, indicating that a decrease in plant height was associated with increased turgor loss and dry matter. Similar linkages were determined by Karimi and Zare (2023).

4. Conclusions

Drought stress has detrimental effects on germination, early or late growth stages, and the growth and yield of crop plants. However, the tolerance level of drought stress varies depending on the growth stage of plants, species, and even cultivars. In this study, different levels of drought severity were induced by PEG 6000, and melon seedlings were grown in the respective conditions. The results demonstrated that drought negatively affected the growth of melon seedlings, with a

notable restriction in root growth. Although increased drought severity reduced the growth of melon, 10% PEG exhibited a particularly pronounced inhibitory effect by disrupting the water balance in tissues and increasing electrolyte leakage. Consequently, electrolyte leakage may serve as an indicator for drought-tolerant melon plants, with 10% PEG representing a critical threshold for drought stress in melon. Further studies connected with the results of field experiments should be recommended to attain a more precise decision on drought tolerance in melon.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Fungal Agents Causing Fruit Rot in Sweet Cherry Orchards and Storages in Isparta Province

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Virulence

Abstract

Fruit rot, causing significant yield losses not only in the orchards, but also in storage conditions, is among the most common diseases of sweet cherries. Isparta Province, an important region for fruit production in Türkiye, ranks fourth in sweet cherry production. In this study, it was aimed to determine the fungal agents causing fruit rot on sweet cherries in the orchards and in the cold storages facilities in Isparta province. Healthy and diseased fruit samples were collected before harvest, from the randomly selected 76 orchards, in the districts of Isparta province where sweet cherry production was made. Fruit samples were also taken after harvest from three cold storages in Eğirdir District, and from one sweet cherry processing center in Uluborlu District. As a result of the isolations, the most common agents on the rotten fruit samples were; *Alternaria alternata*, *Monilinia laxa* and *Botrytis cinerea*, respectively. *A. alternata*, *Aspergillus* sp., *B. cinerea*, *Cladosporium cladosporioides*, *Fusarium oxysporum* and *M. laxa* were isolated from the symptomless fruits indicating latent infections. The most common fungi isolated from the rotten fruits in the cold storages were; *A. alternata*, *Trichoderma* spp., *Penicillium* spp. and *B. cinerea*, while *A. alternata* was also isolated from the healthy looking fruits. *Penicillium* spp., *B. cinerea* and *Rhizopus stolonifer* caused severe rot in the pathogenicity experiment, where *Stemphylium botryosum* and *Fusarium lateritium* were found as the less virulent species. *Fusarium lateritium* and *Trichothecium roseum* were reported as fruit rot agents on sweet cherries for the first time in this study, while *Aspergillus* spp., *F. oxysporum*, *Geotrichum candidum*, *Sclerotinia sclerotiorum*, *Stemphylium botryosum* and *Trichoderma* spp. were first reports for Türkiye.

1. Introduction

Sweet cherry (*Prunus avium* L.), belonging to Rosaceae family, has known to spread throughout the world by birds, other animals and immigrants from its original area between Caspian Sea, South Caucasia and Northeast Anatolia. Cherries are among the important fruits in human nutrition with their high mineral contents (Koç, 2023). Sweet cherry is a product that makes significant contributions to the Turkish economy, as it requires intensive labor in both production and marketing stages, creates extensive job opportunities, and provides significant foreign exchange income in exports (Kaplan et al., 2022). Türkiye ranks first in the world's sweet cherry production with 656 041 tons, while it ranks third in exports (FAOSTAT, 2022). Isparta province has an important place in terms of fruit production in Türkiye, and comes fourth in sweet cherry production with 46 565 tons (TÜİK, 2023).

Pre and post harvest fruit rots cause significant losses in sweet cherry production. Various researchers from different countries mentioned *Penicillium* species such as *P. expansum*, *P. crustosum* and *P. chrysogenum* as the main rot agents (Ceponis, 1987; Lopez et al., 2016; Sanzani et al., 2013; Spotts et al., 1998). Other fungi

commonly causing fruit rot on sweet cherries are; *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium* spp., *Colletotrichum* spp. and *Monilinia* spp. (Barry et al., 2015; Borve and Stensvand, 2015; Tarbath et al., 2014; Thomidis and Exadaktylou, 2012). *Mucor piriformis* was also reported to cause cherry fruit decay by some researchers (Borve et al., 2000; Lopez et al., 2016; Michailides and Spotts, 1990).

Number of studies on the determination of fruit rot agents of sweet cherries is quite limited in Türkiye. In a study on the determination of the effects of modified atmosphere packages on storage and shelf life of sweet cherries, it was found that the main fungal agents causing storage rot were *B. cinerea, Rhizopus stolonifer, Monilinia* spp. and *A. alternata*, while *Penicillium expansum* and *Cladosporium* species were isolated in lower rates (Şen et al., 2016). In another study performed in the Aagean Region during 2015-2017, it was determined that *Monilinia* species (*M. laxa* and *M. fructicola*) caused fruit rot in sweet cherry orchards at rates varying between 5.3 and 10.8%, with increasing rates after harvest (Morca et al., 2022).

Fruit rot is the main problem both in the sweet cherry orchards and in the storages in Isparta province. Since it is well known that the latent pathogens on sweet cherry fruits in the orchards may cause infections after harvest, it is thought that the research on the determination of the fruit rot agents should better be comprised the fruit samples both from the orchards and storages. Thus, determination of the fungal agents causing fruit rot in the sweet cherry orchards and storages in Isparta province was aimed in the present study.

2. Materials and Methods

2.1. Collection of the samples

Surveys were performed in the period of June 19 and July 7, 2023, just before harvest and fruit samples were collected from 76 sweet cherry orchards randomly selected in 13 districts of Isparta province, according to the numbers of sweet cherry trees of the districts (TÜİK, 2021). Number of surveyed orchards increased with the increasing tree numbers, with the districts with less than 25 000 trees only 2 orchards were investigated, while 10 orchards were selected in the districts with tree numbers higher than 200 000 (Table 1). In the orchards, at least 100 representative trees were examined during the period near harvest and fruits with rot symptoms were taken (Figure 1). In addition, to determine the latent infections, healthy-looking fruits from each orchard were also collected. Fruit samples were also taken from 3 cold storages in Eğirdir District and one sweet cherry processing center in Uluborlu District, after harvest. Similarly

Table 1. Sweet cherry tree numbers of the districts of Isparta province (TÜİK, 2021) and number of surveyed orchards.

Districts	Total tree numbers	Number of surveyed orchards
Aksu	2 370	2
Atabey	504 060	10
Eğirdir	41 570	4
Gelendost	10 000	2
Gönen	66 090	6
Keçiborlu	60 110	6
Merkez	336 800	10
Senirkent	419 000	10
Sütçüler	21 030	2
Uluborlu	552 849	10
Yalvaç	49 280	4
Yenişarbademli	6 790	2
Şarkikaraağaç	101 500	8
Total	2 171 449	76

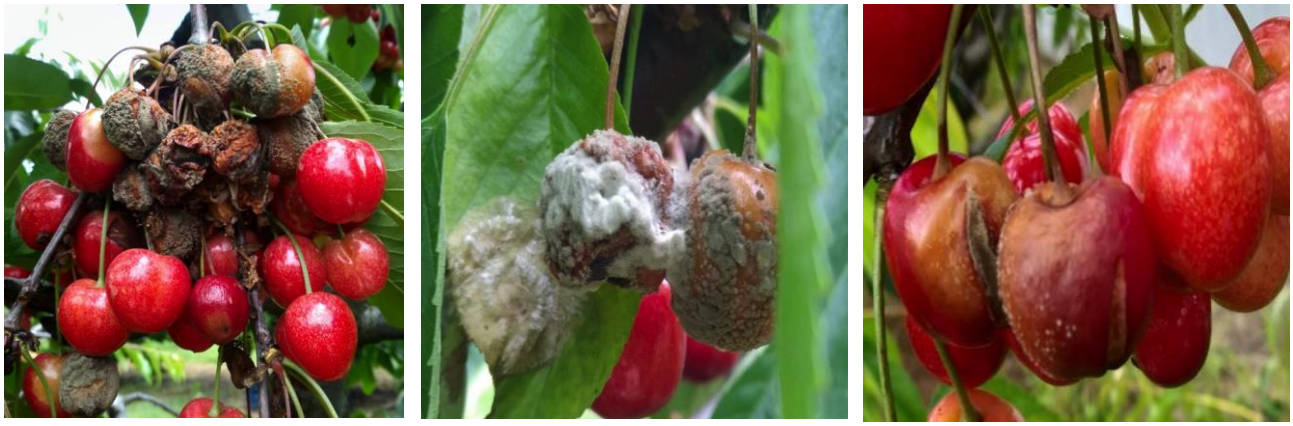

Figure 1. Some rotten fruit samples in the sweet cherry orchards in Isparta province.

rotten and healthy fruit samples were collected from the randomly selected boxes in the storages. Samples were separately packed with necessary labeling and brought to the laboratory in an ice container. They were kept at 4°C and isolations were performed as soon as possible.

2.2. Isolation and identification of the pathogens

Sections of about 3 mm^2, taken from the fruit samples including both healthy and decayed tissue were surface disinfected in 70% ethyl alcohol for 30 seconds. They were washed three times in sterile distilled water, blotted dry and transferred to Potato dextrose agar (PDA: Merck) or Malt extract agar (MEA: Merck) media. Fungal colonies were purified and single spore isolates were kept in two parallel agar slants at 4°C and in 20% glycerol at -80°C for later use in microscopic examinations and pathogenicity test (Kim et al., 2007; Lopez et al., 2016). Identifications of the fungi were done according to their cultural and microscopic features using related literature (Booth, 1971; Ellis, 1971; Houbraken et al., 2011; Kubicek and Harman, 2002; Morca et al., 2022; Pitt and Hocking, 2009; 2016; Samson et al., 1995).

2.3. Pathogenicity tests

To determine the pathogenicity of the fungal isolates, healthy fruits (genotype 219) picked before harvest from the sweet cherry orchard of Fruit Research Institute in Eğirdir District were used. For the pathogenicity test, randomly selected fungal isolates from each identified genus or species were used. Fruits were sterilized in 2% NaOCl solution for 30 seconds, washed two times in sterile distilled water and blotted dry under aseptic conditions. Fungi in stock cultures were activated on PDA and spore suspensions with 10^6 conidia mL^{-1} concentrations were prepared. Three wounds were made with a sterile needle in a small area (3×3 mm) on the dried fruits and inoculated with 10 µl spore suspension of the selected isolate. The same amount of sterile distilled water applied fruits served as controls. Inoculated fruits were kept in sterilized plastic boxes with sterile blotter papers humidified with sterile distilled water. Five fruits were used for each box and 3 replicate boxes were used for each isolate. Boxes were kept at 22°C for seven days and virulence of the isolates were determined by measuring the lesion diameters on the inoculated fruits. Reisolations were made to confirm the similarity with the original isolate (Lopez et al., 2016; Peng et al., 2022). Data obtained by the pathogenicity test were subjected to analyses of variance and lesion diameter means were compared by Tukey's multiple range test ($p \leq 0.05$).

3. Results and Discussion

3.1. Fungi isolated from the sweet cherry orchards in Isparta province

As a result of the isolations made from the rotten sweet cherry fruits taken from 76 orchards in Isparta province, 9 fungi were identified at the species level. In addition, nine fungi, two of them belonging to *Aspergillus*, 5 of them to *Penicillium* and two to *Trichoderma* genera, were also identified according to the related literature (Kubicek and Harman, 2002; Samson et al., 1995;). *Alternaria alternata* was the fungus with the highest prevalence and isolation rates, followed by *M. laxa* and *B. cinerea* (Table 2). Likewise, these fungi were found to be the pathogens causing highest losses in Bulgaria during 1999-2003 (Borovinova, 2004). In addition to these fungi, *R. stolonifer* and *Cladosporium* spp. were isolated from the rotten fruits in Italy (Romanazzi et al., 2008). Similar with our findings, *C. cladosporioides*, *Fusarium oxysporum*, *Geotrichum candidum* (Serradilla et al., 2021), *Sclerotinia sclerotiorum* (Förster and Adaskaveg, 2000; Ruan et al., 2023), *Stemphylium botryosum* (Dugan and Roberts, 1994), *Penicillium* (Borve and Stensvand, 2015; Romanazzi et al., 2008), *Trichoderma* (Serradilla et al., 2021) and *Aspergillus* species (Ali et al., 2024; Thomidis and Exadaktylou, 2012) were isolated from the sweet

Table 2. The prevalence and isolation rates of the fungi isolated from the rotten and healthy fruits in the sweet cherry orchards in Isparta province.

Fungi	Isolations from the rotten fruits		Isolations from the healthy fruits	
	Prevalence rate (%)	Isolation rate (%)	Prevalence rate (%)	Isolation rate (%)
Alternaria alternata	86.84	45.71	18.42	38.77
Aspergillus spp.	5.26	1.94	1.32	2.04
Botrytis cinerea	50.00	16.90	15.79	28.57
Cladosporium cladosporioides	2.63	0.83	1.32	2.04
Fusarium lateritium	1.32	0.28	-	-
Fusarium oxysporum	2.63	0.55	2.63	4.08
Geotrichum candidum	1.32	0.28	-	-
Monilinia laxa	60.53	23.82	13.16	24.48
Penicillium spp.	27.63	7.48	-	-
Sclerotinia sclerotiorum	2.63	0.55	-	-
Stemphylium botryosum	1.32	0.28	-	-
Trichoderma spp.	2.63	1.11	-	-
Trichothecium roseum	1.32	0.28	-	-

Table 3. The prevalence and isolation rates of the fungi isolated from the rotten and healthy sweet cherry fruits in the cold storages in Isparta province.

Fungi	Isolations from the rotten fruits		Isolations from the healthy fruits	
	Prevalence rate (%)	Isolation rate (%)	Prevalence rate (%)	Isolation rate (%)
Alternaria alternata	100	51.83	50	100
Botrytis cinerea	75	8.43	-	-
Fusarium oxysporum	25	2.41	-	-
Monilinia laxa	50	3.62	-	-
Penicillium spp.	100	18.07	-	-
Rhizopus stolonifer	25	2.41	-	-
Stemphylium botryosum	25	1.21	-	-
Trichoderma spp.	75	12.05	-	-

cherry fruits in different countries. However, *Fusarium lateritium* and *Trichothecium roseum* were mentioned for the first time as sweet cherry fruit rot pathogens in this study, while *Aspergillus* spp., *F. oxysporum*, *G. candidum*, *S. sclerotiorum*, *S. botryosum* and *Trichoderma* spp. were first reports for Türkiye.

Alternaria alternata, *M. laxa* and *B. cinerea* had also highest prevalence and isolation rates from the healthy fruit samples taken from the orchards. *Aspergillus* sp., *C. cladosporioides* and *F. oxysporum* were the other fungi causing latent infections (Table 2). Similarly, *A. alternata* and *C. cladosporioides* were among the fungi isolated from symptomless sweet cherry fruits (cv. Bing) and found pathogenic (Dugan and Roberts, 1994). In another study made to determine the latent infections on the raw and mature sweet cherry fruits using specific primers, *M. laxa* and *B. cinerea* were determined (Förster and Adaskaveg, 2000). Similar research made in Spain showed that *A. alternata* and *C. cladosporioides* were among the fungi isolated from the fruit surfaces after harvest (Venturini et al., 2002). Additionally, Tarbath et al. (2014) stated that *B. cinerea* was found on 50% of the healthy fruits, while it was isolated from 94% of the rotten fruits in Tasmania.

3.2. Fungi isolated from the sweet cherry fruits taken from the cold storages in Isparta province

With the isolations made from the rotten fruit samples taken from the three cold storages in Eğirdir District and one sweet cherry processing center in Uluborlu District, 83 fungal isolates were obtained. *Alternaria alternata* had the highest isolation rate and was determined in all four samples taken from different storages (Table 3). *Botrytis cinerea*, *F. oxysporum*, *M. laxa*, *Penicillium* spp., *R. stolonifer*, *S. botryosum* and *Trichoderma* spp. were the other fungi isolated in lower rates, from the rotten sweet cherry fruits from the cold storages. It was determined that only *A. alternata* caused latent infections on the fruits from the two cold storages in Eğirdir District, while no latent infection was found on the healthy fruit samples taken from the sweet cherry processing center in Uluborlu District. Among these agents, *B. cinerea*, *M. laxa*, *Penicillium* spp. and *R. stolonifer* were previously isolated from the fruits in cold storages in Türkiye and other countries (Akbudak et al., 2008; Borve ve Stensvand, 2015; Romanazzi et al., 2008; Şen et al., 2016). *Stemphylium botryosum*, *F. oxysporum* and *Trichoderma* spp. were reported among the fungi colonizing the sweet cherry fruits during the period between petal fall and harvest in eastern Washington, and thought to be the possible agents of storage rot of sweet cherries (Dugan and Roberts, 1994).

3.3. Virulence of the fungal isolates

In the pathogenicity test performed to determine the virulence of the fungi isolated from the rotten and healthy sweet cherry fruits from the orchards and cold storages in Isparta province, statistically significant differences were found among the selected isolates. *Penicillium* sp., *R. stolonifer* and *B. cinerea* caused the largest lesions on the fruits, while the lowest level of virulence was obtained by *F. lateritium* (Table 4). *Penicillium* sp. caused browning starting from the inoculation area, then formed white mycelia and green spores on the lesion and expanded throughout the fruit. *Rhizopus stolonifer* grew all over the fruit forming aerial dark grey mycelia and black sporangia, while *B. cinerea* formed grey mycelia on sunken lesions (Figure 2). *Fusarium lateritium* was isolated from cold-stored Chinese cherry fruits and infected healthy fruits in the pathogenicity test (Wang et al., 2021). It was also among the *Fusarium* species causing leaf spots on sweet cherries in China (Zhou et al., 2022).

4. Conclusion

Sweet cherry is among the fruit species grown and exported in Isparta province, where fruit production is intensive and cold storage facilities are common. Pre and post harvest fruit rot is an important disease causing losses in sweet cherry production. Since sweet cherry fruit has a short storage life depending on its rapid physiological aging and is sensitive to injuries and bruising, it is easier for rot pathogens to develop. In this study, it was aimed to determine the fungal agents causing rot on sweet cherry fruits, to form a basis for studies related to the control of the disease. As a result of

Table 4. Mean lesion diameters on the sweet cherry fruits caused by the fungi isolated from the rotten and healthy sweet cherry fruits in the orchards and cold storages in Isparta province.

Fungi	Mean lesion diameter (mm)
Alternaria alternata	13.80 cf*
Aspergillus sp.	10.33 eg
Botrytis cinerea	22.87 ab
Cladosporium cladosporioides	10.80 dg
Fusarium lateritium	5.33 gh
Fusarium oxysporum	15.73 be
Geotrichum candidum	19.40 ac
Monilinia laxa	15.00 be
Penicillium sp.	27.33 a
Rhizopus stolonifer	26.87 a
Sclerotinia sclerotiorum	13.67 cf
Stemphylium botryosum	6.00 fh
Trichoderma sp.	14.13 ce
Trichothecium roseum	18.47 bd
Control	0.00 h

* Means in the column shown by the same letters are statistically not different from each other according to Tukey's multiple range test ($P \leq 0.05$).

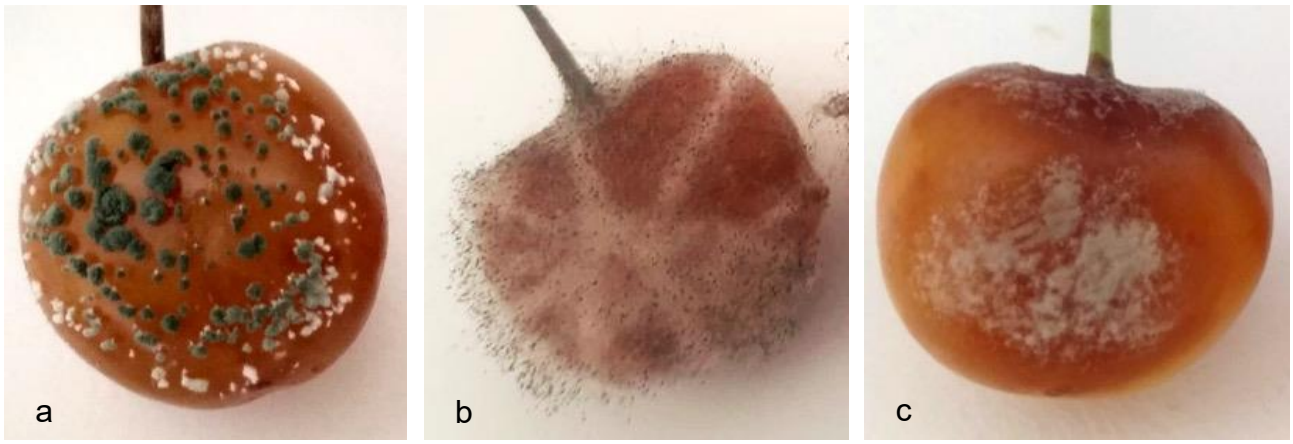

Figure 2. Lesions caused by *Penicillium* sp. (a), *Rhizopus stolonifer* (b) and *Botrytis cinerea* (c) on sweet cherry fruits in the pathogenicity test.

the isolations made from the rotten and symptomless fruit samples taken from the orchards in the province, *Alternaria alternata*, *M. laxa* and *B. cinerea* were the most common agents isolated both from the rotten and symptomless fruits. *A. alternata* was also isolated from the rotten and healthy fruits in the cold storages, while other fungi, mainly *Penicillium* species, were isolated only from the rotten fruits. *Penicillium* species, *R. stolonifer* and *B. cinerea* were found as the most virulent fungi in the pathogenicity test. Some of the pathogens found in the present study were previously reported to cause sweet cherry fruit rot in Türkiye. However *Aspergillus* spp. *F. oxysporum*, *F. lateritium*, *G. candidum*, *S. sclerotiorum*, *S. botryosum*, *Trichoderma* spp. and *T. roseum* were new findings. In addition no information could be found on the isolation and pathogenicity of *F. lateritium* and *T. roseum* on sweet cherry fruits. These findings have shown once again the importance of pre-harvest fruit rot disease in sweet cherry production. Besides, it was found that latent infections of fungi before harvest can also cause rotting in storage conditions resulting serious economic losses. Within the framework of sustainability from orchard to table, it is important to adopt and disseminate alternative control methods instead of commonly used fungicides. Fungi isolated in this study will provide an important material for future studies on alternative control methods.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Effect of Some Colloidal Coating Treatments on the Shelf Life and Quality Characteristics of Strawberry Fruits

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Abstract

Short shelf life after harvest is one of the biggest problems for fresh strawberries. In this study, the effects of next generation edible colloidal coating agents formed from combinations of chitosan, selenium and thyme essential oil obtained by ionic gelation method on the post-harvest quality criteria of strawberries were investigated. The quality parameters (weight loss, decay rate, respiration rate, fruit color, firmness, total soluble solids: TSS, titratable acid: TA, ascorbic acid content, total phenolic content, antioxidant capacity and total anthocyanin content) of fruits of Albion strawberry variety kept at 21°C (room temperature conditions, relative humidity) were measured on days on 0th, 3rd, 5th, 7th and 10th. All coating treatments showed positive effects on quality parameters during shelf life. Edible colloidal coating treatments showed positive effects on the reduction of weight loss, reduction of decay, respiration rate, color values, fruit firmness, titratable acid content, ascorbic acid content, total phenolic content, total anthocyanin content and total antioxidant capacity values during shelf life compared to the control group. Among all the treatments, the most effective treatments in preserving quality properties were Chitosan + Selenium and Chitosan + Selenium + Thyme essential oil treatments. The research of results will provide information on the development of edible colloidal coating materials in related future studies planned on similar topics.

1. Introduction

Strawberry is one of the most consumed fruit species in the world. Strawberry is an important fruit species in human nutrition due to its high vitamin, mineral and nutrient content. Strawberries are consumed intensively both fresh and processed due to their superior sensory properties such as colour, aroma and flavor (Yang and Kim, 2023).

Strawberries spoil quickly due to their high metabolic activity and this situation creates serious problems in the marketing of strawberries (Barikloo and Ahmadi, 2018). Post-harvest physical damage and spoilage caused by microorganisms cause losses in fruit firmness, quality and color of strawberries. Therefore, it is important to determine appropriate methods to preserve the post-harvest quality and nutritional value of strawberries (Fernandez-Leon et al., 2013).

Packaging of foods has an important place in the global economy. However, synthetic plastics, which have superior mechanical and protective properties used in packaging, are the biggest environmental threat nowadays because they are not biodegradable (John et al., 2023). Nowadays, environmentally friendly technologies are important

to reduce post-harvest losses and extend shelf life of agricultural products.

The edible material coating method has gained popularity in recent years in order to extend the storage life and preserve the quality of fruits. Edible coatings provide an ecological advantage compared to plastic-based food packaging because they are biodegradable and environmentally friendly (Şen and Güner, 2023). Edible coatings are packaging materials that preserve food quality, extend shelf life of foods, have antimicrobial activity and are consumed with foods (Jeevahan and Chandrasekaran, 2019). Edible coatings consisting of proteins and polysaccharides are biodegradable substances composed individually or in combination of essential oils, extracts, polyphenols and antioxidant agents (Pham et al., 2023).

Researchers have developed edible colloidal coatings using various components from nanoparticles to microparticles to extend shelf life of foods, maintain freshness, and prevent decay by improving antimicrobial activity (Feyzioglu and Tornuk, 2016; Vera et al., 2018). Colloids (colloidal solutions or colloidal systems) are a mixture dissolved in another substance of microscopic in size insoluble particles of one substance. Colloids, with sizes ranging from 1 to 1000 nm, can be in solid, liquid and gas form. Most colloidal systems are nano-sized or composed of nano-sized particles through colloidal interactions. Therefore, nanoparticles are important (John et al., 2023). The particle size limit value of nanoparticles is known as less than 100 nm. However, large sized particles have been accepted as nano in various studies. The dimensions of chitosan-based nanoparticles were reported as 135–237 nm by Feyzioglu and Tornuk (2016) and 532–716 nm by Keawchaoon and Yoksan (2011). The concentration of the materials and the solvent used in nanoparticles and the pH value of compound are the factors which determine size of particles (Feyzioglu and Tornuk, 2016).

Colloids are effective in providing a large interaction surface area and as preservative, maintain freshness of products from production to consumption and extending shelf life (Kontogeorgis and Kill, 2016). Colloidal systems are an ideal system for the controlled delivery of active ingredients (Moreno and Peinado, 2012). In addition, colloidal systems are low in cost due to need a small amount of active substance (John et al., 2023).

Biopolymers such as enzymes, polysaccharides and proteins are compounds that generally create macromolecular colloids and are used as coatings on packaging surfaces (John et al., 2023). Different biopolymers are used for colloidal coatings. Chitosan is a polysaccharide-based biopolymer commonly used in colloidal coatings (Moustafa et al., 2019). Chitosan is the most abundant polysaccharide in nature after cellulose. It is important among biopolymers due to its antibacterial and antifungal properties (Aider, 2010). The mixture of biologically active compounds such as chitosan and plant essential oils has significant potential in controlling post-harvest decay of fresh fruits (Perdones et al., 2016). Adding plant essential oils and extracts to edible colloidal coatings by increasing the antimicrobial effect, ensuring food safety and food quality against microbes (Li et al., 2024). By adding plant essential oils to colloidal coatings controls diseases that may occur in the fruit by providing active molecules on the fruit surfaces (Aloui et al., 2014). Edible colloidal coatings created by combining chitosan and plant essential oils have strong antimicrobial and antifungal activity that is highly degradable and controls decay in fruits (Kong et al., 2010; Zhang et al., 2011). Especially essential oils, which have antimicrobial effects, are used extensively to prevent microbial spoilage. Plant-derived essential oils and components such as thyme oil, citral, lemon grass oil, cinnamon aldehyde, cinnamon oil can be used in antimicrobial edible coatings (Rojas-Graü et al., 2007).

The main source of Selenium is foods of plant and animal origin (Roman et al., 2014). In recent studies, Selenium nanoparticles, which have anticancer, antimicrobial and antioxidant properties, are used in packaging (Skalickova et al., 2017). Oxidation of foods is prevented by adding selenium to active packaging as an antioxidant source (Vera et al., 2018). One of the best solutions to obtain active food packaging is addition of selenium nanoparticles into the polymeric layer (Palomo-Siguero and Madrid, 2017).

The aim of the study is develop an edible colloidal coating with antimicrobial properties to prevent post-harvest spoilage of short shelf-life strawberries and determine its practical applicability. Various combinations have been obtained by combining chitosan, selenium synthesized from black tea leaf extracts, and thyme oil. The effects of the edible colloidal coatings obtained in the study on the post-harvest quality parameters of Albion strawberry variety fruits kept at room temperature conditions (21°C) were revealed.

2. Material and Methods

In the study, the preparation of chitosan microparticles was carried out according to the ionic gelation method modified by Ilk et al. (2017). Selenium microparticles were obtained by green synthesis method using black tea leaf extract (Mareedu et al., 2021). In the study, four coating solutions were prepared (Chitosan colloidal solution: CsCS, Chitosan colloidal solution + thyme essential oil: CsCS+Oil, Chitosan colloidal solution + Selenium colloidal solution: CsCS+SeCS, and Chitosan colloidal solution + Selenium colloidal solution + thyme essential oil: CsCS+SeCS+Oil) and applied to the fruits of Albion strawberry

species. Strawberry fruits were collected early in the morning and brought to the laboratory quickly. Composites were prepared at 1% active substance rate and essential oil rate was adjusted as 1000 ppm. Fruit samples were kept at room conditions (21°C). The study was planned with three replications and 150 g sample in each replication. Various quality parameters were analyzed on 0th, 3rd, 5th, 7th and 10th days to during shelf life of the samples.

Within the scope of the study, weight loss (%), decay rate (%), respiration rate (mg CO_2 kg^{-1} h^{-1}), fruit color, fruit firmness (N), total soluble solids (TSS) content (%), titratable acid (TA) content (%), vitamin C content (mg 100 g^{-1} AsAE), total phenol content (mg 100 g^{-1} gallic acid), total antioxidant capacity (% inhibition) and total anthocyanin content (µg cy-3-glu g^{-1}) were determined during shelf life of fruits. Respiration rate was determined by measuring the amount of CO_2 spreaded by the fruits to the external environment with a digital carbon dioxide analyzer (Öztürk, 2020) and fruit color was determined with a colorimeter (Minolta, model CR-400, Tokyo, Japan) in terms of CIE L*, chroma and hue (McGuire, 1992). Among the biochemical properties, vitamin C content was determined using the method used by Özdemir and Dündar (2006); total phenol amount was determined using the Folin-Ciocalteu's chemical defined by Singleton and Rossi (1965); total antioxidant capacity was determined using the DPPH method specified by Brand-Williams et al. (1995); and total anthocyanin amount was determined using the pH differential method (Giusti et al., 1999).

The research was carried out according to the random plots experimental design with 3 replicates. Analysis of variance of data was performed by using JMP Pro 17.0 statistical package program and statistically significant parameter values according to the results of analysis of variance were compared with LSD test.

3. Results and Discussion

In the experiment, the effects of the treatment×shelf life time interaction on the weight loss, decay rate and color values (L*, chroma and hue) of strawberries were found statistically significant, while the treatment and shelf life time had statistically significant effects on the respiration rate separately (Table 1).

Weight loss of strawberries increased in parallel with the shelf life in all treatments. The lowest weight loss rate occurred in CsCS+Oil treatment on the 3rd day, CsCS+SeCS+Oil treatment on the 5th day, and CsCS+SeCS treatment on the 7th and 10th days. Our study findings confirm that edible colloidal coatings can significantly reduce weight loss by creating a semi-permeable barrier that reduces transpiration and moisture loss (Jafarzadeh et al., 2018). CsCS+SeCS treatment was the most effective method to reduce weight loss of strawberries. Due to the high synergistic effects of CsCs and SeCS compounds (Dorazilova et al., 2020) the retention of moisture in plant tissues is increased by using these compounds in coatings.

According to the mean of the treatments, the lowest respiration rate occurred in CsCS+SeCS and CsCS+SeCS+Oil treatments. Respiration rate, which is an indicator of the metabolic activities of fresh fruits, is an important factor affecting shelf life. In general, as the respiration rate of fruits and vegetables increases, their storability and shelf life decrease at the same rate (Jafarzadeh et al., 2021). Coatings are known to change gas exchange characteristics of fresh produce, effectively reducing respiration rates and delaying ripening (Iderawumi and Yusuff, 2021).

In strawberry fruits, CsCS+SeCS and CsCS+SeCS+Oil treatments showed lower decay rate than the other treatments starting from the 5th day of shelf life. The 5th day, when the decay rate in fruits is at least 25%, is recommended as the shelf life. These treatments stand out as the most effective methods in delaying decay by preserving the freshness during shelf life of strawberries. The coatings have antimicrobial properties that inhibit microbial contamination and delay decay in fruit and vegetables (Wang et al., 2020). Our study findings may be attributed to the physical barrier formation of coating agents as well as the antimicrobial effect of thyme essential oil (Mith et al., 2014). Chitosan used in coatings acts as a barrier preventing microbial development in fruits (Butler et al., 1996). Many studies have reported that chitosan coatings applied to strawberry fruits reduce decay (Gol et al., 2013; Sangsuwan et al., 2016).

The L* value, which expresses the brightness of fruit color, showed tendency to decrease as the shelf life period increased. Starting from the 5th day of shelf life, CsCS+SeCS+Oil treatment showed higher L* value than other treatments. CsCS+SeCS+Oil treatment was the most effective method to preserving the color of strawberries during shelf life. Chroma and hue values, which are color indicators, showed tendency to decrease as the shelf life period increased. Although there were differences between treatments on these color parameters, in general, treatments preserved chroma and hue values of fruit better compared to control and helped to keep color tone constant during the shelf life. Colloidal coatings, which act as a semi-permeable barrier, control microbial damage and preserve color and texture (Bourtoom, 2008). Coatings slow down the decrease in L*, chroma and hue values of strawberries during shelf life (Sangsuwan et al., 2016; Vargas et al., 2006).

While treatment and shelf life period had a statistically significant effect on fruit firmness separately, this value showed a tendency to decrease during the shelf life. According to the mean of treatments, the highest fruit firmness was

Table 1. Weight loss, respiratory rate, decay rate, L* value, chroma value and hue value of control and coated strawberry during 10 days of 21°C storage.

Coating	Control	CsCS	CsCS+Oil	CsCS+SeCS	CsCS+ SeCS +Oil	Mean
Weight loss (%)						
0	0.00 l	0.00 l	0.00 l	0.00 l	0.00 l	0.00
3	7.35 hı	4.84 j	3.16 k	3.27 k	3.68 k	4.46
5	10.13 c	6.99 ı	6.77 ı	5.73 j	5.19 j	6.96
7	12.19 b	8.10 gh	8.64 eg	8.32 fh	8.39 fg	9.13
10	13.32 a	9.45 ce	9.86 cd	9.00 dg	9.12 cf	10.15
Mean	8.60	5.88	5.69	5.26	5.28	
Respiratory rate (mg CO_2 kg^{-1} h^{-1})						
0	148.90	139.10	133.30	118.40	117.70	131.47 d
3	151.90	141.80	136.00	120.70	120.10	134.10 c
5	154.10	143.80	139.20	123.90	122.80	136.75 b
7	156.40	146.10	140.10	124.30	123.70	138.12 ab
10	158.00	147.60	141.50	125.60	124.90	139.50 a
Mean	153.86 a	143.67 b	138.01 c	122.58 d	121.81 d	
Decay rate (%)						
0	0.00 n	0.00 n	0.00 n	0.00 n	0.00 n	0.00
3	25.00 j	18.75 k	18.75 k	6.25 m	12.50 k	16.25
5	50.00 g	31.25 ı	37.50 h	25.00 j	25.00 j	33.75
7	85.40 b	75.00 d	75.00 d	65.30 e	62.50 f	72.64
10	93.75 a	81.25 c	81.25 c	75.00 d	75.00 d	81.25
Mean	50.83	41.25	42.50	34.31	35.00	
L* value						
0	33.79 ab	33.42 ab	31.21 de	34.07 a	34.15 a	33.33
3	30.66 df	30.14 eg	30.80 df	32.73 bc	33.14 ab	31.49
5	30.76 df	29.27 gı	28.71 hj	30.88 df	31.76 cd	30.28
7	27.93 j	27.98 ıj	28.11 ıj	29.60 fh	29.90 eh	28.70
10	25.34 l	26.55 kl	26.12 l	27.60 jk	27.80 jk	26.68
Mean	29.70	29.47	28.99	30.98	31.35	
Chroma value						
0	33.28 ab	34.01 a	33.41 a	33.71 a	34.07 a	33.70
3	31.28 de	32.29 bc	31.90 cd	31.23 de	32.32 bc	31.80
5	28.37 hj	29.19 gh	30.50 ef	30.61 ef	30.61 ef	29.85
7	27.32 k	28.56 hı	29.66 fg	29.62 fg	28.02 ık	28.64
10	26.17 l	27.87 ık	27.39 jk	28.09 ık	27.99 ık	27.50
Mean	29.28	30.38	30.57	30.65	30.60	
Hue value						
0	35.85 bc	36.68 ab	36.18 ab	36.28 ab	36.84 a	36.37
3	33.85 ef	34.96 cd	34.67 de	33.80 ef	35.09 cd	34.47
5	30.94 km	31.86 ık	33.27 fg	33.18 fh	33.38 fg	32.52
7	29.89 n	31.23 jl	32.43 gı	32.19 hj	30.79 ln	31.31
10	28.74 o	30.54 ln	30.16 mn	30.66 ln	30.76 ln	30.17
Mean	31.85	33.05	33.34	33.22	33.37	

Chitosan colloidal solution: CsCS, Chitosan colloidal solution + thyme essential oil: CsCS+Oil, Chitosan colloidal solution + Selenium colloidal solution: CsCS+SeCS, and Chitosan colloidal solution + Selenium colloidal solution + thyme essential oil: CsCS+SeCS+Oil.
* Mean differences indicated by different letters in the same column are significant ($p < 0.05$). If the interactions were not statistically significant, the differences between the means were compared statistically.

determined in CsCS+SeCS and CsCS+SeCS+Oil treatments, while according to during the shelf life, the highest fruit firmness was determined on day 0th (Table 2). Fruit firmness is a main quality parameter to preserve the freshness and commercial value of strawberries. Edible coatings reduce metabolic activity by reducing oxygen uptake of strawberry fruits, and in this case, slow down the ripening process, that is, softening. Our results are consistent with previous studies on strawberries (Barikloo and Ahmadi, 2018; Sogvar et al., 2016).

The effect of the treatment*shelf life period interaction on the amount of TSS was found statistically significant, and in general the amount of TSS increased during the shelf life. The lowest TSS amount was detected in CsCS+SeCS treatment on the 10th day of shelf life (Table 2). The amount of TSS expressed as a percentage of Brix represents the percentage of dissolved solids and is an indicator of the sweetness and flavor profile in products. While decreases in the amount of TSS may occur as a result of the use of sugars in respiration during the shelf life of fruits, it is more likely that this parameter will increase due to the concentration effect of water losses (Velickova et al., 2013). In addition, coating agents delay synthesis and use of metabolites by reducing the respiration rate in fruits, due to reducing the amount of TSS (Xing et al., 2020).

The effect of shelf life period on TA content was found statistically significant. In the study, it was observed that TA content in strawberry fruits decreased during shelf life. In general, CsCS and CsCS+SeCS treatments were successful in

Table 2. Fruit firmness, total soluble solids, titratable acid, ascorbic acid, total phenols, total antioxidant and total anthocyanin of control and coated strawberry during 10 days of 21°C storage.

Coating	Control	CsCS	CsCS+Oil	CsCS+SeCS	CsCS+ SeCS +Oil	Mean
Fruit firmness (N)						
0	6.77	6.81	6.79	7.50	7.45	7.06 a
3	4.64	4.77	4.77	5.28	5.23	4.94 b
5	3.84	4.00	4.01	4.44	4.39	4.13 c
7	3.04	3.23	3.25	3.61	3.56	3.34 d
10	1.65	1.85	1.83	2.46	2.43	2.05 e
Mean	3.99 c	4.13 bc	4.13 bc	4.66 a	4.61 ab	
Total soluble solids (%)						
0	9.77 ı	9.83 gı	9.77 ı	9.80 hı	9.83 g-ı	9.80
3	9.93 fg	9.93 fg	9.90 fh	9.83 gı	9.93 fg	9.91
5	10.00 ef	9.93 fg	9.93 fg	9.93 fg	9.93 fg	9.95
7	10.23 bc	10.10 de	10.13 cd	10.07 de	10.10 de	10.13
10	10.63 a	10.23 bc	10.27 b	10.13 cd	10.33 b	10.32
Mean	10.11	10.01	10.00	9.95	10.03	
Titratable acid (%)						
0	0.78	0.80	0.78	0.80	0.79	0.79 a
3	0.76	0.78	0.75	0.80	0.77	0.77 ab
5	0.70	0.77	0.71	0.78	0.76	0.75 ac
7	0.68	0.72	0.70	0.75	0.72	0.72 bc
10	0.65	0.71	0.67	0.69	0.69	0.68 c
Mean	0.72	0.76	0.72	0.76	0.75	
Vitamin C (mg 100 g^{-1} AsAE)						
0	51.02 bd	52.01 ab	51.51 bc	53.19 a	52.47 ab	52.04
3	50.08 ce	49.36	49.36 dg	48.46 eh	50.20 cd	49.49
5	47.80 -h	49.60 de	47.09 h	47.75 gh	49.45 df	48.34
7	41.79 lm	44.12 ı	43.86 ıj	43.75 ık	43.54 ık	43.41
10	37.83 n	41.11 lm	40.66 m	42.44 jl	42.14 km	40.84
Mean	45.70	47.24	46.50	47.12	47.56	
Total phenol content (mg 100 g^{-1} gallic acid)						
0	335.95	338.91	335.21	338.54	337.06	337.13 e
3	342.60	342.73	341.99	340.51	340.32	341.63 d
5	360.88	358.66	358.49	352.83	354.95	357.16 c
7	384.16	381.25	376.62	373.64	374.40	378.02 b
10	401.80	399.21	393.84	391.86	393.66	396.08 a
Mean	365.08 a	364.15 a	361.23 b	359.48 b	360.08 b	
Total antioxidant capacity (% inhibition)						
0	68.79	69.19	70.21	71.11	70.40	69.94 a
3	63.72	64.79	66.71	66.87	67.17	65.85 b
5	61.11	62.13	63.77	64.13	64.85	63.20 c
7	58.11	59.09	60.38	60.98	62.18	60.15 d
10	56.31	57.26	58.35	59.09	60.16	58.23 e
Mean	61.61 d	62.49 c	63.88 b	64.44 ab	64.95 a	
Total anthocyanin content (µg cy-3-glu g^{-1})						
0	248.53	249.93	248.17	249.76	249.04	249.09 d
3	251.70	251.77	251.43	250.71	250.62	251.25 d
5	260.52	259.44	259.35	256.65	257.67	258.73 c
7	271.74	270.31	268.08	266.66	267.04	268.77 b
10	280.22	278.97	276.40	275.42	276.30	277.46 a
Mean	262.54	262.09	260.69	259.84	260.13	

Chitosan colloidal solution: CsCS, Chitosan colloidal solution + thyme essential oil: CsCS+Oil, Chitosan colloidal solution + Selenium colloidal solution: CsCS+SeCS, and Chitosan colloidal solution + Selenium colloidal solution + thyme essential oil: CsCS+SeCS+Oil.
* Mean differences indicated by different letters in the same column are significant ($p < 0.05$). If the interactions were not statistically significant, the differences between the means were compared statistically.

preserving TA content, while the lowest values were determined in the control group (Table 2). The reason is that the coatings may be slow down the rate of degradation of organic acids in the product by inhibiting enzyme activity associated with organic acid metabolism. It is stated that the changes in TA content during storage period in fresh fruits are related to respiration, organic acids are consumed by respiration and as a result, acid content decreases (Rivera-Pastrana et al., 2007). It is also known that post-harvest coating agent treatment slows down the metabolic activity in products, and as a result, delays the synthesis and degradation mechanisms (No et al., 2007). Similar findings have been reported in previous studies (Candir et al., 2018; Çınar and Sabır, 2021; Song et al., 2016).

The treatment*shelf life period interaction had a statistically significant effect on vitamin C content. The vitamin C content of strawberries decreased during shelf life. A significant decrease in vitamin C content was observed on the 7th and 10th days

during shelf life. However, CsCS+SeCS treatment has been successful in minimizing vitamin C loss (Table 2). Vitamin C is easily degraded by heat, light and enzymes during storage (Frias and Oliveira, 2001), and the resulting water losses increase oxidation, which can lead to loss of vitamin C content (Nunes et al., 1998). The addition of chitosan to edible coatings can delay vitamin C oxidation of fruits by reducing O_2 diffusion and slowing the respiration rate (Amal et al., 2010). In addition, preservation of vitamin C content by increasing antioxidant activity of selenium colloidal particles can be attributed to their ability to form a light barrier. It is known that edible coating treatments slow down the decrease in vitamin C content of fruits (Emamifar and Mohammadizadeh, 2015; Gol et al., 2013).

Treatment and shelf life period had a statistically significant effects on total phenolic content. According to the study findings, it was observed that total phenolic content increased during shelf life. Total phenolic content was determined the highest in the control group according to mean values. According to the mean of the treatments, the lowest total phenolic content was determined in CsCS+SeCS, CsCS+SeCS+Oil and CsCS+Oil treatments, respectively (Table 2). Phenolic compounds are secondary metabolites which have an important effect on color and taste formation in fruits. Post-harvest changes occur in the phenolic content of fresh fruits and vegetables, and these changes are affected by many factors such as species, variety, harvest time, cultural practices and storage time. Especially in storage studies, phenolic compound contents increase by 40-60% with the extension of storage time (Valero et al., 2011). During shelf life, lower total phenolic content was determined in colloidal coating applications compared to the control. Consistent with our findings, the total phenolic content in many products stored with coating applied was lower than the control (Sogvar et al., 2016; Valizadeh et al., 2021). The suppression of the increase in phenolic content by colloidal coating applications can be attributed to the delay in respiration.

Treatment and shelf life period had statistically significant effects on total antioxidant capacity. A decrease in antioxidant capacity was observed as shelf life increased. In the study, CsCS+SeCS+Oil treatment provided the highest antioxidant capacity and was the method that best preserved the antioxidant capacity. Although a decrease in antioxidant capacity was observed as shelf life increased in all treatments, the treatments were effective in slowing down this decrease. CsCS+SeCS+Oil treatment is application that best preserves antioxidant capacity (Table 2). The total antioxidant capacity value of many fresh fruits and vegetables to which coating was applied was higher than that of control products (Arabpoor et al., 2021; Cid-Lopez et al., 2021; Nguyen et al., 2020). In general, it has been concluded that the applications delay aging by slowing down metabolic activity in products and thus prevent nutritional losses.

Shelf life period had a statistically significant effect on total anthocyanin content. In the study, total anthocyanin content showed a tendency to increase all treatments during shelf life. The least increase in total anthocyanin amount was detected in CsCS+SeCS treatment (Table 2). Anthocyanins are a group of phenolic compounds which are responsible for the red-blue color of fruits and vegetables (Mullen et al., 2002) and have beneficial effects on human health (Garcia-Alonso et al., 2004). Additionally, anthocyanins have strong antioxidant properties. In the study, the increase in total anthocyanin content can be attributed to the decrease in TA content (Sogvar et al., 2016).

4. Conclusion

In this study, the effect of edible coatings consisting of combinations of chitosan, selenium and essential thyme oil, whose particle size was reduced to colloidal limits, on the quality criteria of strawberry fruits during shelf life was investigated. As a result of the study, it was determined that CsCS+SeCS and CsCS+SeCS+Oil treatments from edible colloidal coatings reduced weight loss, respiration rate and decay rate, and were the most effective applications in preserving color values (L*, chroma and hue), fruit firmness, TSS, TA, vitamin C content, total phenol, total anthocyanin and antioxidative capacity. These results indicate that CsCS+SeCS and CsCS+SeCS+Oil treatments can be used as edible coatings to protect fruit quality and extend post-harvest shelf life of strawberries. The results of the study will contribute to the process of obtaining edible colloidal coating agents produced with new technology in extending the shelf life of post-harvest perishable products. In addition, it can be said that the results can be used to shed light on possible future studies on similar topics.

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RESEARCH PAPER

Influence of Different Drying Techniques on Selected Physicochemical and Bioactive Properties of Mushroom Powders

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Abstract

In the present study, the effect of different drying techniques on the mushroom powders were comparatively investigated. Mushroom purees were dried by refractance window drying (RW), oven drying (OD), vacuum drying (VD) and freeze drying (FD) techniques both in their original form and as foams. For foam mat drying (FM) experiments the puree samples were foamed with carboxymethyl cellulose (CMC) and whey protein (WP) with the ratio (1:1) that formed the maximum stable foam. The dried mushrooms were ground to obtain mushroom powder. Some chemical and physical properties of the mushroom powders were determined. Total phenolics content (TPC) of the mushroom powders were determined in the ranges of 0.545-1.293 g GAE 100 g^{-1} dry matter (dm). The highest TPC was determined for the sample dried by VD while the lowest TPC was determined for the sample dried by FM-FD. The L*, chroma and hue angle values of the samples obtained from the FM experiments were higher than those obtained from the three different drying methods (OD, RW and VD) directly applied to the mushroom purees. It was determined that the browning index of the samples especially those subjected to FM was significantly lower than that of the other samples (OD, RW and VD). The study reveals that the FM method generally reduced the drying time in all drying techniques.

1. Introduction

Mushroom is a saprophyte macroscopic fungus from the primitive plant class that has been consumed as food since ancient times (Kotwaliwale et al., 2007). Cultivated *Agaricus bisporus* L. (white cap mushroom, button mushroom) is a round mushroom with a diameter of 5-10 cm with white or brownish caps. These mushrooms are preferred by the consumers and widely cultivated since they are rich in flavour compounds, nutrients, particularly protein and fibre (Kalac, 2013; Manzi et al., 2001; Pei et al., 2014; Zhang et al., 2001).

Mushrooms are highly perishable foods due to the absence of a protective cuticle, their high moisture content and their high respiratory rate. It is well known that the shelf life of fresh mushrooms in desirable quality is not more than a few days under room condition. After harvesting, colour, texture and odour changed rapidly, and the quality of the fresh mushroom began to deteriorate by the effect of enzyme activities such as protease and polyphenol oxidase (Celen et al., 2010; Giri and Prasad, 2007; Giri and Prasad, 2009; Pei et al., 2014).

Mushrooms are mostly sold as fresh in food markets, but they are also processed into different products such as canned, dried, frozen and pickled mushrooms in food industry. However, drying is the most commonly used process in the food industry because it is easy to apply and economical (Arıcı and Mengeş, 2012). High in protein, fibre, vitamins and bioactive constituents such as polysaccharides

and antioxidants, edible mushrooms are a valuable resource for the development of innovative food products. The addition of mushroom powder helps to improve texture, stability and nutritional quality, meeting the growing consumer demand for health-conscious and environmentally sustainable food options (De et al., 2025).

In the food industry, different types of drying processes have been used to dry of many fresh products as whole, sliced or pureed forms. Foam mat drying (FM) is a method in which the liquid forms of foods are converted into a stable foamy structure with various foaming agents and then the drying process is carried out. This method decreases the drying time and allows the food components to dry with less heat exposure. The first step of this process is stable foam formation. In the formation of this foam structure, gums, proteins and various emulsifiers are used as foaming agents. The main reason for the short drying time in the process of drying food in the form of foam is that the water evaporates quickly due to the large surface area of the foam created (Kadam and Balasubramanian, 2011; Sangamithra et al., 2015). Refractance window drying (RW) is a film drying technique used for liquid and semi-liquid food materials such as herbal extract, fruit and vegetable purees, etc. In comparison to most of the other drying techniques, it has several advantages such as short processing time, low energy input and high product quality. In this drying technique, heat is transferred from the water bath to the products by conduction and radiation. This results in less change in the colour, quality characteristics and nutritional value of dried products (Abonyi et al., 2002; Bardakçi and Karacabey, 2024; Nindo et al., 2004; Nindo et al., 2006; Nindo and Tang, 2007; Tontul and Topuz, 2017).

To our knowledge, only one study has reported the drying of *Pleurotus ostreatus* mushrooms using the RW technique (Timaná et al., 2024). However, to our best knowledge, there is no study on drying of mushroom (*Agaricus bisporus*) puree by RW and FM techniques to produce high quality mushroom powder. Therefore, this study aimed to determine the most appropriate drying technique for the mushroom purees obtained from small, deformed, irregularly shaped and broken mushrooms. The purees were dried using RW, oven drying (OD), vacuum drying (VD), and freeze drying (FD), through two different approaches: directly after preparation and in the form of a foamy structure. The products were compared on a number of quality characteristics related to mushroom powders.

2. Material and Methods

2.1. Materials

In the study, deformed mushroom (*Agaricus bisporus* L.) samples were obtained from a local producer in Antalya, Türkiye. The chemicals used in the analyses were purchased from either Sigma-Aldrich (Darmstadt, Germany) or Merck (Darmstadt, Germany). In addition, carboxymethyl cellulose and whey protein used for FM were purchased from local distributors.

2.2. Mushroom puree preparation

After washing with deionized water, the mushroom samples were blended for 3 min with a blender (Waring 51BL30 Laboratory blender, USA) until they were completely pureed. For FM experiments, the puree samples were foamed with carboxymethyl cellulose (CMC) and whey protein (WP) with the ratio (1:1) that formed the maximum stable foam determined by preliminary experiments. Preliminary tests showed that 1 g of CMC as a foaming agent and 1 g of WP powder (for 100 g of puree) provided the maximum stable foam structure. The mushroom puree was blended 3 min in a blender with CMC and WP in proportions determined by preliminary tests. For providing a spreadable structure, the puree samples were diluted with a certain amount of water that provided 6% final moisture content before blending. The mushroom purees were immediately dried using RW, OD, VD and FD methods, individually.

2.3. Drying of mushroom puree

The mushroom purees were dried with and without foam using RW, OD, VD and FD processes. The drying conditions were determined with some preliminary tests. In RW test, mushroom puree was spread as a thickness of 2 mm on the Mylar® plastic film in contact with circulating hot water maintained at 90°C. The drying unit was conditioned before the drying tests. The OD process was carried out in a tray dryer operated at 90°C air temperature and of 2 m s^{-1} air velocity. The mushroom purees were spread to a thickness of 2 mm on a glass plate (30×30 cm). The VD process was similarly carried out on the samples spread to a thickness of 2 mm on a 30×30 cm glass plate (30×30 cm) placed in a vacuum drying chamber operated at 60°C temperature and 0.001 mPa pressure.

For FD process, the mushroom purees were spread as a layer of 2 mm on steel trays and frozen at -80°C for 2 h in a freezer. The frozen samples were immediately placed in a freeze dryer (Operon FDU-7003, South Korea) operated at -70°C and 0.09-0.12 mm Hg absolute pressure. The drying treatments were maintained until the moisture content of the puree decreased below 8%. Drying times of mushroom purees detected in the experiments are given in Table 1. The dried samples were separately ground into a fine powder using a grinder (Sinbo SCM-2934) until they passed through a 35-mesh sieve. The obtained mushroom powders were stored in sealed glass jars at -18°C until the analyses.

Table 1. Drying times of the mushroom purees by different drying techniques.

Drying techniques	Time
Foam-mat-oven drying	75 min
Oven drying	94 min
Foam-mat refractance window drying	26 min
Refractance window drying	56 min
Foam-mat vacuum drying	120 min
Vacuum drying	180 min
Foam-mat freeze drying	48 h
Freeze drying	48 h

2.4. Mushroom powders analyses

2.4.1. Moisture content and water activity

The water activity of the samples was measured using a water activity meter (Aqualab 4TE: Decagon Devices, Pullman, WA) at 25°C. The moisture content was gravimetrically determined with a moisture analyzer (Kern DBS, Balingen, Germany) operated at 105°C until a constant weight.

2.4.2. Bulk and tapped density

To determine the bulk density (ρb) of the powders, 1 g of sample was weighed into a 25 mL graduated cylinder, and the volume was recorded. The tapped density (ρt) was calculated from also the weight/volume ratio, that 1 g of powder was transferred to 25 mL graduated cylinder, and then the graduated cylinder was tapped 30 times on a hard surface until there was no further change in volume (Beristain et al., 2001).

2.4.3. Color analysis

The colors of the mushroom powders were recorded using a chromameter (CR 400; Konica Minolta Corp., Tokyo, Japan) in terms of 'L*' (degree of lightness to darkness), 'a*' (degree of redness to green) and 'b*' (degree of yellow to blue). The hue angle° and chroma values were calculated using Equation 1 and 2, respectively.

$$\text{Hue angle}^\circ = \frac{180}{\pi} \times arctan\frac{b*}{a*} \quad (1)$$

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

where a* and b* are Hunter a *and b* values of the samples, respectively.

2.4.4. Total phenolics content (TPC)

The phenolic compounds from the sample were extracted following the procedure outlined by Dincer et al. (2012). One gram of the sample was mixed with 100 mL of 80% aqueous methanol and homogenized using an Ultraturrax (T 25, IKA Labortechnik, Germany). The extraction was conducted in a water bath (GFL 1092, Germany) maintained at 50°C, with continuous shaking at 150 rpm for 2 hours. After extraction, the mixture was allowed to cool to room temperature and then filtered through Whatman No. 42 filter paper. Total phenolic content (TPC) was determined according to the Folin–Ciocalteu method as described by Škerget et al. (2005). For this analysis, 0.5 mL of the extract was combined with 2.5 mL of Folin–Ciocalteu reagent (0.2 N) and 2 mL of sodium carbonate solution (75 g L^{-1}). The mixture was incubated at 50°C for 5 minutes and then cooled. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-160A, Japan), with 80% aqueous methanol serving as the blank. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample (mg GAE g^{-1} dm).

2.4.5. Radical scavenging activity

The 1,1-diphenyl-2-picryl hydrazil (DPPH) radical scavenging method, as outlined by Fernandez-Leon et al. (2013), was used to evaluate the antioxidant capacity of the mushroom powder. For this purpose, 1 g of the sample was extracted with 100 mL of 80% aqueous methanol. The extraction process was performed in a water bath (GFL 1092, Germany) at 50°C with shaking at 150 rpm for a duration of 2 hours. After extraction, the obtained solution was diluted 1:200 with methanol in a centrifuge tube. Then, 50 µL of the diluted extract was added to 950 µL of DPPH solution, and the mixture was incubated at room temperature in the dark for 30 minutes. Absorbance readings were taken at 517 nm using pure methanol as a blank. The antioxidant activity was calculated and expressed as grams of Trolox equivalent antioxidant activity per gram of dry sample (g TEAA g^{-1} dm).

2.4.6. Phenolic composition

The determination of phenolic compounds in the samples was conducted according to the method described by Dincer et al. (2012). For extract preparation, 0.5 g of the sample was added to 50 mL of 80% aqueous methanol. The extraction process was performed in a water bath (GFL 1092, Germany) at 50°C for 2 hours with constant shaking at 150 rpm. After extraction, the solution was cooled to room temperature, diluted with methanol to a final volume of 200 mL, and filtered through a 0.45 µm nylon membrane filter. Analysis of phenolic compounds was carried out using high-

performance liquid chromatography (HPLC) (Shimadzu LC20 AD, Japan), with an injection volume of 20 µL. Separation was achieved using an AQ 5 C18 column (250 mm×4 mm, 5 µm) maintained at 25°C. The mobile phase consisted of solvent A (0.1% acetic acid in water, v/v) and solvent B (acetonitrile, 100%), with a flow rate of 0.8 mL/min. The gradient elution profile was programmed as follows: 100:0 (A:B) at 0 min, 95:5 at 2 min, 60:40 at 20 min, 20:80 at 22 min, 95:5 at 30 min, and 100:0 at 33 min. Phenolic compounds were identified and quantified using both spiking and external standard calibration techniques. Gallic acid and catechin were detected at 280 nm, while chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid were monitored at 320 nm. The results were expressed as milligrams of compound per 100 grams of dry sample (mg 100 g^{-1} dm).

2.4.7. Browning index

The mushroom powders were suspended in distilled water. Then, the suspensions were centrifuged at 10.000 × g for 10 min. A volume of 7 mL of the supernatant was mixed with 7 mL of aqueous methanol solution (95%) and the mixture was centrifuged under the same conditions for 10 min. The absorbance of the final supernatant was recorded at 420 nm (Gögüs et al., 2000).

2.4.8. Statistical analysis

The drying experiments and analyses were carried out in two replicates. The data were subjected to analysis of variance, and mean separation was performed according to the Duncan's multiple range test using SAS software (SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Moisture content and water activity

Moisture content and water activity of the mushroom powders produced by different dried methods are given in Table 2. The mushroom purees were dried with different drying techniques either as is (OD, RW, VD, and FD) or after foaming (FM-OD, FM-RW, FM-VD, FM-FD) until the final moisture content of 8%. The moisture contents of the mushroom powders were determined in the ranged of 5.62 g 100 g^{-1} to 8.4 g 100 g^{-1}. The water activity results of the mushroom powders produced by the drying techniques ranged between 0.18-0.24. Since food powders or additives are considered to be microbiologically and chemically stable with water activity and moisture content lower than 0.6 and 10 g 100 g^{-1} (Eroğlu et al., 2018), present results of water activity and moisture content of the mushroom powders are suitable for a stable product. Although every care was taken to produce mushroom powder with a target moisture content as close as possible to the drying methods used, inevitable variations occurred in the final moisture content due to the effects of temperature differences and environmental conditions until the product was removed from the dryer.

3.2. Bulk and tapped density

The bulk and tapped densities of the mushroom powders were measured in the ranges between 108.35-498.714 kg m^{-3} and 154.21-619.22 kg m^{-3}, respectively (Table 2). The densities are important parameters determining storage stability and flowing characteristics of powder products. The bulk and tapped densities of the RW-dried and oven dried mushroom powders were significantly ($p<0.05$) higher than the bulk and tapped densities of the other powders. On the contrary, the bulk and tapped densities of the mushroom powders produced from foam mat freeze-drying were determined in the lower ranges. In addition, it was determined that the bulk and tapped density values of the mushroom powders those subjected to FM was lower than that of the control samples (not foamed). The lower bulk density of freeze-dried mushroom powders is highly associated with big pore size of the particles evacuated by sublimation during the drying process (Koc et al., 2008).

3.3. Color analysis

The L*, a*, b*, hue angle and chroma values of the mushroom powder produced by the applied drying methods are given in Table 3.

Table 2.Some physicochemical properties of mushroom powders produced by different drying techniques.

Drying techniques	Moisture content (%)	Water activity	Bulk density (kg m^{-3})	Tapped density (kg m^{-3})
FM-OD	5.98±0.03^{cd}	0.19±0.01^{b}	383.92±0.20^{c}	463.30±2.91^{d}
OD	5.62±0.01^{d}	0.20±0.00^{b}	489.64±6.56^{a}	619.22±13.68^{a}
FM-RW	5.63±0.05^{d}	0.18±0.00^{b}	406.38±8.43^{c}	505.25±8.80^{c}
RW	6.73±0.02^{cd}	0.20±0.00^{b}	488.78±7.93^{a}	554.17±5.41^{b}
FM-VD	8.4±0.27^{a}	0.23±0.00^{a}	403.10±1.47^{c}	454.41±6.95^{d}
VD	6.95±0.03^{bc}	0.20±0.00^{b}	498.71±2.91^{b}	501.25±2.65^{c}
FM-FD	7.07±0.15^{bc}	0.24±0.00^{a}	108.35±4.99^{e}	154.21±5.67^{f}
FD	7.76±0.05^{ab}	0.24±0.00^{a}	135.23±0.80^{d}	220.74±7.09^{e}

The values are mean ± standard error. The values within a column with different superscript letters are significantly ($p<0.05$) different. Foam-mat hot air drying (FM-OD), Oven drying (OD),Foam-mat Refractance Window drying (FM-RW), Refractance Window drying (RW), Foam-mat vacuum drying (FM-VD), Vacuum drying (VD), Foam-mat freeze drying (FM-FD), Freeze drying (FD).

Table 3. Effect of different drying techniques on color of the mushroom powders.

Drying techniques	L*	a*	b*	Hue angle	Chroma
FM-OD	38.75±0.31^{de}	5.94±0.11^{a}	11.62±0.04^{ef}	62.92±0.34^{ef}	13.05±0.08^{cd}
OD	39.36±0.04^{cd}	6.04±0.09^{a}	12.49±0.13^{de}	64.20±0.09^{de}	14.88±0.86^{bc}
FM-RW	43.79±0.01^{b}	6.06±0.04^{a}	13.72±0.11^{c}	66.18±0.03^{b}	15.02±0.10^{bc}
RW	37.56±0.19^{ef}	5.77±0.29^{ab}	11.13±0.52^{f}	62.62±0.06^{f}	12.53±0.61^{d}
FM-VD	40.14±0.43^{c}	5.75±0.00^{ab}	12.84±0.25^{cd}	65.89±0.42^{bc}	14.07±0.23^{cd}
VD	36.75±0.19^{f}	5.67±0.01^{ab}	11.79±0.28^{de}	64.48±0.35^{cd}	13.68±0.54^{cd}
FM-FD	51.39±0.44^{a}	5.23±0.16^{b}	16.09±0.11^{b}	71.81±0.62^{a}	16.94±0.05^{b}
FD	50.29±0.11^{a}	6.23±0.04^{a}	18.04±0.08^{a}	70.79±0.04^{a}	19.10±0.08^{a}

The values are mean ± standard error. The values within a column with different superscript letters are significantly ($p < 0.05$) different. Foam-mat hot air drying (FM-OD), Oven drying (OD),Foam-mat Refractance Window drying (FM-RW), Refractance Window drying (RW), Foam-mat vacuum drying (FM-VD), Vacuum drying (VD), Foam-mat freeze drying (FM-FD), Freeze drying (FD).

Table 4. Effect of different drying techniques on Total phenolics content, radical scavenging activity and browning index of the mushroom powders.

Drying techniques	Total phenolics content (g GAE 100 g^{-1} dm)	Radical scavenging activity (g TEAA 100 g^{-1} dm)	Browning index
FM-OD	0.78±0.01^{d}	0.84±0.00^{c}	77.05±1.94^{c}
OD	1.02±0.03^{b}	1.08±0.00^{a}	109.50±3.89^{a}
FM-RW	0.69±0.03^{e}	0.56±0.03^{e}	45.20±1.27^{e}
RW	0.70±0.01^{e}	0.92±0.01^{b}	73.30±0.28^{cd}
FM-VD	0.89±0.00^{c}	0.77±0.01^{d}	65.35±0.04^{d}
VD	1.29±0.01^{a}	1.06±0.03^{a}	90.60±0.14^{b}
FM-FD	0.55±0.02^{f}	0.62±0.01^{e}	43.65±0.11^{e}
FD	0.64±0.00^{e}	0.75±0.00^{d}	44.89±4.21^{e}

The values are mean ± standard error. The values within a column with different superscript letters are significantly ($p < 0.05$) different. Foam-mat hot air drying (FM-OD), Oven drying (OD),Foam-mat Refractance Window drying (FM-RW), Refractance Window drying (RW), Foam-mat vacuum drying (FM-VD), Vacuum drying (VD), Foam-mat freeze drying (FM-FD), Freeze drying (FD).

The mushroom powders obtained by the FM-FD and FD techniques had the highest L* and Hue angle values with no significant difference ($p > 0.05$) between each other's. The lowest Hue angle and chroma values among the samples were determined in the mushroom obtained by RW. In addition, the highest chroma value among the samples were determine in the mushroom obtained by the FD. In general, the L*, hue angle and chroma values of the samples obtained from the FD treatments were significantly higher than those obtained from the four different drying methods (OD, RW, VD and FD) applied directly to the purees. The reason for these colour differences can be associated with the shorter drying time of the FM process and/or the effect of the foaming agents used. The color results are mostly in agreement with a few previous studies performed on mushrooms similar in nature (Isik and Izlin, 2014; Lee et al., 2007; Qi et al., 2014).

3.4. Total phenolics content

Total phenolics content of the mushroom powder obtained from the drying methods are given in Table 4. The TPC of the mushroom powders were determined in the ranges of 0.55-1.29 g GAE 100 g^{-1} dm, the difference in the TPC results are significantly ($p<0.05$) different from each other. The highest TPC were determined for the sample dried by VD while the lowest TPC was determined for the sample dried by FD. The TPC content of the mushroom powders has been interpreted as highly associated with the drying temperature and time. Indeed, the highest drying temperature and the longest drying time in the drying experiments resulted in more or less high content of TPC, as determined in several previous studies of a similar nature. (Papagiannopoulos et al., 2004; Soontharapirakkul and Kotpat, 2023). Yim et al. (2010) reported TPC of mushroom (*Pleurotus ostreatus*) extract was determined as 798.55 mg GAE 100 g^{-1}. Another study on drying of *P. ostreatus* mushrooms reported that TPC of the mushroom dried by forced air OD, FD and RW changed between 4.62 and 6.82 mg GAE g^{-1} (Timaná et al., 2024). Total phenolics content of the mushrooms dried by freeze and cabinet drying was determined to range between 17.06 and 20.30 mg GAE g^{-1} as well (Shams et al., 2022). The TPC results of a study were found to be between 1.6 and 3.2 mg GAE 100 g^{-1} dm. The results of the current study is generally in agreement with the reviewed results for the mushrooms dried by different drying methods (Tepsongkroh et al., 2019).

3.5. Radical scavenging activity

The radical scavenging activity of the mushroom powders produced by the drying techniques was determined in the ranges of 0.56-1.08 g TEAA 100 g^{-1} dm. The difference in the radical scavenging activity of the samples was significant ($p < 0.05$) (Table 4). The highest radical scavenging activity was determined for the sample dried by OD while the lowest radical scavenging activity was determined for the sample dried by FM-RW. These results are consistent with the findings

Table 5. Phenolic composition of the mushroom powders dried by different drying techniques (mg 100 g^{-1} dm).

Drying techniques	Gallic acid	Catechin	Ferulic acid	ρ-coumaric acid	Caffeic acid	Chlorogenic acid
FM-OD	31.98 ± 0.58^{d}	0.29±0.01^{c}	1.78±0.11^{bc}	0.01±0.00	0.02±0.00	0.09±0.00^{cd}
OD	51.94 ± 0.07^{b}	0.50±0.00^{a}	1.45±0.06^{de}	0.01±0.00	0.02±0.00	0.25±0.01^{a}
FM-RW	25.66±0.44^{e}	0.18±0.01^{d}	1.35±0.02^{de}	n.d	0.01±0.00	0.04±0.00^{e}
RW	40.12±0.09^{c}	0.30±0.01^{b}	1.20±0.02^{e}	n.d	0.01±0.00	0.09±0.00^{cd}
FM-VD	39.11±1.21^{c}	0.14±0.00^{e}	1.82±0.00^{bc}	0.01±0.00	0.02±0.00	0.12±0.01^{c}
VD	58.57±1.27^{a}	0.31±0.00^{b}	4.26±0.06^{a}	0.02±0.00	0.02±0.00	0.17±0.00^{b}
FM-FD	17.77±0.10^{f}	0.21±0.01^{c}	1.57±0.09^{cd}	0.03±0.00	0.01±0.00	0.03±0.00^{e}
FD	15.44±0.18^{f}	0.25±0.00^{c}	1.93±0.03^{b}	0.01±0.00	0.01±0.00	0.07±0.00^{de}

The values are mean ± standard error. The values within a column with different superscript letters are significantly ($p < 0.05$) different. Foam-mat hot air drying (FM-OD), Oven drying (OD), Foam-mat Refractance Window drying (FM-RW), Refractance Window drying (RW), Foam-mat vacuum drying (FM-VD), Vacuum drying (VD), Foam-mat freeze drying (FM-FD), Freeze drying (FD).

of an earlier study (Tepsongkroh et al., 2019). The high radical scavenging activity of the mushroom powder dried by VD can be associated with high concentration of phenolic compounds. It is also related to be amount of brown pigments, low L* values, and melonoidins, which is formed from carbonyl and amine reaction and reported to be responsible for high antioxidant activity (Madrau et al., 2009; Tepsongkroh et al., 2019). The phenolic content of mushroom powders dried by the vacuum drying method was determined to be high and it is expected that these samples have higher antioxidant activity. In addition, the browning index of these samples is also high. It is known that antioxidants are formed as a result of non-enzymatic browning reactions (Quintero Ruiz et al., 2014).

3.6. Browning index

Browning index values of the mushroom powder obtained by the different drying methods are given in Table 4. The results, determined in the ranges of 43.65-109.50, showed a significant difference ($p < 0.05$) between each other. These results are generally in agreement with the findings of a previous study (Timaná et al., 2024). For instance, browning index of mushroom powders was determined between 13.34-69.50 (Zhang et al., 2021). In another study performed with different mushrooms dried by microwave, cabinet and vacuum drying reported that browning index values varied between 29.252 and 84.348 (Siti-Nuramira et al., 2022). The reason for the differences in browning index values between these studies and the current study is the difference in dried mushroom type and drying method. It was determined that the highest browning index belonged to the powders obtained by oven drying method. The differences in the browning index can be related to depending on the drying time and temperature. However, in RW, which is carried out at the same temperature and in a shorter time than OD, the browning index was found to be much lower due to the high drying speed and the short completion time of the process. The lowest browning index was determined in samples obtained by FD, which is not a thermal process. It was determined that the browning index of the mushroom powders, especially those subjected to FM was significantly lower than that of the control samples. It is known that non-enzymatic browning reactions occur through the sugar-amine reaction under the influence of heat. The browning index data obtained increased with high temperatures and long processing times in parallel with this information. The browning index values determined can be explained mostly by non-enzymatic browning reactions (Cernîşev, 2010).

3.7. Phenolic composition

The results of identified phenolic compounds (gallic acid, catechin, ferulic acid, ρ-coumaric acid, caffeic acid, and chlorogenic acid) of the mushroom powders obtained by different drying methods are given in Table 5. Concentration of the individual phenolic compounds in the samples were calculated as mg 100 g^{-1} dm using their calibration curves prepared with standards solutions at different concentrations. The gallic acid, catechin, ferulic acid, ρ-coumaric acid, caffeic acid, and chlorogenic acid of the mushroom powders were determined in the ranges of 15.44-58.57, 0.14-0.50, 1.20-4.26, 0.01-0.03, 0.01-0.02 and 0.03-0.25 mg 100 g^{-1} dm, respectively. Results reflected that the principle phenolic compounds, such as gallic acid, ferulic acid and catechin, were determined to be the highest amount in the samples dried by VD. This can also be associated with combination of the higher drying temperature and longer drying time.The results are generally consistent with the phenolic compounds found in several previous studies conducted on different mushroom species (Palacios et al., 2011). The gallic acid and ferulic acid were determined in the highest amount in the samples dried by VD, while catechin and chlorogenic acid amounts were determined in samples dried by OD, p-coumaric acid was determined in samples dried by FM-FD, and caffeic acid was determined in samples dried by VD and FM-VD.

4. Conclusion

In this study, mushroom purees were dried using the refractance window drying and foam mat refractance window drying for the first time. In order to increase the stability of the bioactive compounds of the mushrooms and to prolong the shelf life of the product, the mushroom purees were comparatively dried by different drying techniques, such as oven drying, vacuum drying and freeze drying, both in the form of foam and directly as is in the form of puree. Various quality characteristics of the dried mushroom powders were tested. The obtained results showed that FD techniques had superior physical characteristics whereas the dried mushroom obtained by RW and VD drying techniques had marked phenolic content thereby higher antiradical scavenging activities. It was specifically found that the colour properties of the products obtained by the FM-RW and FM-FD method gave the best results, and the browning index gave the lowest results. Considering the shortest drying time of FM-RW process which also provides some superior colour and other quality properties in the dried mushroom powders, it can be selected as more practical, preferable and sustainable method. The mushroom powders can be preferably used in a variety of food formulations including soups, sauces, snacks, seasoning blends, and functional foods. Additionally, the high retention of bioactive compounds and improved shelf stability make these powders suitable for nutraceutical products, dietary supplements, and health-oriented food innovations. Overall, the study provides a foundation for the development of energy-efficient and quality-preserving drying processes that can be readily integrated into industrial-scale production.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Synergistic Effects of Biofertilizers and Chemical Fertilizers on Yield and Nutritional Quality of Greenhouse-Grown Lettuce

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Abstract

This study investigates the impact of biofertilizers, chemical fertilizers, and their combined application on lettuce yield and nutrition. A randomized block design experiment was conducted in a greenhouse, comparing control, chemical fertilizer (CF), Bio Veria (BF_1), Bacillus Mix (BF_2), CF+BF_1, and CF+BF_2 treatments. Results showed that combined applications of CF+BF_1 and CF+BF_2 significantly increased lettuce fresh weight by 56% and 61%, respectively, and dry weight by 80% and 112% compared to the control. The CF+BF_2 treatment achieved the highest yield at 34.6 t ha^{-1}, a 61% improvement over the control and a 28% increase compared to CF alone. Furthermore, the mixture treatments demonstrated the highest nitrogen (N), phosphorus (P), and calcium (Ca) contents in lettuce leaves. Specifically, CF+BF_2 had the highest N content, a 29% improvement over the control and a 20% increase over CF. The CF+BF_1 treatment resulted in a 54% improvement in P content over the control and a 21% increase over CF. The CF+BF_2 treatment also produced a 54% improvement in Ca content over the control and a 25% increase over CF. The integrated application of biofertilizers and chemical fertilizers significantly improved lettuce yield and nutritional content, highlighting the potential of biofertilizers containing multi-species in optimizing lettuce production.

1. Introduction

Lettuce, a widely consumed vegetable known for its rich fiber content, folate, iron, and other health-promoting components (Kim et al., 2016), is typically consumed fresh. In greenhouse lettuce cultivation, achieving optimal growth and yield depends on several factors, including irrigation frequency, fertilization rates, incorporation of biological supplements, properties of growth medium, and controlled growth conditions (Liu et al., 2012; Vetrano et al., 2020; Wang and Xing, 2016). In Türkiye, lettuce holds significant agricultural importance, with a total greenhouse production of 154,000 tons in 2023 (TÜİK, 2023). To enhance growth and yield, inorganic mineral fertilizers are commonly applied to lettuce in greenhouses after transplanting (Vetrano et al., 2020; Trinh et al., 2018; Zhao et al., 2010). These fertilizers provide essential nutrients to plants, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), which are crucial for maximizing crop productivity (White and Brown, 2010). However, excessive application of chemical fertilizer may cause nutrient runoff, nitrogen pollution, and leaching losses, which can adversely affect the environment, biodiversity, and human health (Baweja et al., 2020; Zhao et al., 2010). The increasing challenges posed by climate change further threaten crop production and

productivity, requiring innovative approaches to meet the rising demand for food and other agricultural products (Hamid et al., 2020; McLaughlin and Kinzelbach, 2015). Therefore, biofertilizer serves as an alternative to inorganic mineral fertilizers. Biofertilizers are biological products containing living microorganisms that when applied to seed, plant surfaces or soil stimulate growth through a variety of mechanisms including boosting the plant's ability to absorb nutrients, increasing the amount of biomass or root area, and increasing the supply of nutrients (Pylak et al., 2019). These formulations contain beneficial microorganisms that enhance nutrient availability through biological processes. As a result, they contribute to improved soil health and stimulate plant growth (Basu et al., 2021).

Microbes utilized in the production of biofertilizers not only mobilize N and P but also contribute to the natural cultivation of crops. Arbuscular mycorrhizal fungi (AMF) are effective biofertilizers that enhance plant growth and nutrient absorption, particularly in lettuce cultivation (Molaei et al., 2024). This symbiosis significantly improves the uptake of insoluble and immobile phosphate ions from the soil. Arbuscular mycorrhizal fungi produce phosphatases to hydrolyze phosphate from organic sources, boosting crop yield in P-deficient conditions (Lee et al., 2014; Majewska et al., 2017). AMF inoculation has been shown to significantly boost lettuce yield, with studies reporting an average production increase of 186% (Epelde et al., 2020). Furthermore, AMF colonization in greenhouse lettuce can also increase the accumulation of secondary metabolites, vitamins, and minerals, enhancing the intake of beneficial compounds without raising lettuce consumption (Baslam et al., 2013). For instance, *Glomus intraradices*, a well-known arbuscular mycorrhizal fungus, significantly improves nutrient acquisition, water uptake efficiency, and overall biomass production in lettuce (Kohler et al., 2010). Similarly, plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative to inorganic mineral fertilizers by enhancing plant growth and production (Basu et al., 2021). *Bacillus spp*. are one of the most used strains in microbial biofertilizers. Bacillus species enhance plant development through mechanisms such as phytohormone production, nutrient solubilization, antibiotic synthesis, and promotion of systemic resistance to pathogens (Tejera et al., 2013). Vetrano et al. (2020) reported that applying *Bacillus spp*. to lettuce seedlings improved growth, yield, and nitrate content in greenhouse and field experiments, while maintaining the natural rhizosphere microbiome (Kröber et al., 2014).

Despite the well-documented advantages of biofertilizers in improving yield, biomass, water retention capacity, and nutrient availability (Cipriano et al., 2016), there is a lack of knowledge regarding the combined use of chemical and biofertilizers in lettuce cultivation. Addressing this gap is essential for optimizing nutrient management strategies that balance productivity and environmental sustainability. Accordingly, this study aimed to investigate the impact of biofertilizers, chemical fertilizer, and their combined application on the yield and nutritional content of lettuce, thereby contributing to a more sustainable and productive greenhouse cultivation system.

2. Materials and Methods

2.1. Study site

The experiment was conducted in the autumn of 2021 in a glass greenhouse located at the Batı Akdeniz Agricultural Research Institute (Serik, Antalya, Türkiye), on silty loam (61% sand, 21% clay, and 18%) soil characterized by slightly alkaline pH (7.8), nonesaline (EC: 1.1 dS m^{-1}), and low lime content (3.3%). The soil also contained 1.65% organic matter (Table 1). The region has a mean annual temperature of 18.9°C and an average annual precipitation of 1039.8 mm (Türkiye State Meteorological Service, 2024).

Table 1. The physical and chemical properties of the experimental soil.

Parameters	Values
pH (1:2.5)	7.80
Lime (%)	3.30
EC (dS m^{-1})	1.10
Sand (%)	61.00
Clay (%)	21.00
Silt (%)	18.00
Organic matter (%)	1.65
Phosphate (ppm)	70.00
Potassium (ppm)	365.00
Calcium (ppm)	3700.00
Magnesium (ppm)	285.00
Iron (ppm)	4.40
Manganese (ppm)	12.00
Zinc (ppm)	2.10
Copper (ppm)	8.00

Table 2. Ingredients of biofertilizers used in the experiment.

Bio veria	Bacillus Mix
Glomus intraradices (0.001 progagule g^{-1})	*Glomus intaradices* (0.01 progagule g^{-1})
Bacillus licheniformis (10^7 CFU ml^{-1})	*Bacillus licheniformis* (10^9 CFU ml^{-1})
	Bacillus megaterium (10^9 CFU ml^{-1})

2.2. Biofertilizer and plant material

The lettuce (*Lactuca sativa* L.), hybrid AG Tohum Caipira®, was used in the experiment. The commercial biofertilizers applied were Bio Veria® (liquid) and Bacillus Mix® (solid), both manufactured by ED&F MAN (UK). The composition of these biofertilizers is provided in Table 2.

2.3. Experimental design and setup

The experiment was arranged in a randomized block design with four replications. The following fertilizer treatments were applied: Control (no fertilization), chemical fertilizer (CF) at the recommended dose, Bio Veria (BF_1), Bacillus Mix (BF_2), Chemical fertilizer + Bio Veria ($CF+BF_1$), and Chemical fertilizer + Bacillus Mix ($CF+BF_2$). The chemical fertilizers used were urea (46% N), monoammonium phosphate (MAP) (12% N, 61% P_2O_5), and potassium nitrate (13% N, 45% K_2O). The application rates of chemical fertilizers were determined based on plant growth stages. Fertigation was applied on average every four days, with intervals adjusted based on soil moisture. The fertigation solution was maintained at a pH of 6.5-7.0 and an electrical conductivity (EC) of 1.5-2.0 dS m^{-1}, depending on the crop developmental stage.

A drip irrigation system was used, and chemical fertilizers were applied through this system. Bio Veria and Bacillus Mix applications were split into two doses: the first at sowing and the second 14 days after sowing. The total biofertilizer application per hectare was 10 liters for Bio Veria and 2500 grams for Bacillus Mix.

Planting was carried out on October 14, 2021. The experimental unit consisted of two rows, each 4.6 m in length, with 0.4 m row spacing and 0.5 m in-row spacing, accommodating 20 plants per plot. This resulted in a theoretical plant population density of 60,000 plants per hectare.

2.4. Plant sampling and analysis

Lettuce plants were harvested 52 days after planting, and four plants were collected from each plot to determine fresh and dry weights. The total yield per plot was measured, and the data were converted to tons per hectare (t ha^{-1}).

Leaf samples were dried at 65°C, ground, and prepared for chemical analysis. In the filtrates obtained from wet digestion using a nitric:perchloric acid mixture ($4HNO_3 + HClO_4$), the concentrations of P, K, Ca, Mg, Fe, Zn, Mn, and Cu were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Nitrogen (N) content in lettuce leaves was analyzed using the modified Kjeldahl method (Kacar and İnal, 2008).

2.5. Statistical analysis

All statistical analyses were performed using RStudio® (RStudio Team, 2020) with R version 4.2.2. A randomized block design (RBD) with four replications was used to assess the effects of fertilizer treatments on plant growth and nutrient content. The data were subjected to analysis of variance (ANOVA) using the aov function from the base R package. Data visualization and summary statistics were performed using the ggplot2 (Wickham, 2016) and dplyr (Wickham et al., 2023) packages. The statistical model used for the analysis was:

$$Y_{ijk} = \mu + T_i + B_j + \varepsilon_{ijk}$$

where:

Y_{ijk} represents the observed response variable (e.g., fresh weight, dry weight, total yield, or nutrient content) for the i-th fertilizer treatment in the j -th block (replication),
μ is the overall mean,
T_i is the fixed effect of the i-th fertilizer treatment (i=1, 2,...,6),
B_j is the random effect of the j-th block (replication) (j=1, 2, 3, 4),
ε_{ijk} is the random error term, assumed to follow a normal distribution with mean 0 and variance σ^2 ($\varepsilon_{ijk} \sim N(0, \sigma^2)$).

Analysis of variance (ANOVA) was conducted to determine the statistical significance of treatment effects on all measured parameters. When significant differences were detected, Fisher's least significant difference (LSD) test was applied to compare treatment means at a 5% significance level ($p < 0.05$).

3. Results and Discussion

3.1. Effects of fertilizers on plant growth

The effects of fertilizers on growth parameters, including fresh weight (FW), dry weight (DW), and total yield, are shown in Figure 1. The application of BF individually and combined with CF significantly

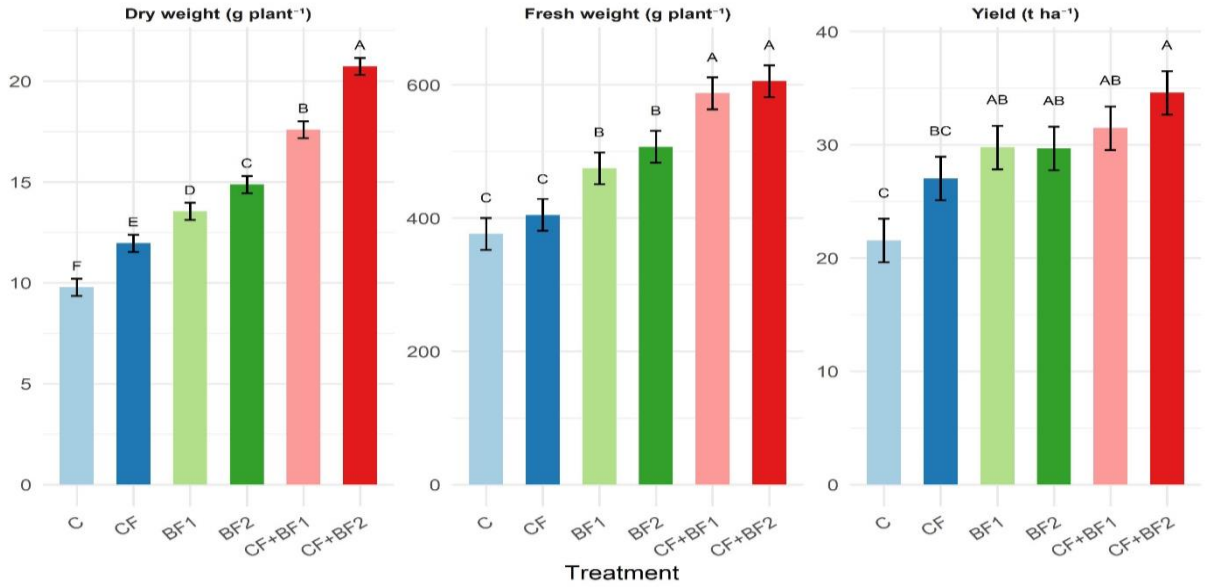

Figure 1. Effect of biofertilizer and chemical fertilizer treatments on dry and fresh weights and yield (Values are the means of four replicates and error bars represent the standard errors, n=4. Significant differences from Fisher's LSD test at $p<0.05$ are shown as different letters. C: Control, CF: Chemical Fertilizer, BF1: Bio veria, BF2: Bacillus mix, CF+BF1: Chemical Fertilizer+ Bio veria, CF+BF2: Chemical Fertilizer+Bacillus mix.

increased the FW of lettuce compared to control plants. The dual application of CF+BF_1 and CF+BF_2 resulted in the highest values, reaching 587.6 g $plant^{-1}$ and 605.7 g $plant^{-1}$, respectively. Compared with the control, CF+BF_1 and CF+BF_2 increased FW by 56% and 61%, respectively. Moreover, the combination of BFs and CF significantly enhanced lettuce FW compared to single applications of CF and BF, while no significant differences were observed between CF+BF_1 and CF+BF_2. The results obtained are consistent with Stoll et al. (2018) and they found a notable increase in the FW when bacterial strains were inoculated into lettuce seedlings, resulting in up to 30% more weight compared to the control.

Regarding lettuce DW, the obtained results revealed that all treatments significantly increased DW compared to control plants (Figure 1). The highest increases were observed in plants treated with CF+BF_1 and CF+BF_2, which improved the DW by 80% and 112%, respectively, compared to the control. These dual applications also resulted in significantly higher DW values than single fertilizer applications. Similar findings were reported by Sánchez et al. (2014) who assessed consortia of bacterial strains in lettuce (*L. sativa*) plants and found that bacterial consortium treatment increased DW by 102% compared to CF alone, likely due to improved root growth and nutrient uptake.

Biofertilizers may enhance root penetration and water retention capacity leading to increased plant growth (Bhardwaj et al., 2014). The remarkable 112% increases in DW observed in the CF+BF_2 treatment may be attributed to improved soil conditions, which facilitate better moisture and nutrient absorption by plant roots. The significant increases in FW and DW, particularly in the CF+BF_2 treatment, could be closely linked to the beneficial properties of PGPR and AMF, including nutrient solubilization and enhanced nutrient uptake. PGPR are well known for their ability to solubilize essential nutrients, particularly P, a key nutrient often limited in bioavailability in soils. The substantial increase FW (605.7 g $plant^{-1}$) and DW (20.7 g $plant^{-1}$) in the CF+BF_2 treatment likely resulted from improved nutrient uptake facilitated by PGPR. Aini et al. (2019) found that AMF and PGPR increase root surface area and improve nutrient uptake, especially P, leading to increased biomass.

3.2. Total yield response to fertilizer treatments

The total yield across all treatments showed significant improvements compared to the control (Figure 1). The control plants had the lowest yield (21.5 t ha^{-1}). The application of biofertilizers alone or in combination with chemical fertilizers significantly increased yield. BF_1 resulted in a yield of 29.8 t ha^{-1}, outperforming both the control and CF treatments, while BF_2 produced similar results to BF_1. The highest yields were obtained from combined applications, with CF+BF_1 yielding 31.5 t ha^{-1} and CF+BF_2 yielding 34.6 t ha^{-1}. CF+BF_2 exhibited the highest yield, representing a 61% increase over the control and a 28% increase compared to CF alone.

These results underscore the significant impact of integrated nutrient management strategies on crop productivity. Our findings align with literature emphasizing the benefits of combining chemical fertilizers with biofertilizers to enhance agricultural yields. Chatzistathis et al. (2024) conducted a greenhouse experiment to investigate the effects of PGPR and AMF on growth, nutrient uptake, and physiological performance of Batavia lettuce (*Lactuca sativa* L. var. longifolia) and found that

PGPR and AMF applications with inorganic NPK fertilizer resulted in higher total biomass compared to the inorganic fertilizer treatment. Other studies have similarly demonstrated that the integration of chemical and biofertilizers enhances crop yields across diverse agricultural systems. Oktaviani and Patiung (2024) highlighted the role of biofertilizers in increasing nutrient uptake and improving soil fertility, showing that biofertilizers can partially replace chemical fertilizers without compromising yield. Our results support this, as both biofertilizer treatments (BF_1 and BF_2) increased yields by 38.1% and 37.7%, respectively, compared to the control.

3.3. Nutrient content in lettuce leaves

In addition to yield improvements, nutrient concentrations in leaves were significantly influenced by fertilizer treatments (Figures 2 and 3). N content ranged from 3.52% to 4.55% among treatments, with the highest values recorded in the $CF+BF_1$ (4.22%) and $CF+BF_2$ (4.55%) treatments. The $CF+BF_2$ treatment resulted in a 29% increase over the control and a 20% increase compared to CF alone. P content varied between 0.40% and 0.61%, with $CF+BF_1$ (0.61%) and $CF+BF_2$ (0.59%) showing the highest levels. The $CF+BF_1$ treatment

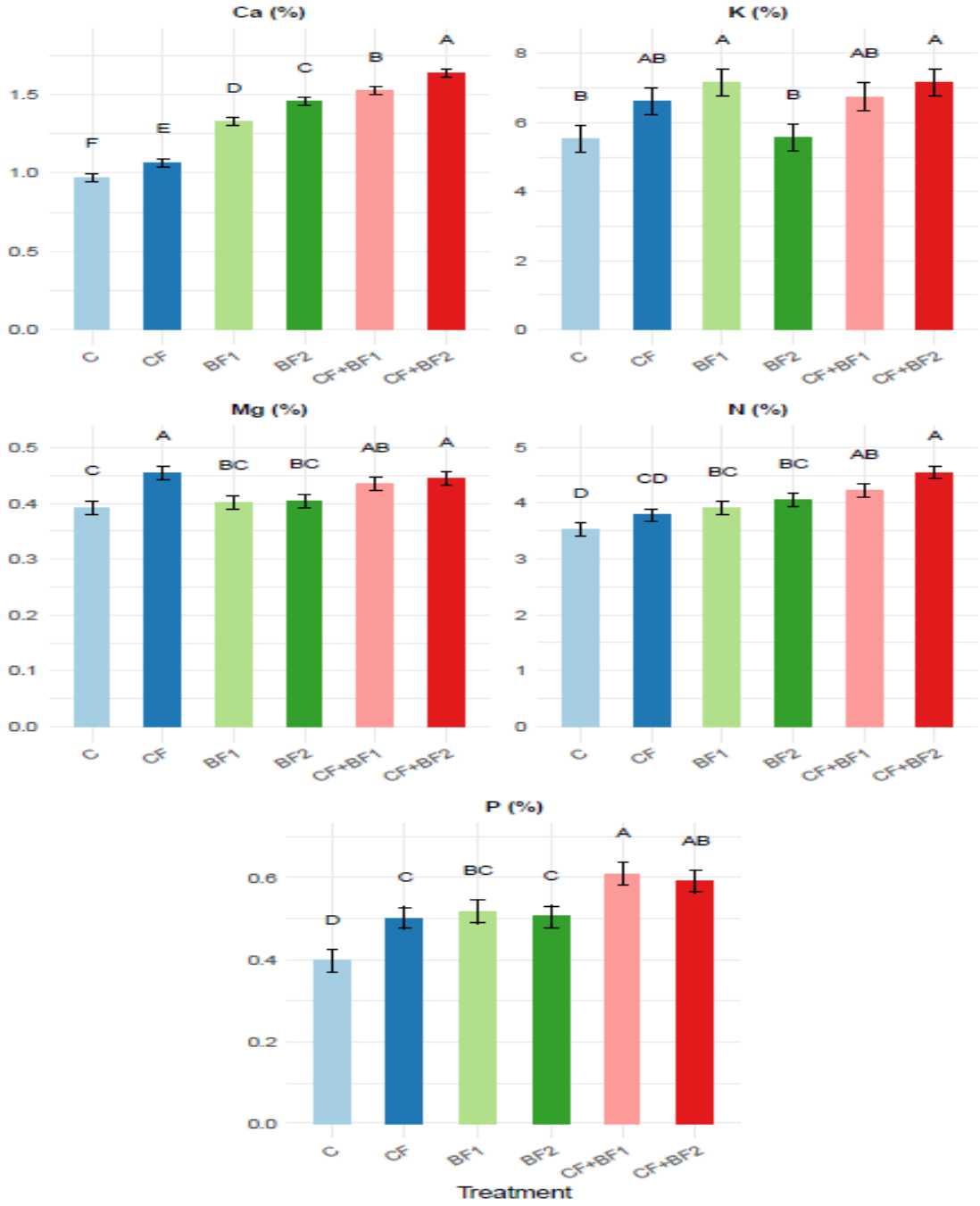

Figure 2. Effect of biofertilizer and chemical fertilizer treatments on macronutrients of lettuce leaves (Values are the means of four replicates and error bars represent the standard errors, n=4. Significant differences from Fisher's LSD test at $p<0.05$ are shown as different letters). C: Control, CF: Chemical Fertilizer, BF_1: Bio veria, BF_2: Bacillus mix, $CF+BF_1$: Chemical Fertilizer+ Bio veria, $CF+BF_2$: Chemical Fertilizer+Bacillus mix.

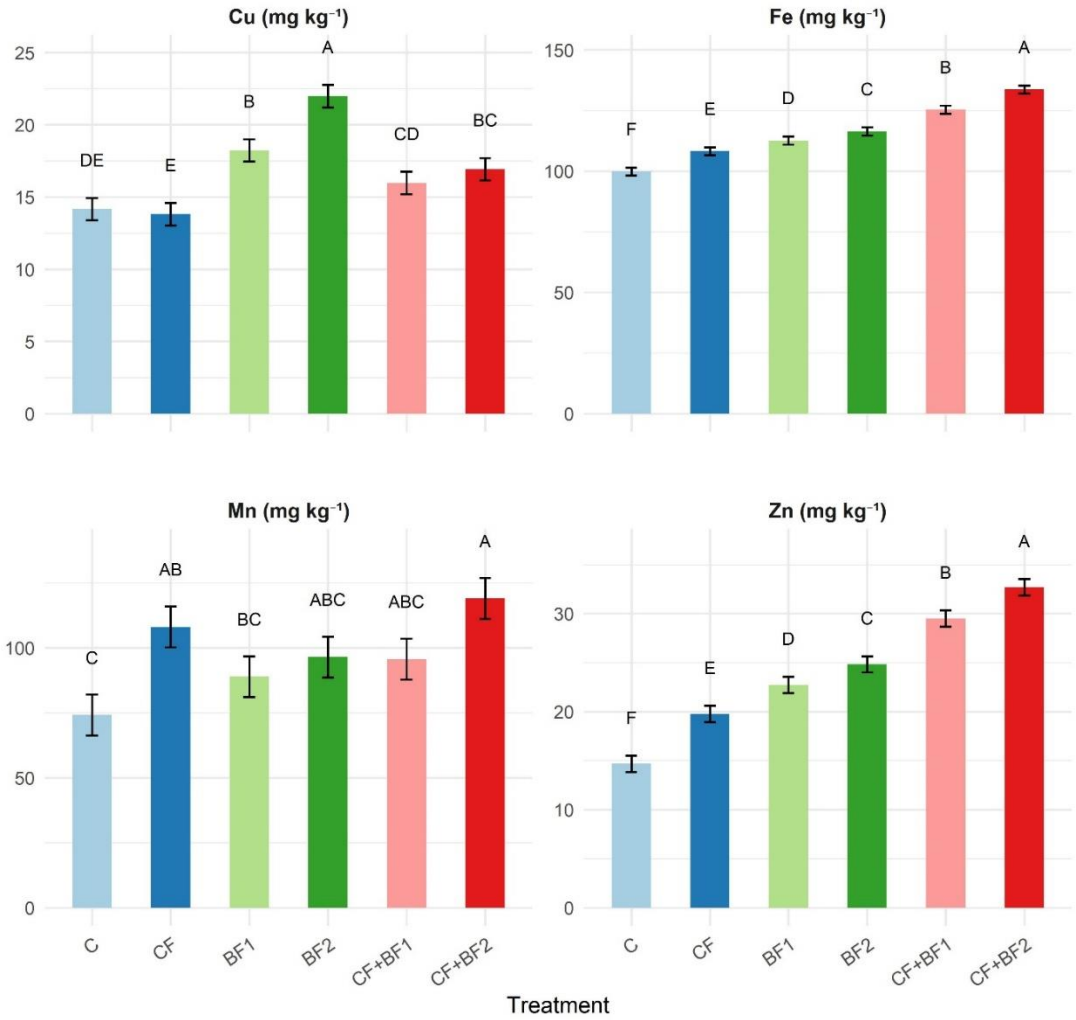

Figure 3. Effect of biofertilizer and chemical fertilizer treatments on micronutrients of lettuce leaves (Values are the means of four replicates and error bars represent the standard errors, n=4. Significant differences from Fisher's LSD test at $p<0.05$ are shown as different letters). C: Control, CF: Chemical Fertilizer, BF_1: Bio veria, BF_2: Bacillus mix, $CF+BF_1$: Chemical Fertilizer+ Bio veria, $CF+BF_2$: Chemical Fertilizer+Bacillus mix.

increased P content by 54% over the control and by 21% compared to CF. Potassium content ranged from 5.52% to 7.17%. The highest values were observed in $CF+BF_1$ (7.10%) and $CF+BF_2$ (7.15%), with $CF+BF_2$ increasing K content by 30% over the control. The Ca content of lettuce leaves ranged between 0.97% and 2.85% among treatments. The $CF+BF_2$ treatment produced a 54% improvement over the control and a 25% increase compared to CF. The Mg content of plant leaves varied from 0.39% to 0.46% between treatments. The dual applications did not significantly increase the Mg content in the leaves compared with the CF.

Among micronutrients, Fe content ranged from 99.87 mg kg^{-1} to 133.64 mg kg^{-1}, with $CF+BF_2$ achieving the highest value, 34% higher than the control. Manganese content varied from 74.16 mg kg^{-1} to 118.99 mg kg^{-1}, with $CF+BF_2$ showing the highest increase (60% over the control). Zinc concentrations ranged between 14.69 mg kg^{-1} and 32.69 mg kg^{-1}, with $CF+BF_2$ improving Zn content by 122% over the control and 20% over CF alone. The single application of biofertilizers significantly influenced the Cu concentration of the leaves, and the Cu content of the lettuce leaves varied from 13.82 mg kg^{-1} to 19.94 mg kg^{-1} between treatments.

3.4. Mechanisms behind improved nutrient uptake

The results demonstrate that combining BF with CF creates a synergistic effect, enhancing nutrient uptake. Various studies support this. Scuderi et al. (2011) found that inoculating lettuce plants with beneficial rhizosphere microorganisms significantly increased N content, likely due to improved root architecture and nutrient absorption. Hestrin et al., (2019) showed that synergistic interactions between AMF and soil microbial communities enhance nitrogen acquisition, leading to a tenfold increase in N uptake compared to non-mycorrhizal plants. Kalamulla and Yapa (2024) demonstrated that combining AMF with PGPR enhances nutrient acquisition, improving plant growth and productivity compared to single inoculants. Barea et al. (2002)

reported that the combination of AMF, phosphate-solubilizing bacteria, and nitrogen- fixing rhizobia significantly increased the availability of rock phosphate for legumes, a principle applicable to lettuce cultivation. Similar to our findings, the use of *Bacillus spp.* as a biofertilizer significantly increased the uptake of macronutrients (N, P, and K) and micronutrients (Fe, Zn, Cu, and B) in lettuce, when applied in combination with chemical fertilizers (Pagliarini et al., 2023).

The present study confirms the enhanced P uptake of plants through biofertilizers, containing particularly AMF and PGPR. The mechanisms involved in more P uptake of plants are producing organic acids and enzymes, solubilizing insoluble phosphate compounds in the soil by PGPR (Saxena et al., 2013; Widada et al., 2007) and extending root system through their hyphal network, allowing plants to access P from a larger soil volume by AMF (Santoyo et al., 2021; Xu et al., 2024). In addition, our results showed that Fe and Zn content in the leaves following the combination of biofertilizers and chemical fertilizers. This is likely due to siderophore-producing and solubilizing ability of PGPR and extension of the root system by AMF that enable better transportation and uptake of Fe and Zn (Nguyen et al., 2019; Widada et al., 2007).

These findings support the use of integrated nutrient management strategies to improve plant growth, yield, and nutrient uptake in lettuce cultivation.

4. Conclusion

This study provides strong evidence that integrating biofertilizers with chemical fertilizers significantly enhances both the yield and nutritional quality of lettuce. The combined use of Bio Veria® and Bacillus Mix® with chemical fertilizers demonstrated a synergistic effect, leading to substantial improvements in fresh and dry weight, and overall yield compared to individual fertilizer applications or the control. Among the tested treatments, CF+BF_2 demonstrated the greatest effectiveness the potential of multi-species containing biofertilizers in optimizing lettuce production since it outperforms other treatments by achieving the highest yield and nutrient uptake.

Moreover, the observed increases in macronutrient (N, P, K, Ca, and Mg) and micronutrient (Fe, Zn, Mn, and Cu) content in the leaves further highlight the benefits of this integrating biofertilizers with conventional fertilization strategies. The enhanced nutrient uptake can be attributed to the complementary mechanisms of PGPR and AMF, including nutrient solubilization and improved root architecture. However, future studies are needed to explore the long-term effects of this combined application on soil health and agro-ecosystems.

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