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Enhancing growth and flowering of petunia (*Petunia hybrida* L.) through the application of jujube biochar and vermicompost

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ABSTRACT

Purpose: This study investigates the enhancing growth and flowering traits of petunia (*Petunia hybrida* L.) through the application of biochar and vermicompost. **Research Method:** The experiment employed a completely randomized design with four replications in a greenhouse setting during the years of 2022-2023. The experimental treatments comprised the control group (without vermicompost and biochar), vermicompost at 5% by weight, jujube biochar at 2% by weight, or combination of vermicompost with biochar. **Findings:** Results revealed that the application of vermicompost significantly increased the dry weight of root, shoot, and total biomass by 23%, 51%, and 46%, respectively, compared to the control. Additionally, the vermicompost treatment yielded the highest number of leaves and plant height, while the biochar treatment resulted in the maximum number of flowers per plant. The durability of flowers on the plant varied, with biochar treatment exhibiting the highest durability (6.75 days) while control treatment gave the lowest durability (4.75 days). Biochar-treated plants also displayed the highest levels of total chlorophyll and relative water content in the leaves, exhibiting increases of 29% and 14%, respectively, compared to the control. Leaf nutrient content demonstrated significant changes, with the biochar + vermicompost treatment exhibiting the highest nitrogen and potassium content, demonstrating a 34% and 19% increase, respectively, compared to the control. **Research limitations:** No limitations were identified**. Originality/Value:** In summary, the findings underscore the positive influence of biochar and vermicompost fertilizers on the growth, ornamental features, and physiological characteristics of petunia. Notably, biochar demonstrated superior effectiveness in enhancing ornamental parameters compared to vermicompost. Biochar and vermicompost can be used as organic fertilizers to reduce the use of chemical fertilizers and increase the production and yield of petunia plant.

INTRODUCTION

The petunia (*Petunia hybrida* L.) plant is a perennial ornamental species belonging to the Solanaceae family (Sahu et al., 2023). Indigenous to South America, the petunia has gained widespread popularity as one of the most favored seasonal plants for urban green spaces, owing to its undemanding nature and ease of cultivation (Chen et al., 2017). Renowned for its aesthetically pleasing features, including a captivatingly beautiful form, flowers with vibrant colors, and extended flowering duration, the petunia holds a distinguished position among the world's finest plants (Keykha et al., 2016).

Nutrition management plays a crucial role in enhancing ornamental plants' yield and quality (Davoudi & Bayat, 2024). An optimal bed for these plants should possess attributes such as high water-holding capacity, proper ventilation, effective drainage, and the incorporation of organic matter. Utilizing organic fertilizers stands out as a key method to foster the growth and overall performance of ornamental plants, leading to the production of flowers with desirable quality. In soil fertility and quality, organic matter emerges as a paramount factor. Particularly in arid and semi-arid regions, the deficiency of organic matter is more pronounced. In such instances, farmers often prioritize using organic matter to augment crop production. This practice is especially prevalent in areas characterized by higher organic matter poverty (Zhang et al., 2020; Lachkar et al., 2021; Yan et al., 2022). Organic matter stands out as a pivotal factor influencing the fertility of agricultural land. Typically, intensive agricultural practices reliant on chemical fertilizers lead to a reduction in soil organic content, consequently diminishing the quality of agricultural products (Vahidi et al., 2022; Yan et al., 2022). Moreover, the excessive use of mineral fertilizers to boost crop production poses heightened risks to human health and contributes to environmental challenges, such as water and soil pollution (Suthar, 2021). Consequently, the adoption of organic fertilizers has been recognized as a viable solution to promote sustainable agriculture (Lehmann & Joseph, 2015; Jatuwong et al., 2024). Within the realm of sustainable agriculture, there has been a notable focus on the utilization of biochar and vermicompost as innovative alternatives to chemical fertilizers. These methods serve as sustainable modifiers to enhance soil fertility and reduce reliance on chemical fertilizers in agriculture (Mak-Mensah et al., 2021). Biochar, a product derived from the pyrolysis of natural organic materials in the absence or limited presence of oxygen, has demonstrated diverse mechanisms for improving plant growth. These include the augmentation and preservation of nutrients (You et al., 2021), enhanced availability of elements for plants (Xi et al., 2020), and alterations in the soil microbial population (Zheng et al., 2018). Additionally, biochar has been observed to enhance plant tolerance to abiotic stresses such as drought, salinity, and heat (Yoo et al., 2020; Liang et al., 2021; Fedeli et al., 2024). The distinctive properties of biochar position it as a valuable soil conditioner. It not only provides essential elements to plants but also contributes to the removal of mineral pollutants from water and soil, mitigating the adverse effects of heavy metals or pesticides (Du et al., 2021; Vahidi et al., 2023). Moreover, biochar plays a role in increasing carbon sequestration in the soil, reducing the release of greenhouse gases, and enhancing nutrient consumption efficiency, thereby promoting plant growth and performance (Ginebra et al., 2022; Bhatia et al., 2021). Similarly, vermicompost, as an organic fertilizer, exerts positive effects on the physical, chemical, and biological properties of the soil. Serving as a rich source of vitamins, essential and trace elements, growth-stimulating hormones, and enzymes, vermicompost enhances the soil microbial community, fostering nutrient absorption for sustainable plant growth in agriculture (Manzoor et al., 2024). In recent years, numerous studies have investigated the impact of biochar on soil fertility and the yield and quality of various crops, displaying its effectiveness across a range

of plant species. Notable examples include its positive effects on lettuce (*Lactuca sativa* L.) (Jabborova et al., 2021), mullein (*Verbascum thapsus*) (Esfahani et al., 2023), and periwinkle (*Catharanthus roseus* L.) (Mohammadi Kabari et al., 2024). Similarly, vermicompost has demonstrated its ability to significantly enhance the growth and yield of diverse plant varieties, including cucumber (Piri & Rashki, 2019) and tomato (Zucco et al., 2015). A growing trend in agricultural research is the exploration of combined biochar and vermicompost applications to restore soil fertility, improve overall plant growth, and yield (EL-Mogy et al., 2024). Several studies have indicated that the simultaneous use of biochar and vermicompost positively influences the physicochemical and microbial properties of the soil, thereby contributing to increased plant growth and performance (Zhang et al., 2016; Lu et al., 2020). Notably, findings from Liu et al. (2022) demonstrate the positive impact of biochar and vermicompost on tomato yield. Additionally, research by Lin et al. (2015) reveals increased bean biomass and seed yield associated with the application of biochar and vermicompost.

The jujube plant, (*Ziziphus jujuba* Mill.), is indigenous to China and is found in various regions of Iran with a cultivated area spanning 3621 hectares and an annual production of 5460 tons of dry jujube; Iran ranks as the third-largest producer of this medicinal and important fruit tree after China and South Korea (Ebrahimi et al., 2022; Zeraatgar et al., 2019). This tree has gained significance in arid and semi-arid lands due to its remarkable resilience and adaptation to drought, as well as infertile and saline soil (Liu et al., 2022; Vahidi, 2020). Despite its agricultural importance, a substantial amount of pruned jujube foliage is annually discarded as waste without any specific or planned utilization in nature. Consequently, there is a pressing need to develop innovative methods for repurposing this waste (Vahidi et al., 2022). The waste, comprising jujube branches, leaves, and cores, can be immediately utilized as raw material for biochar production (Vahidi et al., 2022; Zhang et al., 2020). Biochar derived from jujube residues proves to be an effective and economical solution for enhancing soil properties, mitigating erosion, and managing waste (Vahidi et al., 2022; Al Wabel et al., 2021). The primary objective of incorporating biochar into soil is to facilitate the recycling of organic waste and improve soil conditions. Given the rich composition of both high and low-use nutrients in biochar and vermicompost, these fertilizers play a crucial role in enhancing plant growth and performance. Despite various studies on the subject, there is a notable absence of experiments exploring the impact of jujube biochar on the growth and performance of plants. Therefore, the focus of this study is to investigate the influence of jujube biochar and vermicompost on the growth, ornamental features, and physiological characteristics of the petunia plant.

MATERIALS AND METHODS

Plant material and treatments

This study seeks to examine the impact of biochar derived from the remnants of the jujube plant (*Ziziphus jujuba* Mill.) and vermicompost on the morphological, physiological, and biochemical traits of the petunia plant. The investigation was conducted using a completely randomized design with four replications in the research greenhouse of University of Birjand, Iran during the period 2022-2023. The experimental treatments comprised four groups: the control group (without the application of biochar or vermicompost), vermicompost at a 5% weight ratio, jujube biochar at a 2% weight ratio, or a combination of biochar at 2% with vermicompost at 5% by weight. Each replication involved four pots, each housing one petunia plant. The experiment utilized sixteen pots with a diameter and height of 14 cm. prior to use, the soil for the experiment underwent air drying and was subsequently sieved through a 2 mm

mesh. The physical and chemical properties of the soil were then assessed using standard methods (Wilke, 2005) (Table 1).

Petunia seeds (*Petunia hybrida* L.) were sourced from Poponik Company in Tehran, Iran. The cultivation process began with the seeds being planted in 105-hole seedling trays, each with a diameter of 2.3 cm and a height of 5 cm. The trays contained a blend of cocopeat and peat in a 2:1 ratio. After 45 days of seed germination, individual seedlings at the 6-8 leaf stage were transplanted into separate pots. Throughout the experiment, irrigation was conducted twice a week, providing 250 ml of water per pot, tailored to the plant's specific needs. Subsequent to the seedling establishment (14 days after transplantation), key growth parameters, including the number of leaves and plant height, were measured at 10-day intervals. The comprehensive assessment of growth, reproductive, and physiological parameters took place at the conclusion of the experiment, 155 days post-seed sowing. The biochar utilized in the experiment was prepared using pruned leaves from the jujube tree. After air-drying, the leaves were crushed, and each sample was subjected to 24 hours of exposure in an oven at 105 degrees Celsius. Following this, the material was further crushed into smaller fragments. During the thermal decomposition inside the furnace, vapors were released, and a stainless metal container was employed to generate biochar. The furnace environment's oxygen was restricted with the use of a candle. The chopped jujube pieces were then subjected to a temperature of 300 degrees Celsius for 2 hours, resulting in the production of biochar. The final product was sieved through a 2 mm mesh. (Table 1) provides some physiochemical properties of the produced biochar. Additionally, (Table 1) presents selected chemical characteristics of the vermicompost used in the experiment.

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

	Initial Jujube	Jujube biochar	Vermicompost	Soil
Oxygen %	23.4	12.4	33.9	
pH	7.2	8.9	6.9	7.9
Saturation percentage (Sp)				27
EC (mmho/cm)	6.8	4.5	1.58	2.3
Carbon (%)			11.88	
Hydrogen(%)	7.2	3.4	4.22	
Nitrogen(%)	0.42	0.76	1.02	0.78
Phosphorous(%)	0.19	0.42	0.3	13.8
Calcium(%)	3.55	6.23	0.17	5.13
Potassium(%)	0.42	0.66		0.53
Magnesium(%)	0.06	0.17	0.06	2.53
Ca+Mg				8
C/N	125.48	92.37	11.64	
Organic carbon(%)	52.7	76.2		
Sodium(%)	0.06	0.12		
Bulk density	0.57	0.68	0.54	1.81
Biochar performance (%)		29		2.65
Ash biochar (%)		14.2		
Sand				76
Silt				18
Clay				6
Organic matter				0.2
Textural				Loamy Sand

Measurements

Arnon's approach involved the quantification of chlorophyll and carotenoid content (Arnon, 1949). The absorbance levels were then assessed at 470, 645, and 663 nm wavelengths using a spectrophotometer (Model Unico 2100, China).

Leaf relative water content (RWC) was determined according to Galmes et al (2007) method. Sampling involved collecting newly matured leaves, followed by the measurement of their fresh weight (FW). Subsequently, the samples were immersed in distilled water for 24 hours, and the saturated weight (SW) of the leaves was recorded. To determine the dry weight (DW), the samples were then subjected to an oven at a temperature of 70°C for 24 hours. The calculation of RWC was performed using the formula (1):

$$RWC = \frac{(FW-DW)}{(SW-DW)} \times 100 \quad (1)$$

To determine nitrogen, phosphorus, potassium, calcium, and magnesium concentrations, leaf samples collected during the flowering stage underwent a series of processes. After drying and pulverizing, phosphorus, potassium, calcium, and magnesium elements were digested using concentrated hydrochloric acid. Potassium concentration was measured using a Film photometer device (Model JENWAY-PFP7), and phosphorus concentration was determined with a spectrophotometer at a wavelength of 660 nm. Magnesium and calcium concentrations were assessed using the EDTA complexometric method. The detailed methodology, including specific procedures and equipment, follows protocols outlined by Jones (2005) for potassium, magnesium and calcium. Nitrogen content was determined using the Kjeldahl method, wherein 1 gram of plant sample and 6 grams of catalyst were placed into Kjeldahl digestion balloons. Subsequently, 15 ml of concentrated sulfuric acid (98%) was added. Following digestion and cooling, 100 ml of distilled water was introduced into each balloon. Erlenmeyer flasks were then prepared corresponding to the number of balloons, and 20 ml of reagent was added to each flask. Each balloon, accompanied by its dedicated flask, was positioned within the distillation apparatus. Upon completion of the distillation process, the solution in the flasks underwent titration with 0.05 normal sulfuric acid until a pink coloration emerged (Jones, 2005).

Data analysis

Data were subjected to analysis of variance (ANOVA) using the JMP 13 software (SAS Campus, Cary, NC, USA), and graphical representations were generated using Excel software. Mean comparisons were performed employing the Least Significant Difference (LSD) Test at a 5% probability level.

RESULTS

Growth and reproduction traits

The analysis of variance results indicated that the fertilizer treatments significantly influenced various growth and reproductive traits (Table 2). Comparing the average growth parameters (Table 2) revealed a 32% increase in leaf length with biochar application compared to the control. The highest number of branches was observed in the control, vermicompost, or biochar + vermicompost treatments, while the lowest value was observed in biochar treatment. The maximum stem diameter recorded in the biochar treatment, which showing a 28% increase compared to the control. Stem diameter was greater in the vermicompost or biochar treatments compared to control or biochar + vermicompost treatments. Root length

values were highest in the control or biochar treatments, followed by vermicompost or biochar + vermicompost treatments, which exhibited the lowest root length values. Biochar application led to an 11% increase in root fresh weight. Plants treated with vermicompost showed the highest root dry weight, reflecting a 23% increase. The fresh weight of shoots was greatest in plants treated with biochar, indicating a 35% increase compared to the control. Aerial parts' dry weight in plants treated with vermicompost surpassed the control or vermicompost + biochar treatments, with the highest dry weight recorded in the vermicompost treatment, showing 51% increase. The total dry weight of plants treated with vermicompost or biochar exceeded that control or vermicompost + biochar treatments, with 46% increase in dry weight for the vermicompost treatment and 38% increase for the biochar treatment (Table 2). During the observation period for leaf number changes, plants treated with vermicompost exhibited more leaves than other treatments, followed by biochar or biochar + vermicompost treatments (Fig. 1). The trend in plant height changes indicated that plants treated with vermicompost were taller than other treatments, followed by biochar or control treatments (Fig. 2).

Table 2. Effects of vermicompost or jujube biochar or their combination on growth parameters of petunia (*Petunia hybrida* L.).

Treatment	Leaf width (cm)	Leaf length (cm)	Number of branch per plant	Stem diameter (mm)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Total dry weight (g)
Control	3.22 a	6.55 b	5.25 a	3.66 b	30.25 a	29.02 a	2.49 b	67.68 c	6.41 b	8.91 b
Vermicompost	3.35 a	7.75 ab	5.00 a	4.31 a	22.25 b	27.89 a	3.07 a	78.58 b	9.96 a	13.03 a
Jujube biochar	3.17 a	8.67 a	3.00 b	4.70 a	28.00 a	32.17 a	2.64 b	91.79 a	9.69 a	12.34 a
Vermicompost+ Jujube biochar	3.12 a	7.37 ab	5.25 a	3.55 b	21.25 b	18.53 b	1.63 c	65.97 c	6.11 b	7.74 b
Significance level	ns	*	**	**	**	**	**	**	**	**

*, **, ns: Significance at %5 and %1 probability levels and non-significance, respectively. The LSD test indicates that there is no significant difference between the means in the columns that are followed by the same letter at P < 0.05.

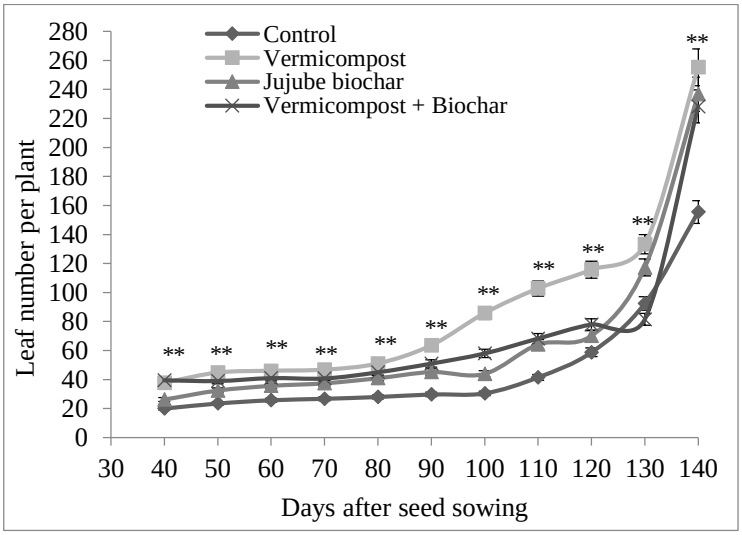

Fig. 1. Effects of vermicompost, jujube biochar or their combination on leaf number of petunia (*Petunia hybrida* L.) during the experiment period. **: Significance at %1 probability level.

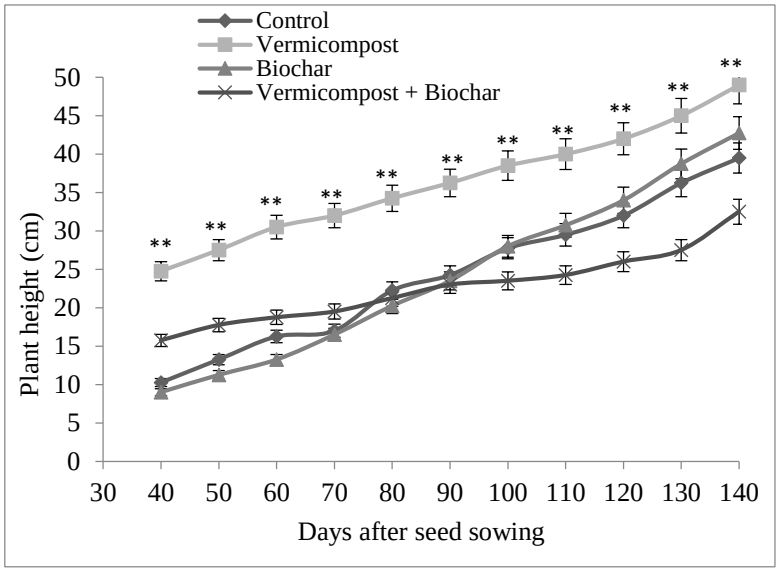

Fig. 2. Effects of vermicompost, jujube biochar or their combination on plant height of petunia (*Petunia hybrida* L.) during the experiment period. **: Significance at %1 probability level. Vertical bars indicate ± SE.

The results of the analysis of variance indicated that the flowering traits were statistically significant at a 1% probability level. The longest flower durability on the plant was observed in the biochar treatment (6.75 days), while the shortest was in the control treatment (4.75 days) (Fig. 3). Additionally, the highest (122 days) and lowest (104 days) number of days until flowering were recorded in the biochar and vermicompost treatments, respectively (Fig. 4). Investigation into the changes in the number of flowers revealed that the biochar treatment recorded the highest number, followed by vermicompost, control, or biochar + vermicompost treatments, respectively (Fig. 5).

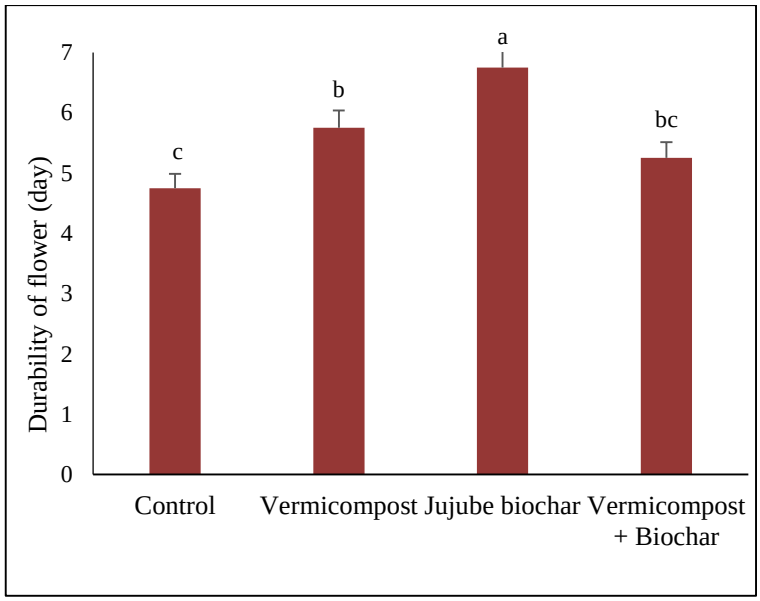

Fig. 3. Effects of vermicompost, jujube biochar or their combination on flower durability of petunia (*Petunia hybrida* L.). The LSD test indicates that there is no significant difference between the means in the columns that are followed by the same letter at $P < 0.05$. Vertical bars indicate ± SE.

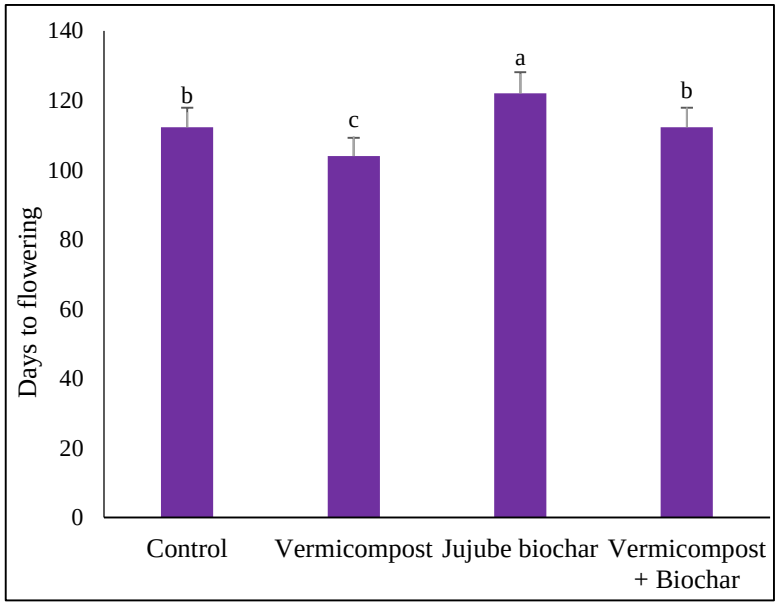

Fig. 4. Effects of vermicompost, jujube biochar or their combination on flower durability of petunia (*Petunia hybrida* L.). The LSD test indicates that there is no significant difference between the means in the columns that are followed by the same letter at $P < 0.05$. Vertical bars indicate ± SE.

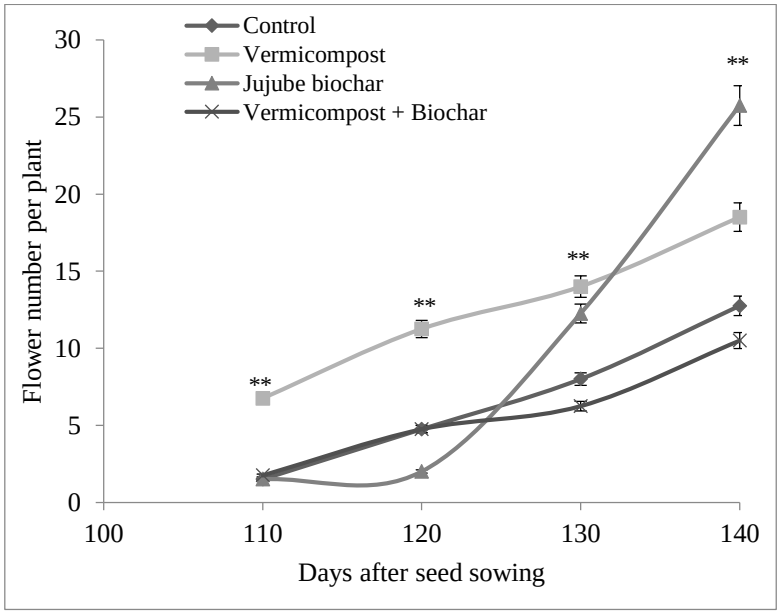

Fig. 5. Effects of vermicompost, jujube biochar or their combination on flower number per plant of petunia (*Petunia hybrida* L.) during the experiment period. **: Significance at %1 probability level. Vertical bars indicate ± SE.

Table 3. Effects of vermicompost, jujube biochar or their combination on chlorophylls, carotenoids, and relative water content (RWC) of petunia (*Petunia hybrida* L.).

Treatment	Chlorophyll a (mg. g FW^{-1})	Chlorophyll b (mg. g FW^{-1})	Total Chlorophylls (mg. g FW^{-1})	Carotenoids (mg. g FW^{-1})	RWC (%)
Control	0.63 b	0.21 b	0.84 b	0.19 b	73.01 c
Vermicompost	0.77 ab	0.29 a	1.06 a	0.28 a	80.75 b
Jujube biochar	0.82 a	0.26 a	1.09 a	0.24 a	87.52 a
Vermicompost+ Jujube biochar	0.44 c	0.19 b	0.63 c	0.14 c	71.05 c
Significance level	**	**	**	**	**

**: Significance at %1 probability level. The LSD test indicates that there is no significant difference between the means in the columns that are followed by the same letter at P < 0.05.

Table 4. Effects of vermicompost, jujube biochar or their combination on leaf nutrient content of petunia (*Petunia hybrida* L.).

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Control	2.49 c	0.27 b	4.25 b	1.62 a	0.34 c
Vermicompost	2.85 b	0.42 a	4.31 b	1.20 bc	0.63 b
Jujube Biochar	2.50 c	0.14 d	3.86 b	1.45 ab	0.42 c
Vermicompost+ Jujube biochar	3.36 a	0.21 c	5.07 a	1.17 c	0.79 a
Significance level	**	**	**	**	**

**: Significance at %1 probability level. The LSD test indicates that there is no significant difference between the means in the columns that are followed by the same letter at P < 0.05.

Physiological traits

The results of analysis variance for photosynthetic pigments exhibited statistical significance at the 1% probability level (Table 3). According to the average comparison results (Table 3), the biochar treatment yielded the highest chlorophyll content, surpassing the control by 30%. Similarly, plants treated with vermicompost showed the highest chlorophyll b content, representing a 38% increase. The biochar treatment also resulted in the highest total chlorophyll content, demonstrating a 29% increase. Carotenoid levels in plants treated with vermicompost or biochar surpassed those in the control or biochar + vermicompost treatments. Specifically, the highest carotenoid content was observed in the vermicompost treatment, indicating a 47% increase. The RWC in plants treated with biochar or vermicompost exceeded that of other treatments. Notably, the use of biochar led to a 14% increase in RWC (Table 3).

Nutrient elements

The results of the variance analysis regarding the impact of fertilizer treatments on leaf nutrient content were statistically significant at the 1% probability level (Table 4). According to the average comparison results (Table 4), plants treated with vermicompost + biochar exhibited the highest nitrogen content, representing a 34% increase. The application of vermicompost led to a 55% increase in phosphorus content. The highest potassium content was observed in plants treated with vermicompost + biochar, showing a 19% increase with respect to control. Regarding calcium content, plants treated with the control or biochar had significantly higher levels compared to other treatments. The highest magnesium content was recorded in the vermicompost + biochar treatment, showing an increase of 2.3 times with respect to control (Table 4).

DISCUSSION

The outcomes of this experiment demonstrate that treating petunia plants with jujube biochar or vermicompost enhances both vegetative and reproductive indicators. Previous studies support these findings, with research by Jabborova et al. (2021) noting significant improvements in plant height, leaf length, leaf number, and root length in lettuce treated with cherry biochar. Similarly, Zulfiqar et al. (2021) reported enhanced growth parameters in ginger plants (*Zingiber officinale*) following the application of wheat biochar. Additionally, Safari et al. (2023) found that a 10% weight application of rice husk biochar positively impacted the growth indicators of *Lolium perenne* L. The use of biochar or vermicompost, in addition to directly improving nutritional conditions by adding nutrients to the soil, has positive effects on the environmental conditions of plant growth and nutrition. These effects can also be indirectly facilitated through increasing soil porosity and promoting root growth. Consequently, they contribute to enhancing the growth and reproductive characteristics of plants, aiding in their overall development and expansion (Roy et al., 2022; Sahu et al., 2023; Safari et al., 2023). The benefits of biochar are not limited to petunia plants, as evidenced by the work of Reddy et al (2023), who reported that rice husk biochar improved the dry weight and ornamental parameters of African parsley (*Tagetes erecta* L). Likewise, Conversa et al. (2015) observed increased leaf and flower numbers in *Pelargonium zonale* L. with the use of *Abies alba* mill biochar. Furthermore, Goswami et al. (2017) found that vermicompost fertilizer significantly influenced the growth parameters of cabbage. It appears that due to the presence of essential nutrients, especially nitrogen, phosphorus, calcium, and trace elements, biochar plays a significant role in plant growth and flowering. Its ability to provide these nutrients, along with its improvement of soil conditions, leads to increased plant growth and development (Reddy et al., 2023). In various studies, vermicompost has shown positive effects on plant growth and flowering. Esfahani et al. (2023) reported increased reproductive parameters in the mullein (*Verbascum thapsus*) plant, while Kural & Coşkan (2023) found that vermicompost application enhanced flower life, daily flower yield, flower bloom, and flower count in the Oily Rose plant. The use of vermicompost in plant growth substrates, by modifying substrate characteristics and improving the accessibility and uptake of nutrients, leads to enhanced plant growth (Farjana et al., 2019; Manzoor et al., 2024). In this study, it also resulted in improvements in growth and reproductive indices in the petunia plant. Both vermicompost and biochar positively influence petunia growth and flowering by enhancing soil physical and chemical structures, increasing nutrient availability, and improving water retention capacity. Notably, the current study suggests that the effect of biochar surpasses that of vermicompost in improving reproductive traits. The combined application of biochar and vermicompost did not yield favorable outcomes, which may be due to the nutrient toxicity and disruption of the physicochemical properties of the soil (Li et al., 2022).

The outcomes of this experiment revealed that the addition of biochar or vermicompost to the soil augmented the chlorophylls, carotenoid, and RWC in the leaves of petunia plants. These findings align with the results reported by Younis et al. (2016), who observed enhanced chlorophylls and carotenoid levels in spinach (*Spinacia oleracea* L.) plants with the application of ear cleaning biochar (ear cotton). In lettuce (*Lactuca sativa* L.), Jabborova et al. (2021) found that the application of 3% cherry biochar led to increased chlorophyll and carotenoid content. Altaf et al. (2021) reported heightened chlorophyll levels in the leaves of *(Matthiola incana* and *Pelargonium* spp.) with the application of plant waste biochar. Additionally, Esfahani et al. (2023) observed increased chlorophyll content in mullein plants with the application of 8 kg of vermicompost per square meter. The positive impact of vermicompost and biochar on photosynthetic pigments can be inferred from their role in

providing low-use and high-use nutrients to these pigments. The application of biochar and vermicompost enriches the soil and plants with these essential elements, ultimately leading to an increase in chlorophyll content in the plant (Theunissen et al., 2010). Furthermore, nitrogen, a vital component of all amino acids in proteins and fats, serves as a structural compound for chloroplasts (Farhan et al., 2024). The findings of this report align with those of Jabborova et al. (2021), who reported an increase in the RWC in lettuce with the use of cherry biochar. Additionally, Yoo et al. (2021) observed an augmentation in the RWC of spinach leaves with the application of biochar derived from the waste of a local Korean brewing company, Jeongnim-dong. The capacity of biochar and vermicompost to absorb and retain water in the soil may be the reason for the observed improvement in RWC in plants. This suggests that soils modified with biochar exhibit enhanced water retention capabilities, providing more optimal humidity conditions for plant growth throughout the growth period (Esfahani et al., 2023).

The application of biochar or vermicompost has influenced the nutrient content in petunia leaves. The treatment involving biochar + vermicompost resulted in the highest levels of nitrogen, potassium, and magnesium, while the highest phosphorus content was observed with vermicompost alone. Similar findings were observed in rapeseed treated with vermicompost + biochar, where potassium concentration in aerial plant parts was highest compared to other treatments (Mamnabia et al., 2020). Vermicompost, known for promoting nitrogen fixation and phosphorus solubilization, enhances the availability of these nutrients (Zucco et al., 2015). The organic matter in vermicompost, coupled with its ability to stimulate soil microorganisms, facilitates the plant's access to otherwise unavailable resources, particularly phosphorus. Contrarily, a study by Karimi et al. (2020) reported that the use of biochar did not significantly affect calcium levels in marigolds. The impacts of biochar and vermicompost on soil calcium and sodium are intricate and depend on various factors. While biochar may contribute to calcium content, its impact on sodium absorption is not well-established. Vermicompost, being nutrient-rich, likely enhances calcium availability without introducing significant sodium, though the specific outcomes hinge on factors such as the type and quality of biochar and vermicompost, soil composition, and environmental conditions. The combined use of vermicompost and biochar, owing to their high specific surface area, cation exchange capacity, and soluble solutes, has improved nutrient availability for petunia plants, potentially enhancing overall plant performance (Kulczycki et al., 2020). The gradual release of nutrients from vermicompost and biochar contributes to sustained availability, resulting in increased nutrient levels in the petunia plant (Lehmann et al., 2011). The use of biochar and vermicompost in soils deficient in organic matter can improve the physical, chemical, and biological properties of the soil, leading to increased levels of calcium and magnesium in the soil. However, a significant increase in these elements in plants may not be observed. This could be attributed to factors such as the precipitation of these elements in the soil or competition with other elements. Biochar and vermicompost typically contain various amounts of nutrients such as calcium, magnesium, potassium, and other elements. Their use can enhance plant access to these nutrients, thereby improving plant quality and performance (Zucco et al., 2015; Karimi et al., 2020)

CONCLUSION

The current study's findings highlight the positive impact of employing biochar or vermicompost fertilizers on enhancing the growth, ornamental features, and physiological attributes of the petunia plant. However, the effectiveness of biochar, particularly in improving ornamental parameters, surpassed that of vermicompost. The combined application

of biochar and vermicompost did not yield favorable outcomes, so further research on their concentrations can be conducted in the future. Consequently, the use of these fertilizers emerges as a viable solution to diminish reliance on chemical fertilizers, stabilize soil resources, mitigate environmental pollution, and ultimately yield a healthier product.

Conflict of interest

The authors declare no conflict of interest.

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Foliar application of L-phenylalanine, sodium selenate, and nitroxine biological fertilizer can improve antioxidant and phytochemical properties of goji berry (*Lycium barbarum* L.)

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ABSTRACT

Purpose: *Lycium barbarum* berries can be a source of natural antioxidants for human food production. **Research method:** To increase the antioxidant activity of secondary metabolites in goji berry seedlings, we applied amino acid L-phenylalanine (Phe: 0.5, 1, and 1.5 mM), sodium selenate (Se: 0.25, 0.5, and 1 mg. L^{-1}), and nitroxine biological fertilizer (170, 330, and 500 µL.L^{-1}) at three levels. Distilled water was the control treatment. The experiment took place at the research farm of Ferdowsi University of Mashhad during 2021-2022. **Findings**: The results revealed that the treatments significantly affected goji berry plants regarding physiological and chemical attributes. Phenylalanine, selenium, and nitroxine substantially affected photosynthetic pigments, including chlorophyll and carotenoid, antioxidant, and catalase during the two years of foliar application. The results showed that phenylalanine with selenium increased the amounts of flavonoids, anthocyanins, and carbohydrates in goji berry plants. Applying phenylalanine alone had a positive, more potent effect on the amount of phenylalanine ammonia-lyase enzyme, which shows the impact of this substance on the phenylpropanoid pathway. Using it with nitroxine enhanced the phenol content and superoxide dismutase activity significantly. **Research limitations:** There was no limitation. **Originality/Value:** According to the results of this experiment, during the two years of 2021 and 2022, phenylalanine improved antioxidant enzyme activity and other traits significantly. Using phenylalanine and sodium selenate at low concentrations increased all antioxidant compounds and improved plant growth.

INTRODUCTION

The Lycium genus of the Solanaceae family has excellent nutritional and medicinal value. Two species *Lycium barbarum* L. and *Lycium chinense* comprise the majority of the genus. The plant bears a fruit often called wolfberry or goji berry (Jiang et al., 2021). The red goji berry (*Lycium barbarum* L.) is a deciduous perennial shrub that grows in northwest China and the Mediterranean region (Xin et al., 2017). It has oval leaves with a curved spear-shaped tip. Its leaves and shoots appear in opposite groups on the branches. Its leaves are 7 cm long and 3.5 cm wide (Oğuz et al., 2022). Goji berry fruits can be a source of natural antioxidants in producing functional foods. So far, most studies have focused on phenolic compounds in *Lycium* leaves and root bark (Qian et al., 2017; Jiang et al., 2021). Studies have shown that fruits contain polysaccharides, carotenoids, flavonoids, vitamins, and essential oils. These compounds cause hepatic, hypoglycemic, lipid-lowering, anticancer, immune-stimulant, anti-fatigue, and neuroprotective properties (Ozkan et al., 2018). Among the dozens of *Lycium* species distributed worldwide today, approximately 90% of commercial goji berry (wolfberry) products appear in nutritional supplements, flavored teas, juices, jams, snacks, soups, and other foods (Yossa Nzeuwa et al., 2019).

Using amino acids for horticultural crops is a worldwide practice, and amino acids constitute organic fertilizers while acting as biostimulants (Colla et al., 2015). The effect of amino acids on plants depends on the type of amino acid supplied and the type of plant (Khan et al., 2019). Amino acids are biostimulants that promote plant growth, improve nutrient availability, and enhance plant performance (Khan et al., 2019). Amino acids also directly or indirectly affect performance (Abd El-Aal et al., 2010). Amino acids are a part of plant proteins that partake in biological and functional roles (Shetta & Zayed, 2016). They are the primary chains in protein structures and facilitate plant growth (Hashem et al., 2016). These protein subunits crucially function in enzymes, vitamins, alkaloids, terpenoids, etc., and other plant metabolites.

Phenylalanine is a precursor to many energetic secondary metabolites, such as phenylpropanoids, flavonoids, lignin, and anthocyanins. The phenylpropanoid metabolic structure has many features, especially for protecting against abiotic and biotic stresses (Tzin & Galili, 2010; Heydarnajad Giglou et al., 2024). Phenylalanine helps in producing fragrant compounds, antioxidants, lignin, anthocyanins, phenols, and cellulose as the primary tissue of the plant cell wall, as an ethanol carrier, and in the first segment of anthocyanin biosynthesis, phenylpropanoids, and flavonoids.

In metabolic tactics of plant cells, compared to previous techniques, iron is less expensive and helpful in disposing of anthocyanins (Edahiro et al., 2005). Research by Sarojnee et al. (2009) indicated that treating hot pepper (*Capsicum annum* L.) plants with amino acids sizably increased the plant canopy size, stem diameter, range of branches, and dry shoots, fruit length, fruit diameter, dry fruit percentage, and ascorbic acid content. Phenylalanine, tyrosine, and tryptophan are crucial components for plant protein synthesis, but they are precursors to various secondary metabolites essential for plant growth and human nutrition (Sarojnee et al., 2009). According to Watanabe et al. (2017), phenylalanine is a crucial element that can increase the folic acid content in spinach by two-fold. Amino acid administrations (20%) reportedly multiplied Chl and CLB contents. This increase contributed to leaf mineral enhancements, mainly by improving the photosynthetic mechanism (Garcia et al., 2011). Also, including amino acids in the nutrient solution improved the vegetative vigor of tomato plants while boosting the chlorophyll content and activity of antioxidant enzymes (Zhang et al., 2009).

Selenium (Se) is an essential component of selenoproteins and seleno-amino acids, contributing significantly to cellular growth and biological functions in both animals and humans. Due to its chemical similarity to sulfur, Se can substitute for S within biochemical systems, where it plays comparable roles. The absorption, transportation of simulated toxins, and replacement of sulfur with selenium can result in Se isotopes, which subsequently elevate Se concentrations within cells (Puccinelli et al., 2017). Abbas (2012) observed that low concentrations of selenate stimulated increases in chlorophylls, anthocyanins, sugars, proline, ascorbic acid, and enzymatic activities. Selenite also raised enzymatic antioxidant activities—such as ascorbic acid peroxidase and guaiacol peroxidase—and non-enzymatic antioxidants, including ascorbic acid and carotenoids, in sorghum seedlings. Moreover, Se enhanced carbohydrate and bioactive compound levels, including total flavonoid content, glutathione, and vitamins C and E in *Solanum lycopersicum* cv. Provence (Zhu et al., 2018). Overall, Se heightened antioxidant activity across plants, humans, animals, and microorganisms (Ramos et al., 2010). Sources of selenium, like selenate in low concentrations, have demonstrated a beneficial role in plant enhancement by acting as an antioxidant to promote growth; however, at elevated levels, Se adversely affects plant performance.

Biological fertilizers, comprising one or more beneficial soil organisms within a suitable carrier, play a crucial role in agriculture. Such fertilizers often include microorganisms capable of transforming nutrients from inaccessible to accessible forms through biological processes (Zahedyan et al., 2022). Zahedyan et al. (2022) confirmed that the application of nitroxine significantly influenced nutrient content, relative water content (RWC), and total antioxidant (TA) capacity. Additionally, plants treated with nitroxine showed notable increases in fresh and dry leaf and stem weights, as well as in chlorophyll a and b, carotenoids, and anthocyanin concentrations (Rahi, 2013).

Goji berry, specifically the GB1 variety, is widely cultivated in Iran and demonstrates compatibility with the country's diverse climates. Given the high economic value of goji berries compared to indigenous alternatives like blueberries and raspberries, cultivation efforts have intensified, particularly in Khorasan province. This study aims to assess the feasibility of goji berry cultivation in the region, optimizing growing conditions and exploring methods to enhance yield. Specifically, this research evaluates the effects of pre-harvest applications of the amino acid phenylalanine, selenium, and nitroxine biofertilizer on *Lycium barbarum* L.

MATERIALS AND METHODS

Plant materials

This experiment took place from 2021 to 2022 at a research farm at Ferdowsi University of Mashhad, Iran. In early May, we planted goji berry seedlings under field conditions to evaluate the effects of L-phenylalanine (Phe), sodium selenate (Se), and nitroxine on plant growth and yield. We applied these treatments through foliar spraying before harvest and throughout the growth period.

We planted two-year-old goji berry seedlings, cultivar GB1, sourced from Mashhad Seedling Company in Razavi Khorasan province, with 150 cm spacing between both rows and seedlings. We used a strip-drip irrigation system tailored to local climatic conditions, watering every two days, and performed weeding consistently throughout the growing season. We used a randomized complete block design (RCBD) with five replications to assess the effects of L-phenylalanine, selenium, and nitroxine. The experimental factors included three concentrations of L-phenylalanine (Phe: 0.5, 1, and 1.5 mM), sodium selenate (Se: 0.25, 0.5, and 1 mg L^{-1}), and nitroxine biological fertilizer (170, 330, and 500 µL L^{-1}), while distilled

water served as the control. Each row in the field represented one block for each repetition, and we established ten treatment groups, including the control. Following initial plant establishment, we applied foliar sprays every 15 days across three growth stages.

Soil preparation included treatment adjustments based on pre-planting soil nitrogen levels, as determined through soil analysis (Table 1). To enhance soil fertility, we applied vermicompost at a rate of 7.8 tons per hectare. After ensuring initial plant establishment, we administered the first foliar spray containing amino acid phenylalanine, selenium, and nitroxine, with subsequent applications at 15-day intervals across three stages. We sprayed all treatments at the same times and used distilled water for the control plants' foliar applications. After completing three stages of foliar applications, we conducted analyses to determine chlorophyll, carotenoid, carbohydrate, phenol, and flavonoid content. We also measured antioxidant activity, antioxidant enzyme activities (phenylalanine ammonia-lyase [PAL], superoxide dismutase [SOD], and catalase [CAT]), and anthocyanin content across all seedlings. Table 1 presents the soil analysis results.

Leaf area and specific leaf area

Leaf surface measurements involved using a leaf surface measuring device (CID-CI2002 made in America) to calculate the average leaf surface in square centimeters (Gong et al., 2013). The specific goji berry leaf area was calculated by the following equation (1):

$$SLA = LA / LDW \quad (1)$$

Where LA: leaf area and LDW: leaf dry weight.

Photosynthetic pigments

To evaluate photosynthetic pigment contents, we collected three leaves from three plants per replicate in each treatment. We extracted photosynthetic pigments from 0.5 g of fresh leaves using 80% acetone. Then, we measured the absorbance of the extracts at 663 and 645 nm for chlorophyll and at 480 and 510 nm for carotenoids, using a spectrophotometer (ND One, Thermo Fisher, USA). We expressed concentrations of total chlorophyll and carotenoids as mg g^{-1} fresh weight and calculated them using the formula (2 & 3) reported by Arnon (1967):

$$\text{Chlorophyll contents (mg g}^{-1}\text{ FW)} = [20.2\ (A_{645}) + 8.02\ (A_{663})] \times V/(W \times 1000) \quad (2)$$

$$\text{Carotenoids (mg g}^{-1}\text{ FW)} = [7.6\ (A_{480}) - 1.49\ (A_{510})] \times V/(W \times 1000) \quad (3)$$

Soluble sugars

We measured total sugar content following the modified method of Liu et al. (2015). For this analysis, we mixed 0.1 mg of powdered sample with 4 mL of 80% (v/v) ethanol and incubated it in a water bath at 80°C for 20 minutes. After incubation, we centrifuged the mixture at 5000 rpm for 10 minutes, and then added 1 mL of 5% (v/v) phenol solution to 1 mL of the resulting extract. We further added 5 mL of sulfuric acid to this solution, thoroughly mixed it, and maintained the solution at room temperature for 30 minutes to cool. We then measured absorbance at 490 nm using a spectrophotometer (ND One, Thermo Fisher, USA).

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

Depth	Soil Texture	Sand	Clay	Loam	pH	EC (dS m^{-1})	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
0-30	Sandy loam	40	33	27	7.5	1.3	610	60.6	625.1

Total phenolic content (TPC) and total flavonoid content (TFC)

To determine the TPC, we combined each extract with 80% methanol, and then added 100 µL of this mixture to 2.8 mL of distilled water. Following this, we added 2 mL of 2% sodium carbonate (Na_2CO_3) and 100 µL of 50% Folin-Ciocalteu reagent, allowing the mixture to incubate for 30 minutes. We measured the absorbance at 720 nm relative to the control, expressing the total phenolic content of extracts as mg gallic acid equivalents per gram of plant weight (mg GAE/g) (Namvar et al., 2018). For TFC determination, we followed the colorimetric method of Kaijv et al. (2006) and measured absorbance at 510 nm, reporting results in millimoles of quercetin equivalents per 100 g fresh weight (mM QE/100 g FW).

DPPH radical scavenging activity

We evaluated DPPH radical scavenging activity following the modified protocol of Sanchez-Moreno et al. (1998). To start, we added 100 µL of extract or control (methanol instead of extract) to 100 µL of a prepared 0.2 mM DPPH solution in methanol. We recorded absorbance at 517 nm after a 15-minute reaction period. Results for DPPH radical scavenging activity are presented as percentages (Sanchez-Moreno et al., 1999) (4).

$$\text{Radical scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100 \quad (4)$$

A: absorbance.

Antioxidant enzymes activity

Phenylalanine ammonia-lyase changed into extracted by way of 5 ml, 100 mM sodium borate buffer (pH 8.8, containing 1mM EDTA, five mM β-mercaptoethanol, and 0.1% polyvinylpyrrolidone), and its activity changed into measured in line with the approach of Kovács et al. (2014). Soluble protein content material turned into results as described by Bradford (1976). Catalase pastime changed into measured data as by Aebi (1984), with slight modifications. We monitored the destruction of H_2O_2 at 240 nm absorbance while using a spectrophotometer (ND One, Thermo Fisher, USA) for 1 min. The response changed into 3 ml reaction aggregate containing 2.78 ml phosphate buffer (pH 7), 0.1 mL enzyme extract, and 30µl of 15 mM H_2O_2 (Aebi, 1984). Enzyme pastime was calculable via $\varepsilon = 0.28$ mM^{-1} cm^{-1} and expressed. We measured SOD by the nitro blue tetrazolium technique (Giannopolitis and Ries, 1977). We mixed the enzyme extract (0.1 mL) of one hundred mM phosphate buffer (pH7.6) with 1.5 mM Na2CO3, 2.25 mM NBT, two hundred mM methionine, three mM ethylene diamine tetraacetic acid (EDTA), 0.06 mM riboflavin, and distilled water. The response combos without illumination were usable as blank. The absorbance of the response combination became measurable at 560 nm with a UV spectrophotometer (ND One, Thermo Fisher, United States of America). Enzymes were expressable as units consistent with milligrams of protein (U mg^{-1} protein).

Anthocyanin

Anthocyanin changed into quantified data via the Wagner et al. (1979) method. To determine the concentration of anthocyanins, we extracted 0.25 g sparkling leaves in 15 ml glass centrifuge tubes containing 3 ml of acidified methanol (methanol: HCl 99: 1 v:v) and

maintained them overnight in the darkish. We delivered the samples and measured the absorbance at 550 nm. Anthocyanin concentration was calculable via an extinction coefficient of 33000 mol^{-1} cm^{-1} (Krizek et al., 1998).

Statistical analysis

The experiments operated on a Randomized Complete Block design (RCBD). We applied each treatment to three replications on one plant. We analyzed variance (ANOVA) via SAS. V.9.1 Statistical Program (SAS Institute Inc., Cary, NC, USA). Differences between mean values were determined by Duncan's test, with differences considered significant at $P \leq 0.05$.

RESULTS

Morphological characteristics

The study of annual effects (2021-2022) on leaf area (LA) and specific leaf area (SLA) revealed that both indicators reached their highest values in 2022, with LA and SLA increasing by 10.10% and 11.01%, respectively, compared to 2021 (Table 2). The results showed that treatments with nitroxine, phenylalanine, and selenium significantly influenced both indicators compared to control conditions. Specifically, goji berry plants treated with nitroxine at a concentration of 500 µL exhibited the highest LA and SLA, representing increases of 19.58% and 21.93%, respectively, relative to control values (Table 2).

Chlorophyll and carotenoid contents

Figure 1 presents the total chlorophyll content of goji berry plants treated with varying concentrations of Phe, Se, and nitroxine across both years. In 2022, total chlorophyll levels were significantly higher than in 2021. The nitroxine treatment positively influenced chlorophyll content across all concentrations, with the highest content (1.84 mg g^{-1} FW) observed in plants treated with 333 µL L^{-1} nitroxine in 2022 (Fig. 1A & B). Maximum carotenoid content resulted from Se at 1 mg L^{-1}, and data indicated that plants grown in 2021 contained significantly higher carotenoid levels than those in 2022.

Table 2. Changes in the amount of LA and LSA during the 2021-2022 and the treatments used in goji berry leaves.

Year	leaf area (LA)	specific leaf area (SLA)
2021	13.37 ^{b}	7.11 ^{b}
2022	15.022 ^{a}	7.99 ^{a}
Treat		
Control	13.001 ^{d}	7.01 ^{cd}
Phe0.5mM	14.022 ^{cd}	7.40 ^{cd}
Phe1mM	14.48 ^{bc}	7.51 ^{c}
Phe1.5mM	13.23 ^{cd}	7.17 ^{cd}
Se 0.25mg.L^{-1}	13.19 ^{cd}	6.91 ^{cd}
Se 0.5mg.L^{-1}	14.13 ^{cd}	6.70 ^{d}
Se 1mg.L^{-1}	13.48 ^{cd}	7.39 ^{cd}
N 167µL	13.92 ^{cd}	7.46 ^{cd}
N 333µL	15.61 ^{b}	8.30 ^{b}

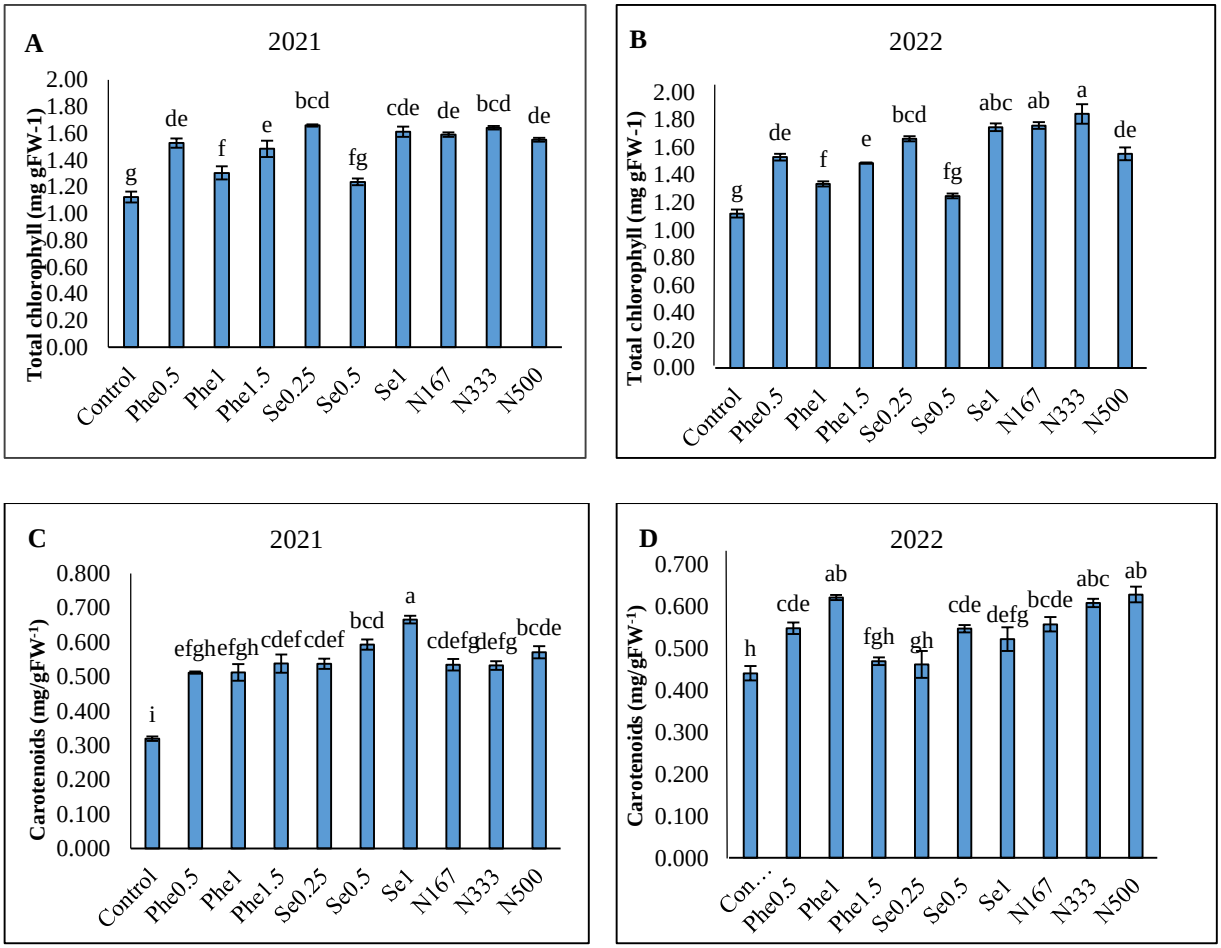

Fig. 1. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on total chlorophyll (A and B) and carotenoids (C and D) contents of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$

Carbohydrate content

Figure 2 illustrates the carbohydrate content in plants treated with different concentrations of Phe, Se, and nitroxine over the two study years. The carbohydrate content in 2021 significantly exceeded that of 2022 (Fig. 2A & B). Our findings confirmed that foliar applications of Phe, Se, and nitroxine increased carbohydrate levels compared to the control, with the highest carbohydrate values recorded in plants treated with 0.5 mg L^{-1} Se and 1 mM Phe in 2021.

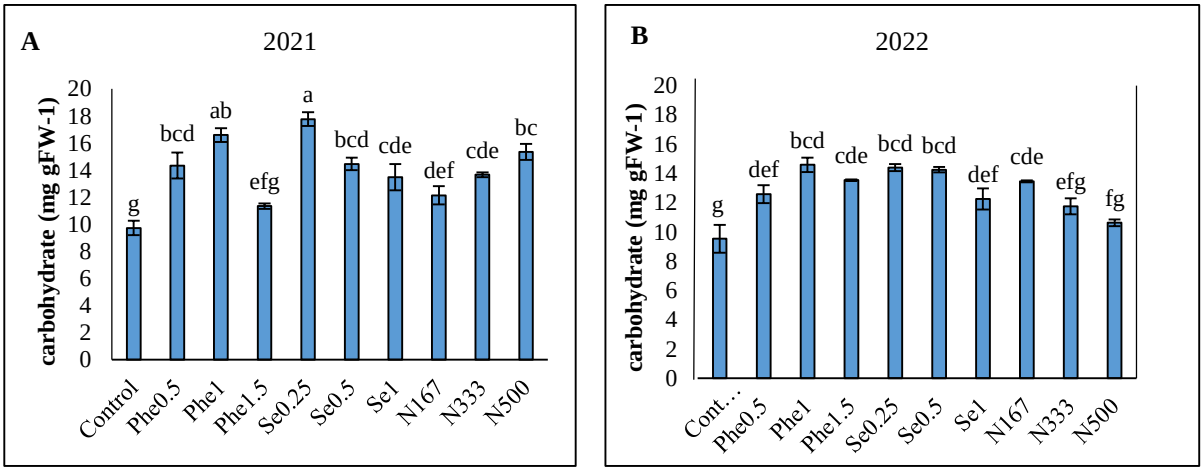

Fig. 2. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on carbohydrate (A and B) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

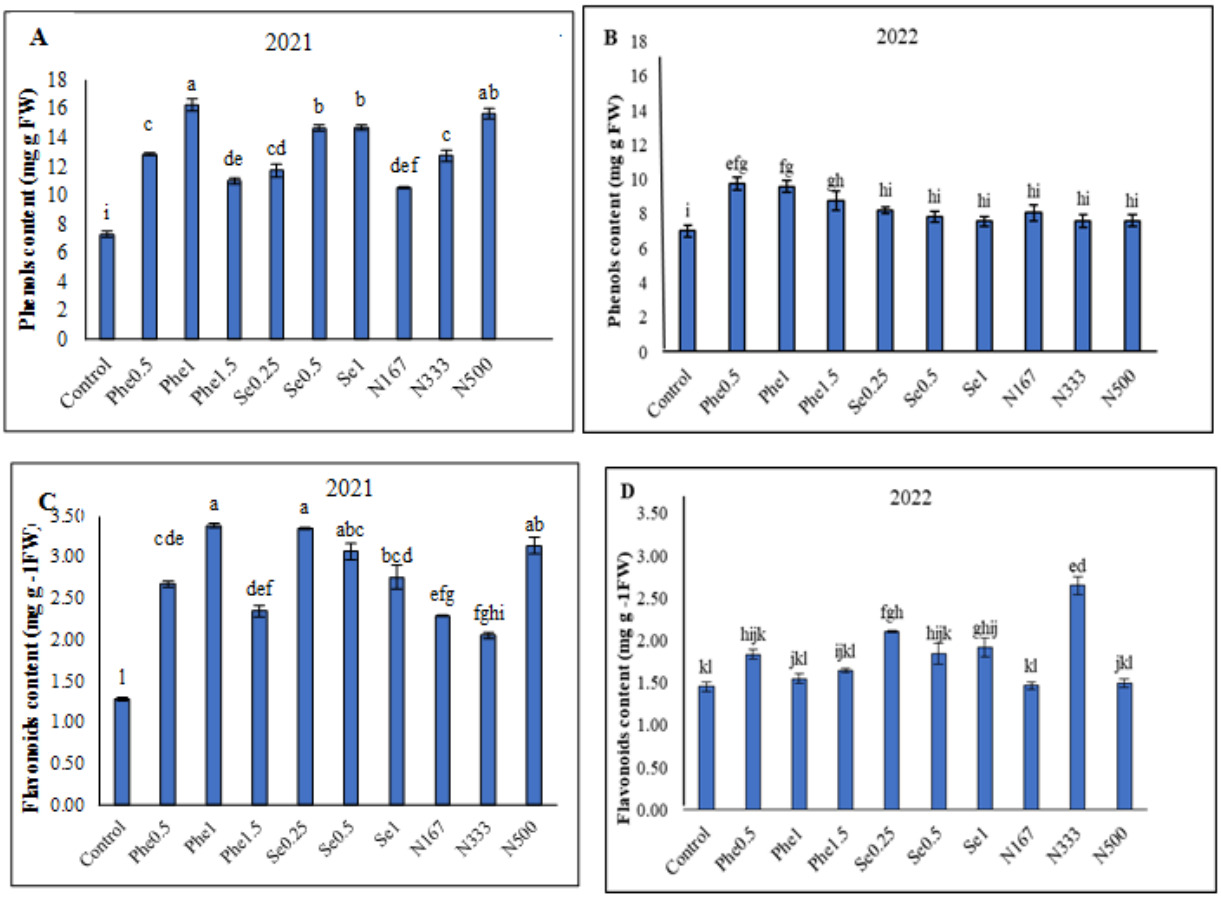

Fig. 3. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar s pray on Phenols content (A and B) and Flavonoids content (C and D) of Goji berry in two years (2021 and 2022. Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

Flavonoid and phenol content

Figure 3 indicated significant treatment effects on flavonoid and phenol content in goji berry. The highest flavonoid content occurred in the 1 mM Phe treatment (Fig. 3C & d). Foliar applications of Phe, Se, and nitroxine notably increased flavonoid content, with levels in 2021 being significantly higher than in 2022. The maximum phenol content resulted from nitroxine at 500 µL L^{-1}, with values in 2021 significantly exceeding those of 2022 (Fig 3A & B).

Antioxidant capacity

As shown in Figure 4, nitroxine and phenylalanine applications substantially influenced antioxidant capacity throughout the growth period. The highest antioxidant capacity occurred in 2021, with 2022 showing the lowest values. The nitroxine treatment at 500 µL L^{-1} had a significant impact on antioxidant capacity. However, values showed no significant difference when compared to 1 mg L^{-1} selenium and 1.5 mM Phe. Higher treatment concentrations generally proved more effective than lower concentrations in enhancing antioxidant capacity.

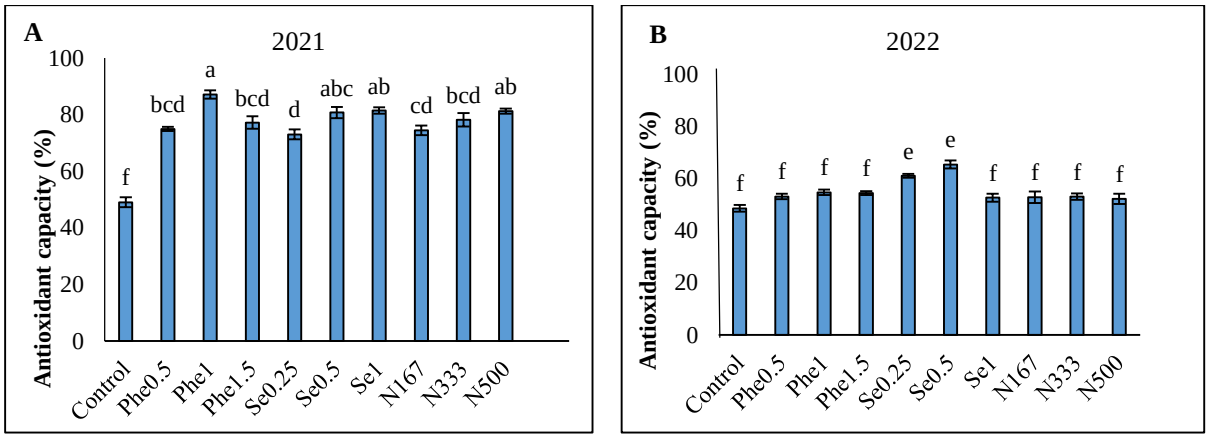

Fig. 4. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on antioxidant capacity (A and B) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

Enzymatic antioxidant activity (PAL, SOD, CAT)

The exogenous application of Phe, Se, and nitroxine significantly enhanced phenylalanine ammonia-lyase (PAL) enzyme activity in goji berry plants (Fig. 5). Notably, plants grown in 2022 exhibited significantly higher PAL enzyme activity than those in 2021 ($p \leq 0.01$), though no significant variation appeared between the years in specific treatment responses. Treatments with 1.5 mM Phe in 2022 produced the maximum PAL content, with no substantial difference between the 1.5 mM Phe treatments in the two years (Fig. 5A & B). Similarly, Phe, Se, and nitroxine applications considerably impacted superoxide dismutase (SOD) activity. The SOD enzyme levels increased with Phe application (Table 1). Foliar application of 1 mM Phe in 2021 notably boosted SOD activity, with the highest recorded values of 16.46 and 15.90 protein units in plants treated with 1 mM Phe and 500 µL L^{-1} nitroxine, respectively (Fig. 5C & D).

Phe, Se, and nitroxine applications enhanced catalase (CAT) activity in 2021 and 2022 (Table 1 & Fig. 5). CAT activity was significantly higher ($P \leq 0.01$) in 2021 compared to 2022. The highest CAT activity values of 2.72, 2.42, and 2.40 protein units appeared in treatments with 1 mg L^{-1} Se, 1.5 mM Phe, and 167 µL L^{-1} nitroxine in 2021 and 2022, respectively (Fig. 5E & F).

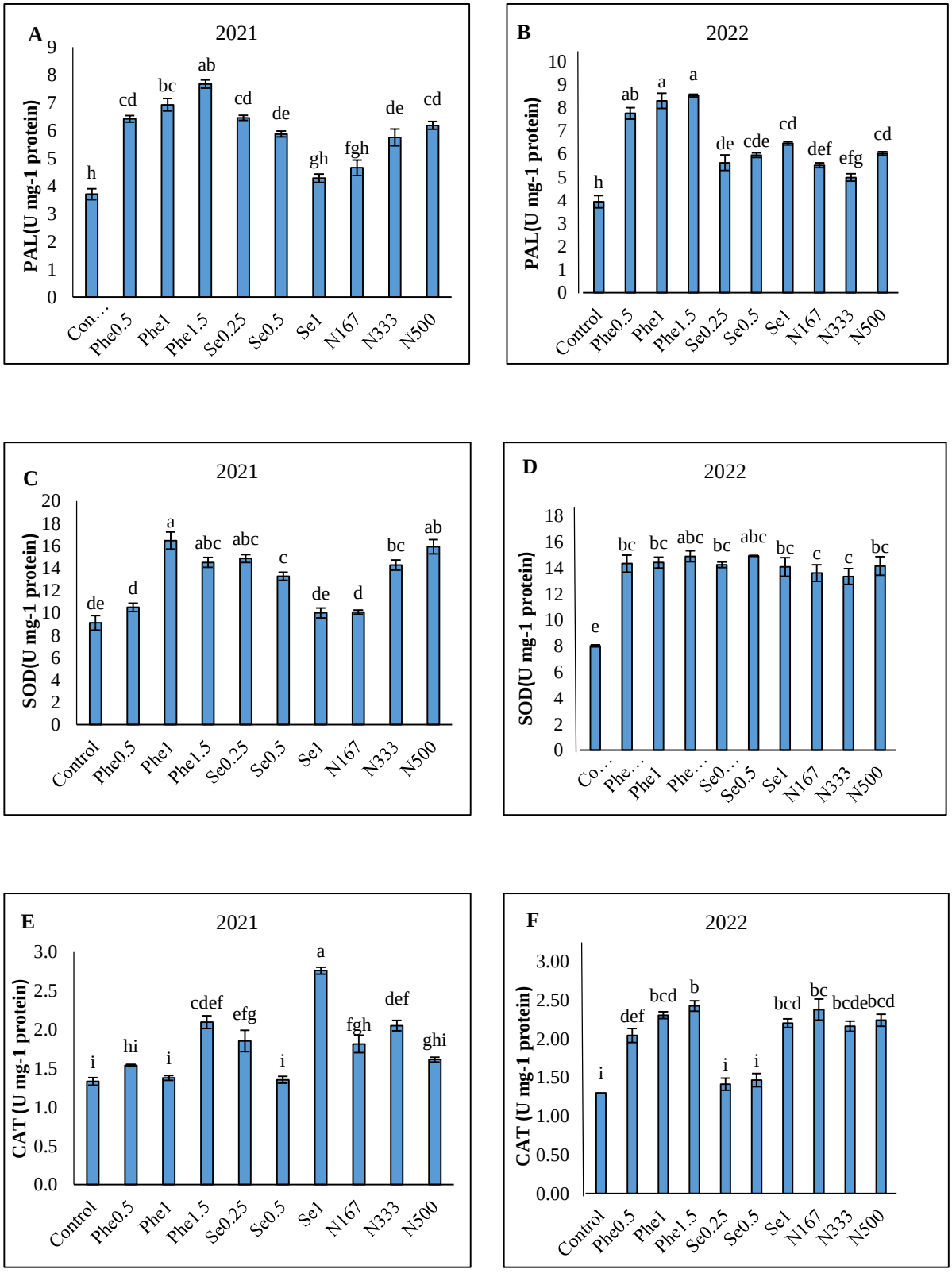

Fig. 5. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on phenylalanine ammonia-lyase (PAL) enzyme (A and B), Superoxide dismutase (SOD) enzyme (C and D) and Catalaz (CAT) enzyme (E and F) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

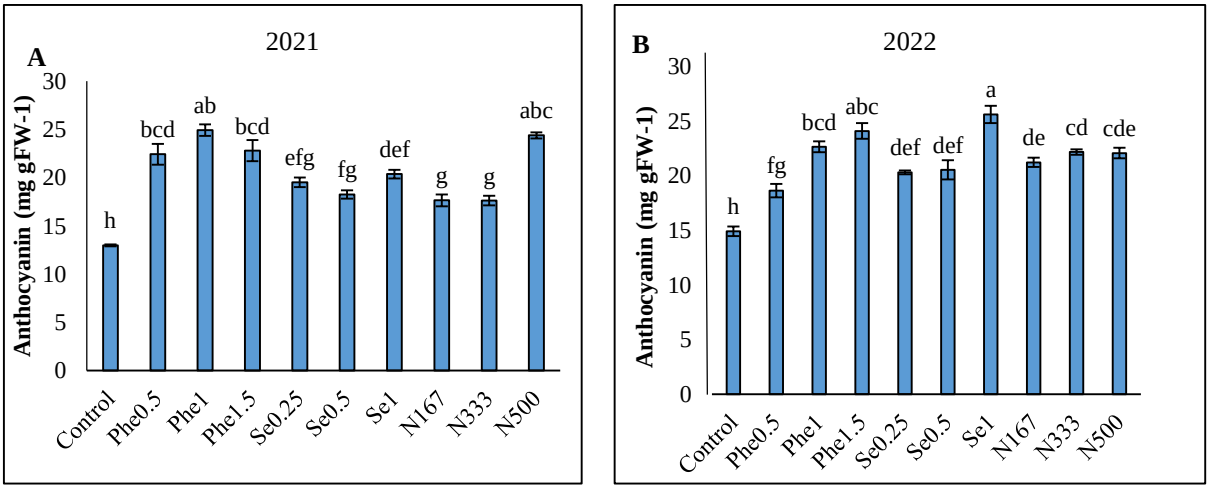

Fig. 6. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on anthocyanin (A and B) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

Anthocyanin content

Anthocyanin levels in goji berry plants varied across different concentrations of Phe, Se, and nitroxine over the two years (Fig. 6A & B). The anthocyanin content in control plants was significantly lower ($P \leq 0.01$) than in all treated plants. Treatments with Phe, Se, and nitroxine markedly increased anthocyanin content compared to the control, demonstrating the beneficial effects of these applications.

DISCUSSION

Increasing antioxidant system function enhances total antioxidant capacity, governed by the content of low-molecular antioxidants and the activity of antioxidant enzymes. Key low-molecular antioxidants, including ascorbic acid, glutathione, tocopherol, carotenoids, anthocyanins, endogenous metal chelators, TPC, TFC, and alkaloids, play essential roles in this capacity (Radyuk et al., 2009; Giglou et al., 2023). Nitroxine, which binds atmospheric nitrogen, aids in nutrient balance within plants by promoting the secretion of amino acids, antibiotics, hydrogen cyanide, and siderophores, which stimulate root and shoot growth and protect roots from pathogens, thereby increasing yield (Shoaei et al., 2012; Fahramand et al., 2013). Research by Zahedian et al. (2022) identified the highest chlorophyll content (1.84 mg g^{-1} FW) following the nitroxine treatment.

Anthocyanins, beneficial secondary metabolites and natural pigments, demonstrate medicinal properties. L-phenylalanine, a pivotal precursor in anthocyanin biosynthesis, activates the phenylpropanoid pathway, promoting TFC accumulation. Due to its relative affordability and high efficiency, Phe is valuable for inducing pigment production (Edahiro et al., 2005). Endogenous Phe, derived from the shikimic acid pathway, or exogenously supplied Phe stimulates the phenylpropanoid pathway and PAL activity, crucial for accumulating phenols, flavonoids, and anthocyanins. This process also underscores the necessity of ROS accumulation, an essential plant response (Akkad et al., 2019). Anthocyanins, a subset of flavonoids in plant vacuoles, epidermal, and mesophyll cells, shield chlorophyll from light-induced oxidation, making them reliable indicators of plant oxidative stress (Abbas, 2012; Hatier & Gould, 2008).

Supporting these findings, Garavand et al. (2019) observed that Se treatment increased phenolic acids and anthocyanins in red and green lettuce. Application of Se on sweet basil

leaves enhanced anthocyanin pigments by 73% compared to the control, while selenate application significantly increased anthocyanins (Abbas, 2012). Nitroxine also promoted chlorophyll and carotenoid production in celery grown in Iraq and Iran, leading to linear improvements in photosynthetic pigments (Dahham, 2021; Rahi, 2013).

Increased carbohydrate content in sprayed plants may relate to higher atmospheric CO_2 levels due to improved stomatal permeability or activation of enzymes integral to CO_2 absorption (Hajiboland et al., 2015). Turakainen et al. (2015) highlighted carbohydrate accumulation and senescence processes in potato roots and tubers, attributing the rise in soluble sugar in selenium-treated alfalfa to fructose 1,6-bisphosphatase, a key enzyme in carbohydrate metabolism. Simojoki et al. (2003) noted that selenium supplementation enhances root efficiency, positively impacting carbohydrate production and accumulation.

Amino acids, essential components of plant proteins, contribute to numerous biochemical and physiological processes (Kandil et al., 2017). Phe, as a precursor for secondary metabolites, supports phenylpropanoid, flavonoid, and other essential metabolic pathways (Tzin and Galili, 2010). Port et al. (2015) found that foliar application of L-phenylalanine increased anthocyanin and phenolic compound levels in grapefruit, aligning with Pakkish and Mohammadrezakhani (2021) research, which demonstrated that amino acid sprays on mango trees elevated anthocyanin, carotenoid, and phenolic contents. Additionally, bio-nitroxide fertilizer application increased total phenolic content in cantaloupe fruits (Zahedian et al., 2022), and Jalil Shishbehra et al. (2022) confirmed that nitroxine treatment significantly elevated phenolic yields in purple conifer plants.

Applying nitroxine enhances antioxidant enzyme activity and facilitates the accumulation of compatible osmolytes in black cumin, ultimately boosting biological performance. Similarly, biological fertilizers have been shown to increase antioxidant activity in sweet sorghum (Wang et al., 2019). Treatments with biofertilizers also increased total phenolic content (TPC), total flavonoid content (TFC), and DPPH activity in *Cephalaria syriaca* (L.). Foliar applications of nitroxine and iron (Fe) significantly raised the antioxidant capacity in goji berry plants, with high flavonoid and phenolic compound levels correlating strongly with antioxidant activity, as previously established between TFC and antioxidant capacity (Ghasemzadeh et al., 2012). Amino acids, such as phenylalanine, significantly influence plant antioxidant activity (Khaki et al., 2020).

Phenylalanine is essential in synthesizing aromatic compounds, antioxidants, lignin, anthocyanins, and other phenolic compounds, playing a role in cellulosic ethanol production as well (Edahiro et al., 2005). Studies indicate that applying amino acids, including phenylalanine, positively impacts antioxidant metabolism in soybean plants, benefiting both seeds and leaves (Teixeira et al., 2017). In goji berry plants, antioxidant enzymes such as phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and catalase (CAT) increased in response to Phe, Se, and nitroxine treatments. Plants naturally produce an antioxidant system comprising enzymes like SOD, CAT, and PAL and metabolites such as ascorbic acid, glutathione, α-tocopherol, carotenoids, and flavonoids (El-Desouky et al., 2011). Various stimulants and environmental conditions can enhance these antioxidant components in plants.

Phenylalanine, an aromatic amino acid, is vital for synthesizing phenolic compounds via the phenylpropanoid pathway. PAL, the initial enzyme in this pathway, catalyzes phenylalanine's conversion to flavonoids, phenols, and anthocyanins. This pathway activation is often a response to biotic and abiotic stress, resulting in the accumulation of bioactive compounds (Aghdam et al., 2019). Studies have consistently demonstrated that phenylalanine effectively boosts PAL activity and phenolic compound accumulation (Aghdam et al., 2019; Sogvar et al., 2020). Foliar and seed treatments with phenylalanine enhanced PAL activity in

soybean plants (Teixeira et al., 2017; Wu et al., 2011). Increased SOD activity, induced by environmental stress, can result in H_2O_2 accumulation, a signaling molecule that further stimulates the phenylpropanoid pathway and promotes phenol production, enhancing the plant's oxygen radical absorbance capacity (Jacobo-Velázquez et al., 2011).

Overall, phenylalanine remains one of the most effective treatments for increasing phenolic compound buildup (Aghdam et al., 2019; Sogvar et al., 2020). Additionally, it plays a critical role in anthocyanin biosynthesis by activating PAL, leading to heightened anthocyanin accumulation (Wu et al., 2011; Heydarnajad Giglou & Torabi Giglou, 2023).

In the available literature, amino acid application on soybean, both during processing and as a foliar spray, increased the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes, respectively (Teixeira et al., 2017). Similarly, Ulianych et al. (2020) reported an upward trend in SOD and CAT activities in garlic plants following amino acid treatments. Our findings align with those of Aghdam et al. (2019), who observed that tomato fruits treated with 0.5 mM phenylalanine (Phe) exhibited higher total phenolic content (TPC), total flavonoid content (TFC), and phenylalanine ammonia-lyase (PAL) activity compared to untreated fruits. The treated fruits also showed elevated CAT and SOD levels, underscoring the role of amino acid intake in enhancing both enzymatic and non-enzymatic antioxidant activity. For instance, exogenous amino acids were shown to significantly increase SOD levels in tomato leaves (Liu et al., 2015).

Adequate selenium (Se) concentrations are also essential for optimal plant growth, antioxidant function, photosynthesis, and osmoregulation. Selenium adjusts the antioxidant system primarily through three mechanisms: it promotes the conversion of superoxide (O_2^-) to H_2O_2 independent of SOD enzymatic catalysis, it quenches reactive oxygen species (O_2^- and OH^-) via selenium compounds, and it directly regulates the activity of antioxidant enzymes (Chongping et al., 2022). These functions highlight Se's antioxidative effects on plants (Huang et al., 2018). Research shows that Se can boost plant growth in challenging environments, such as by enhancing root development in chili plants (Mozafariyan et al., 2014). Low Se concentrations have also been associated with increased antioxidant enzyme activity and non-enzymatic antioxidants in lettuce plants (Ríos et al., 2009). Additionally, Rady et al. (2020) found that semi-cured tomatoes exhibit increased activity of several antioxidant enzymes, including SOD and CAT, confirming Se's role in enhancing antioxidant defenses. El-Ramady et al. (2016) demonstrated that Se impacts the growth of groundnut cultivars by modulating photosynthetic pigments, catalase activity, phenolic content, and total flavonoid levels.

Nitroxine, a biological fertilizer rich in microorganisms, further stimulates antioxidant enzyme activity. Studies show that treatments with Phe, Se, and nitroxine in goji berries elevate PAL, SOD, and CAT activities. Biofertilizers, in particular, have been found to significantly increase CAT activity, enhancing cellular stability (Hashem et al., 2016). Numerous studies have indicated that biofertilizers promote CAT and SOD activity, providing superior protection to the photosynthetic apparatus and, subsequently, improving plant growth (Weng et al., 2015). The highest CAT levels occurred in plants subjected to foliar applications of nitroxine, which likely contributed to increased CAT activity in the leaves. This effect may be linked to the nitrogen supply facilitated by nitrogen-fixing bacteria like *Azotobacter* and *Azospirillum* (Najafi et al., 2021).

CONCLUSION

Our findings indicated that phenylalanine and selenium treatments enhanced quality traits in goji berry seedlings, resulting in increased anthocyanins, carbohydrates, chlorophyll, and antioxidant compounds. Foliar applications of phenylalanine, selenium, and nitroxine stimulated the biosynthesis of photosynthetic pigments and total phenolic content, which elevated antioxidant and phytoactive compound levels. Over two years, these treatments demonstrated positive effects on various traits, particularly by promoting the biosynthetic pathways for active substances and bolstering plant defenses. Specifically, applying Phe (0.5 mM) and Se (0.5 mg L^{-1}) substantially improved both enzymatic and non-enzymatic antioxidant mechanisms.

Conflict of interest

The authors have reviewed the journal's policies and declare no conflicts of interest. This manuscript is original, has not been published, and is not under consideration elsewhere.

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Detection of zygotic and apomictic embryonic origin in *Citrus sinensis* Osbeck based on RAPD markers

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ABSTRACT

Purpose: Citrus plant exhibits a unique trait called polyembryony. In open-pollinated plants the pollen source of the plant remains unknown but it is assumed that the apomictic nucellar embryos mimic the genetic architecture of the mother plant. This assumption was exploited in the detection of true-to the type seedlings of polyembryonic *Citrus sinensis* plants for fruit quality retention and smooth maintenance of the orchards. **Research Method:** The randomly amplified polymorphic DNA (RAPD) technique was employed to distinguish nucellar and zygotic seedlings obtained from a selected *Citrus sinensis* plant marked in South 24- Parganas district of state of West Bengal in India. The embryos were extracted from a fruit and seedlings were raised in poly-house. To identify DNA marker for tracing the embryonic origin, ten vigorous seedlings marked in poly-house were used for RAPD analysis. DNA was extracted from three-month-old seedlings along with the mother plant and RAPD analysis was performed with 25 arbitrary decamer primers with a negative control. **Findings:** Four decamer primers OPQ15, OPAH02, OPAA02 and OPA11 were able to differentiate the sexual seedlings from the apomictic nucellar types. The total study took 48 hours for tracing the embryonic origin of the seedlings. **Research limitations:** This study could be extended with inclusion of more primers and screening of more fruits from diverse locations of India. **Originality/Value:** This process could act as a fast technique for preliminary identification of true-to the-type plants for quality control of Citrus fruit industry and sustainable nursery management.

INTRODUCTION

Apomixis is a unique phenomenon of embryo development found in a couple of families of angiosperm. Apomixis is detected in more than 400 species of flowering plants (Carman, 1997) including important horticultural plant families represented by citrus (Wakana & Uemoto, 1987), mango (Aron et al., 1998), walnut (Peng-fei et al., 2007) and pepper (Nowaczyk, 1987). Apomixis is divided into gametophytic and sporophytic type where both display potential usefulness in plant breeding. Sporophytic apomixis also known as adventitious embryony is a process in which the embryo arises directly from the nucellus or the integument part of the ovular tissue. Sporophytic apomixis occurs commonly in citrus species but rarely found in any renowned cereal crop plants. The nucellar apomixis is very promising in permanent fixation of superior genetic architecture of female plants (Xu et al., 2021). The valuable heterotic potential present in highly cross-pollinated crops could be preserved through apomictic embryos.

In Citrus, the apomictic nucellar embryos could be utilized as explants for *ex-vivo* clonal propagation or as superior scion in grafting operation. The sexual reproduction in cross pollinated crops may involve integration of foreign genes from pollen donor. The fertilization event from unknown pollen source often deteriorates the quality of fruits, field performance and affects disease resistant properties of the sexual progeny plants in citrus. Whereas the nucellar apomictic progeny population developed from an elite well performing genotype could ensure uniformity in progeny population with equivalent performance as the mother progenitor (Xu et al., 2022).

The apomictic trait promotes a short-circuited life cycle developing embryos identical to the mother plant. The early screening of nucellar embryos helps in retention of fruit quality along with effective utilization of financial resource, man-power, and farm-input in orchard management programme. Scientists applied several techniques including the low cost *in-vitro* embryo germination, isozyme and DNA marking techniques for discrimination of nucellar apomictic embryos in citrus. The isozyme system displays many limitations such as excessive dependence on tissue age, environmental factors, accuracy of the enzyme system and efficacy of the researcher (Ashari et al., 1988). The constraints in the application of isozyme markers to differentiate the embryos created room for application of strong molecular markers such as direct involvement of DNA based molecular marker.

The DNA based marking is devoid of any direct influence from the tissue age, type and environment. The random amplified polymorphic DNA markers (RAPD) has been widely used in fruit crops for solving diverse problems due to their phenotypic neutrality, high polymorphism, low-cost and fast result. The versatile technique was used for genotype identification, genome analysis, duplicate identification, phylogenetic studies, mapping and mutant identification (Babu et al., 2021; Pillay et al., 2000; Subudhi et al., 2016; Zarei et al., 2017; Li et al., 2019; Abdein et al., 2022; Wahyudi et al., 2020). The RAPD technique does not require previous information about the targeted DNA and may reveal immense polymorphism with easy, simple method of operation suitable to conduct in a moderate laboratory set-up (Bardakci, 2001).

In the present study RAPD marking technique is used for molecular detection of the apomictic seedlings from sexual one with an assumption of early detection of seedlings similar to the mother plant. The results of this preliminary trial will help to develop advance markers for identification of desired apomictic embryos for Citrus propagation. The detection of reproducible RAPD markers in long run could be converted into robust markers for the study of sporophytic polyembryony in other horticultural crop species and tracing of elite plant types for quality control in fruit business.

MATERIALS AND METHODS

Selection of plant and embryo extraction

One open pollinated sweet orange (*Citrus sinensis)* plant from Krishnanagar, Amtala at South 24 Parganas district of West Bengal, India was marked for high productivity, regular bearing and excellent fruit quality. Mature fruits were collected and brought to the laboratory of the Biochemistry and Crop Physiology department of School of Agriculture & Allied Sciences, The Neotia University. Seeds collected from five representative fruits from the plant were surface sterilized with 0.1% mercuric chloride solution, placed between two layers of sterile moist sterile cotton pad in Petri dishes, and incubated for 5 to 7 days at 30-32°C to germinate in laboratory incubator. Upon swelling of the seeds, the germinating nucellar and zygotic embryos were identified following the procedure standardized by Tisserat (Tisserat, 1985). Under aseptic conditions, the integument of the mature seed was carefully rolled away by making a longitudinal incision with a fine scalpel from the micropylar end. The germinating embryo holding the two original cotyledons originating from the micropylar end was considered as the zygotic embryo. All other germinating embryos under the integument, each with two newly differentiating tiny cotyledons or globular shaped embryos were considered as nucellar embryos (Dubey et al., 2020). The number of embryos and seedlings originating from the zygotes and from nucellar tissue were carefully examined.

Establishment of seedling progeny

After observation on polyembryony, the germinating seeds were allowed to grow in aseptic conditions on a cotton bed for another 10 to 12 days, and then put into a sterile soil-sand-organic matter mixture (2:1:1) under controlled conditions with high humidity for further growth of the seedlings, and were marked separately according to their origin. The growth pattern of different seedlings was carefully noted and recorded. The most vigorous seedlings developed from a single fruit were grown for three months in poly-house. As all the embryos were selected from the same fruit, it was expected that the developed seedlings will be genetically similar in majority of the characters except few ones. The mother plant was also included in the experiment for marking of the apomictic nucellar seedlings.

DNA Extraction

Genomic DNA was extracted from the soft leaves of the seedlings and mother plant using the Plant DNA CTAB Extraction procedure (Schenk et al., 2023). The quantity and amount of DNA were determined using intact bacteriophage lambda DNA in 1% agarose gel (Kahangi et al., 2002).

Primer selection and RAPD Analysis

The PCR amplification was achieved by the protocol outlined by Williams 1990 with slight modifications (Williams, 1990). Ingredients of each reaction included template 25–30 ng DNA, 200 µM dNTPs each, 1.5 unit Taq DNA polymerase, 2 mM $MgCl_2$, 10X Taq Polymerase buffer´(Bangalore Genei) and 15 ng of decamer primers (Nalbiogen, India) in a total volume of 25 µL. The amplification was performed in a thermocycler (Gene Amp PCR System 9700, Applied BioSystems). Total reaction consisted of 45 cycles, each cycle comprising three steps (denaturation at 92°C for 30 seconds; annealing at 38°C for 30 seconds; extension at 72°C for 1 minute), with an initial denaturation at 94°C for 30 seconds and a final extension at 72°C for 5 minutes, followed by cooling at 4°C. RAPD analysis was carried with 25 Operon decamer primers selected by preliminary screening to give

polymorphism and reproducible fragment patterns in the species using mother plant DNA and analysis of polyembryony in *Citrus reticulata* (Mondal et al., 2015).

Horizontal gel electrophoresis and amplicon detection

Amplified fragments were separated on 2% agarose (Merck-Genei) gels containing ethidium bromide (0.5 µg per mL of agarose) at 60 V for 6 hours in Tris Borate EDTA buffer. The gel was visualized and photographed under UV excitation using an electronic dual wave transilluminator system (Ultra. Lum Inc., USA). Amplified fragments from all the primers were scored by the Total Lab gel documentation software (Ultra. Lum Inc., USA). The size of the amplicons (DNA fragments) in base pairs (molecular weight) was estimated by using a 100-bp ladder marker (Bangalore Genei), which was run along with the amplified products. The primers that could generate differential banding pattern of the seedlings of different origin (apomictic and sexual) of a fruit were noted by comparing with the DNA profile of the mother plant. The experiment randomly included a positive and negative control in 10% of the PCR run. One of the selected apomictic seedlings was used as positive control and pure de-ionised water was used as negative control in PCR reactions.

RESULTS & DISCUSSION

The phenomenon of apomixis is very common in Citrus. Addition to the presence of the single normal sexual embryo, small, plural embryos were also observed. In further course of development these embryos compete for nutrient resource. In *Citrus sinensis* several embryonic anomalies were noticed in this experiment. Figure 1 revealed the presence of several embryos per seed along with presence of zygotic twin, triplets, and anomalies such as fused radical or fused plumule of two seedlings. The number of twin and triplet seedlings was significantly less in comparison to large number of polyembryonic seeds.

For the molecular experiment a single fruit was used for extraction of all the seeds. The embryos extracted from the seeds were screened on the basis of morphological character and utilized for raising the seedlings. The embryos were carefully kept in poly-house with high humidity for seedling development. During the period of seedling growth, DNA was isolated from the leaves of the mother plant and was used for primer screening. In total 50 primers belonging to the Operon series were selected for RAPD analysis. The primer selection was based on previous literature search and ongoing research of the laboratory on citrus. The three-month-old seedlings with 7-8 leaves were used for DNA extraction using CTAB Plant DNA extraction method. After preliminary screening, 25 primers yielding strong, intense, unambiguous and reproducible DNA fragments were selected and utilized for conducting the reactions. Out of which four primers were able to trace the embryonic origin of the seedlings. The details of decamer primer able to differentiate the zygotic and apomictic embryos in sweet orange were as shown in Table 1. Out of 25 primers four primers, OPQ15, OPAH02, OPAA02 and OPA11were able to detect variation in DNA profile among the selected seedlings. The seedlings showing banding pattern dissimilar to the mother plant were marked as the sexual or zygotic types. Out of the ten seedlings two displayed difference in banding pattern from the mother plant (Fig. 2).

In horticulture industry the fruit quality parameter plays an instrumental role in the profitability and growth of the business. The early detection of a high performing plant could assist in meaningful orchard management. Nowadays the scientists are relying on diverse rapid and novel techniques for early diagnosis of internal and external quality of fruits. In majority of the cases the non-destructive sensing methods are regarded as excellent tool for advance quality assessment of the fruits before marketing of the produce (Li et al., 2018). In a

study led by a group of scientists of Portugal, non-destructive near infrared spectroscopy method was used to diagnose the quality of Citrus fruits. The method was able to measure several biochemical parameters using NIR spectra without damaging the fruits. These new-age techniques are regarded as low cost, environment friendly, accurate, easy-to-use measures by the scientist for quality control studies (Magwaza et al., 2012; El Khaled et al., 2017; Santos et al., 2021).

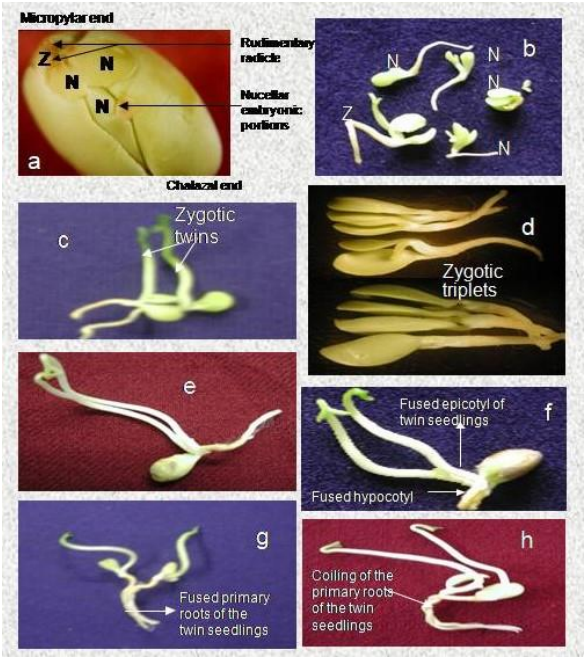

Fig. 1. a. Position of zygotic and nucellar embryo inside a dissected seed, b. extracted embryos, c. zygotic twins, d. zygotic triplets, e., f., g., h – diverse anomalies in embryonic development.

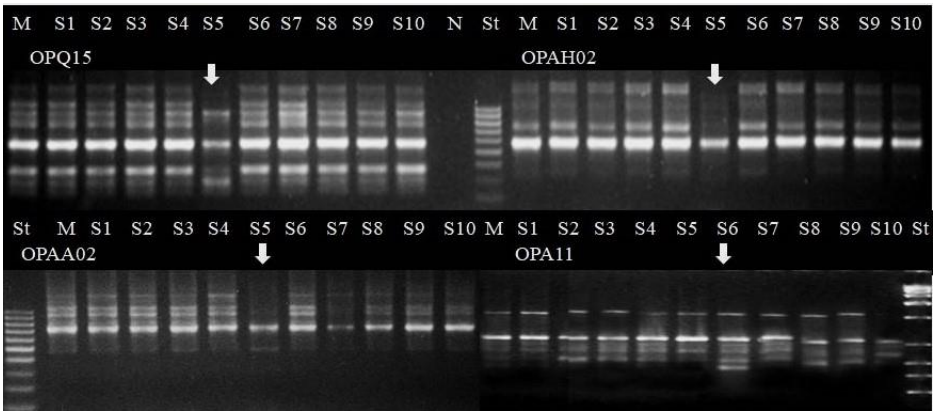

Fig. 2. The RAPD profile of the seedlings developed from a single fruit, M= mother plant, S1-S10= seedlings, N= negative control, St= 100 bp ladder.

Table 1. The details of decamer primers able to differentiate sexual and apomictic seedlings

Primer	Sequence (5'-3')	Total number of amplicon	Polymorphic amplicon	Range of amplicon	Size of unique amplicon (Approximate)
OPQ15	GGGTAACGTC	8	2	175 bp - 1.4 kb	1.3 kb, 1.4 kb
OPAH02	CACTTCCGCT	5	4	350 bp - 1.2 kb	1.2kb,1.1 kb,1.0 kb, 600 bp
OPAA02	GAGACCAGAC	5	3	200 bp – 1.12 kb	1.12 kb, 900 bp, 700bp
OPA11	CAATCGCCGT	6	2	280 bp – 2.1 kb	300 bp, 250 bp

This article emphasizes the scope of using molecular techniques as an efficient tool for quality control of fruits at a very early stage of seedling development. The RAPD based molecular technique utilized in this research experiment could detect quality of plants just after establishment of the seedlings. The Horticultural business sector requires sizable land for rearing and maintenance of the plants. In Citrus usually the flowering and fruiting occurs after 5-6 years due to the prevalence of a long juvenile period. The maintenance of low quality non-performing plant types for five to six year could become a costly venture for orchard owners and growers. The rouging of the plants at the age of 5-6 year will generate a problem of agricultural waste removal. The DNA based molecular technique discussed here could detect the true-to-the-type seedlings within 48 hours without destruction of the plant in net house. The process requires only 400 mg of leaf tissue that could be obtained from a 2-3 month old seedling. In this experiment one to two leaves from the three month old seedlings were used for RAPD analysis. Considering the same by three months of establishment of the seedling the PCR based molecular technique could identify nucellar plants in a sustainable way without destruction of the germplasm. The research described above could be used by the horticulturist for quality control of fruits in lucrative Citrus industry with a nominal input.

CONCLUSION

The unidentified pollen source may significantly influence the performance of an elite genotype. The contribution of an undesirable allele may negatively alter the fruit quality of the extracted embryos. The initial detection of off-type plant in nursery and orchard may assist in quality control of fruits and production of uniform progeny population for citrus industry. The DNA fingerprinting process described in this article could trace the embryonic origin of the seedlings within 48 hours. This technique could be used as a rapid, low cost molecular technique for fruit quality control.

Conflict of interest

The author declares no conflict of interest regarding publication of this work.

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Effects of methylcellulose coating with citrus essential oils on the quality of tomato fruits during storage

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ABSTRACT

Purpose: Highly perishable tomatoes face rapid deterioration at postharvest. This study investigated the effect of methylcellulose (MC) edible coating and citrus essential oil (EO) on disease control and postharvest quality preservation of tomatoes. **Research Method:** The experimental factors included MC at three levels (0, 0.5, and 1% (w/v)), citrus EO (control, orange, and sour orange EO, at concentration of (1 g/L), and studying time (ST) (7, 14, and 21 days). The treated fruits were stored at 10°C with RH over 80±5% and evaluated for disease severity and other fruit quality attributes during storage. **Findings:** The results showed that both MC and EO treatments effectively controlled tomato fruit disease and maintained its marketability throughout the experiment, with the combination of these treatments yielding better results. The applied treatments, especially 1% MC, reduced weight loss compared to control. The results indicated increase in coloring of samples during the experiment. The firmness of the fruit tissue decreased over time, and the EO treatment proved to be more effective than MC in preserving fruit firmness. Applying MC and EO treatments, either alone or in combination, preserved total soluble solids compared to the control samples. **Research limitations:** No limitations were encountered. **Originality/Value:** Based on the results of this experiment, incorporating EO into MC edible coating showed promise in extending the shelf life of tomatoes by controlling weight loss, rate of metabolism, and disease severity. This approach offers a sustainable and effective alternative to traditional chemical treatments while providing consumers with a healthier and more flavorful product.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a climacteric fruit with a short shelf life after ripening (Park et al., 2018). Storing some tomato production for off seasons becomes necessary since they cannot all be sold during the production season. However, tomato fruits are prone to severe weight loss, quality deterioration, and fungal contamination after harvest. Therefore, there is a need for treatments that can control weight and quality loss while also having antimicrobial properties (Thole et al., 2020). Postharvest diseases of fresh produce can occur during harvesting, sorting, packaging, storage, and transportation to markets. These diseases can develop at normal room temperatures or even in refrigeration. If the produce is stored in refrigeration, the diseases continue to expand until the product is consumed (Elik et al., 2019). In most cases, postharvest diseases are caused by fungi of the genera *Botrytis*, *Aspergillus*, and *Penicillium*. Even in areas with advanced warehouses using high technology, these fungi can still damage fruits, sometimes causing up to 50% damage (Alegbeleye et al., 2022).

The use of natural preservatives as an alternative to chemical preservatives has grown due to consumers wanting to use natural and safer products (Mesías et al., 2021; Moradinezhad & Firdous, 2025). Among the natural substances that can be used as preservatives in food are essential oils (EOs) and plant extracts. EOs, produced by plants as part of their secondary metabolism, possess many biological effects, including the potential to kill bacteria, fungi, and yeasts (Angane et al., 2022). In the natural world, EOs defend against biotic stresses while attracting pollinating insects, contributing to plant survival and evolution (Raguso, 2020). The active ingredient in plant EOs, is less than 1% of the dry weight of the plant (Ni et al., 2021). Plant EOs are mixtures of compounds produced by living organisms and are obtained through physical means such as distillation from the whole plant or parts of the plant. These fragrant, oily, and easily evaporated plant compounds are produced and stored in specialized secretory structures within various plant parts, such as leaves, flowers, fruits, buds, and stems. They are not always chemically uniform and often involve terpenes (Butnariu, 2021). Citrus EO is considered one of the most important raw materials for flavoring foods and beverages. A total of 21 compounds have been identified in citrus EO, with limonene (94.3%), myrcene (1.5%), linalool (0.9%), decanal (0.5%), alpha-pinene (0.4%), and octanol (0.3%) being the most prominent (González-Mas et al., 2019). Research shows that citrus EO may inhibit pathogen growth in postharvest diseases (Simas et al., 2017). Recently, limonene, a natural citrus compound, has gained attraction due to its versatile applications in food flavoring, green chemistry, pharmaceuticals, and sustainable pathogen and pest control. It's a biodegradable and non-toxic alternative to traditional chemicals, offering eco-friendly solutions (Satari & Karimi, 2018).

Proper packaging is key to reducing waste and preserving the quality and shelf life of horticultural crops during postharvest handling (Rahman et al., 2024). Covering fruits and vegetables with various films and edible coatings is important for packaging. Edible coatings form thin, protective layers that control food's moisture, oxygen, and solute transfer. Likewise, reducing moisture, oxidation, and respiration helps maintain quality and extend the shelf life of fresh products (Iñiguez-Moreno et al., 2024). Methylcellulose (MC) is a natural, colorless, odorless, and non-toxic polysaccharide coating (Kocira et al., 2021). MC edible coating has been applied to peaches, nectarines, apricots, peppers, avocados, citrus fruits, berries, and green beans (Suhag et al., 2020a). Edible coatings have emerged as a promising alternative to traditional chemical preservatives, offering a natural and environmentally friendly approach to extending shelf life and maintaining quality (Perez-Vazquez et al., 2023). Some studies have revealed that coatings, either alone or with natural compounds, extended

the shelf life and maintained the quality of postharvest the particularly perishable foods including, tomatoes (Barbosa et al., 2021; Suhag et al., 2020b; Zhang et al., 2019). To our knowledge, no research has examined the effects of citrus EO-infused MC coating on postharvest tomato quality during storage. This study aims to investigate the effectiveness of the coating in prolonging shelf life and provide fundamental understanding for tomato preservation.

MATERIALS AND METHODS

Preparing samples, coating formulation and application

The experiment was carried out in the postharvest physiology and technology laboratory at Shahed University in 2022. Tomatoes (variety; SV8320TD, Seminis Company) at mature green stage were harvested and washed with tap water. Healthy and uniform fruits were selected for the experiment (Fig. 1). The fruit quality traits at harvest are presented in Table 1. The treatments applied included MC edible coating at three levels (zero as a control, 0.5%, and 1%) and EO at three levels (distilled water as a control, orange (Thomson Navel variety) and sour orange peel EOs, each one at a concentration of 1 g/L), as well as their combination. The required amount of MC powder was weighed and completely dissolved in one liter of 70% methanol while heating to prepare the edible coating. After cooling, the EO was dissolved in the edible coating solution and used for treatment. The EOs from fresh citrus peels (40 g) were prepared by hydrodistillation using a Clevenger-type apparatus (Aria Exir, Iran), for 3 hours. To remove any remaining moisture, the obtained EOs underwent dehydration with the aid of sodium sulfate and was subsequently stored at a temperature of 4°C (Chanthaphon et al., 2018). The EOs extracted from citrus peel exhibited a distinct yellow color, and the yield of the extracted EOs was determined to be 1.2% (w/w).

The fruits were immersed in the solutions prepared from the edible coating of MC and EOs for 10 minutes. They were then transferred to a dry room at 10°C with a relative humidity above 80%. At 7, 14, and 21 days, 21 fruits from each treatment were taken out of storage as three replicates and examined for disease percentage and quality indicators.

Evaluation of characters

The disease of tomato fruits was visually graded based on the severity of each fruit's disease, ranging from one (indicating the lowest amount of disease) to four (indicating the highest amount of disease). Similarly, the marketability of tomatoes was visually graded based on the appearance of each fruit, with scores ranging from one (indicating the lowest degree of marketability) to four (indicating the highest degree of marketability) (Jiang et al., 2010) (1):

$$\text{Marketability index} = \sum [(\text{degree of marketability}) \times (\text{number of fruits in each degree of marketability})] / (4 \times \text{total number of fruits in the treatment}) \quad (1)$$

Table 1. Traits of tomato fruit at the initial time before treatments.

Traits	L*	Hue angle	Firmness (kg/cm^2)	TSS (Brix°)	TA (%)
value	59	128	6.6	6.2	0.81

TSS: total soluble solids, TA: titratable acidity.

Fig. 1. Tomato fruit sample used in this experiment.

To measure the percentage of weight loss, a digital scale with an accuracy of 0.01 g was used to measure and record the initial weight of tomatoes on the day of the treatment. On the day of the investigation, each tomato was weighed again using the same digital scale, and the secondary weight was recorded. Then, the weight loss percentage was calculated using the following equation (2):

$$\text{Weight loss percentage} = (\text{initial weight} - \text{secondary weight}) / \text{initial weight} \times 100 \qquad (2)$$

A colorimeter (model 135 TES, Taiwan) was used to measure the color indices L*, a*, and b*. Random points on each tomato were measured using the colorimeter, and hue angle was calculated by the following equation (3) (Khademi et al., 2013):

$$\text{Hue}^* = \tan^{-1}(b^*/a^*) \qquad (3)$$

The texture firmness of each tomato was measured using a handheld firmness tester (GY-3 model) with a 4 mm diameter. The average texture firmness of the tomatoes was recorded and expressed as Kg/cm^{-2}.

A refractometer (model VBR80, Taiwan) was used to measure the TSS. A drop of tomato juice was placed on the prism of the refractometer, the amount of TSS was recorded and expressed in Brix°.

To measure the TA, 10 ml of filtered fruit extract was mixed with 90 ml of distilled water, bringing it to a final volume of 100 ml. The solution was then titrated with 0.1 N sodium hydroxide until it reached a pH of 8.2. The TA was calculated based on the predominance of citric acid using the relevant formula and expressed as percentage (Barzegar et al., 2018).

Data analysis

The experiment was conducted as a three-factor factorial in a completely randomized design with three replications. The experimental factors included edible coating treatment at three levels, EO treatment at three levels, and studying time (ST), at three levels. After checking the data for normality using SAS software (version 3.9), the data was analyzed, and the difference between the means was compared using the Least Significant Difference (LSD, P=0.05) test. Additionally, the standard deviation of the means was calculated.

RESULTS AND DISCUSSION

Disease

Based on the results of the analysis of variance (Table 2), the main effects of ST, edible coating, and EO, as well as the interaction effects between ST and EO, the interaction between edible coating and EO, and the triple interaction between the factors, on the percentage of disease were significant. However, the interaction effect between ST and edible coating on the disease percentage was insignificant.

The results showed that almost no disease was observed in most treatments on day 7 of the study. However, on day 14, the disease spread under the effect of most treatments, significantly increasing with the increase of storage time to 21 days. Samples without edible coating and EO treatment (control) had the highest disease percentage. On the other hand, applying EO and edible coating treatments reduced the disease percentage of tomato fruit. The lowest rate of disease was observed on tomatoes treated with 1% MC coating in combination with orange and sour orange EOs. In general, orange EO was more effective than sour orange EO in controlling tomato disease in this experiment (Fig. 2-A, Fig. 3).

Tomatoes can suffer from microbial decay due to fungal and bacterial rots, compromising quality and food safety. Edible coatings and the inclusion of antimicrobial compounds, such as plant extracts and EOs, have proven effective in minimizing this decay. For example, applying a chitosan-based coating with *Ruta graveolens* EO to 'Chonto' tomatoes preserved their quality by preventing mold growth, minimizing weight loss, and lowering the decay index during 14 days of storage compared to control (Peralta-Ruiz et al., 2020). A chitosan-grape seed extract coating effectively reduced disease by inhibiting *Salmonella* and total mesophilic aerobes and expanded the shelf life of cherry tomatoes (Won et al., 2018). According to Robledo et al. (2018) thymol incorporated into an edible film of quinoa protein/chitosan effectively inhibited the growth of *Botrytis cinerea* on tomatoes. It was also shown that *Aloe vera* gel alone or combined with sage EO decreased tomato fruit decay symptoms with more pronounced effects on low EO concentration (i.e., 0.1%) after 14 days of storage (Tzortzakis et al., 2019). In addition, the application of the *Aloe vera* gel with *Zataria multiflora* essential oil as a new edible coating on the apple fruit surface resulted in a delay in the severity and occurrence of diseases caused by *Botrytis cinerea* and *Penicillium expansum* (Oraee et al., 2025).

Table 2. Analysis of variance of the effect of MC coating and citrus EO on disease control and preserving tomato fruit quality during storage.

		Mean of squares							
Source of variations	df	Disease	Marketability	L*	Hue	Weight loss	firmness	TSS	TA
Studying time (ST)	2	2177.7**	0.11**	2600.5**	18216.4**	789.4**	3.4**	1.07**	0.43**
Methylcellulose (MC)	2	2088.6**	0.21**	28.3^{ns}	1324.3**	275.8**	1.4*	1.76**	0.06*
Essential oil (EO)	2	1944.4**	0.16**	53.2*	529.1*	418.1**	7.6**	1.52**	0.002^{ns}
MC×ST	4	677.7^{ns}	0.021*	123.1**	1576.3**	141.9**	4.5**	1.72**	0.11**
EO×ST	4	1044.4**	0.057**	43.9**	913.7**	126.5**	0.4^{ns}	0.35**	0.013^{ns}
EO×MC	4	1644.4**	0.073**	35.3*	312.7^{ns}	251.1**	7.5**	2.16**	0.068**
EO×MC×ST	8	894.4**	0.06**	35.2**	199.5^{ns}	79.5**	1.6**	1.28**	0.030*
Error	54	288.8	0.008	8.2	20.4	20.1	0.4	0.089	0.013

ns, *, and ** indicate non-significant, significant at 5% and 1% probability levels, respectively.

EOs are concentrated hydrophobic liquids that may inhibit microbes' growth by attaching to the phospholipid bilayer of their cell membrane through hydrophobic interactions, causing structural damage that ultimately leads to the death of cells. In addition, limonene, a principal constituent of citrus EO, possesses antimicrobial properties that should not be overlooked. Its lipophilic nature allows it to disrupt proteins and lipid layers, altering cell wall function and properties and causing the leakage of intracellular components, ultimately leading to cell death (Tzortzakis et al., 2019).

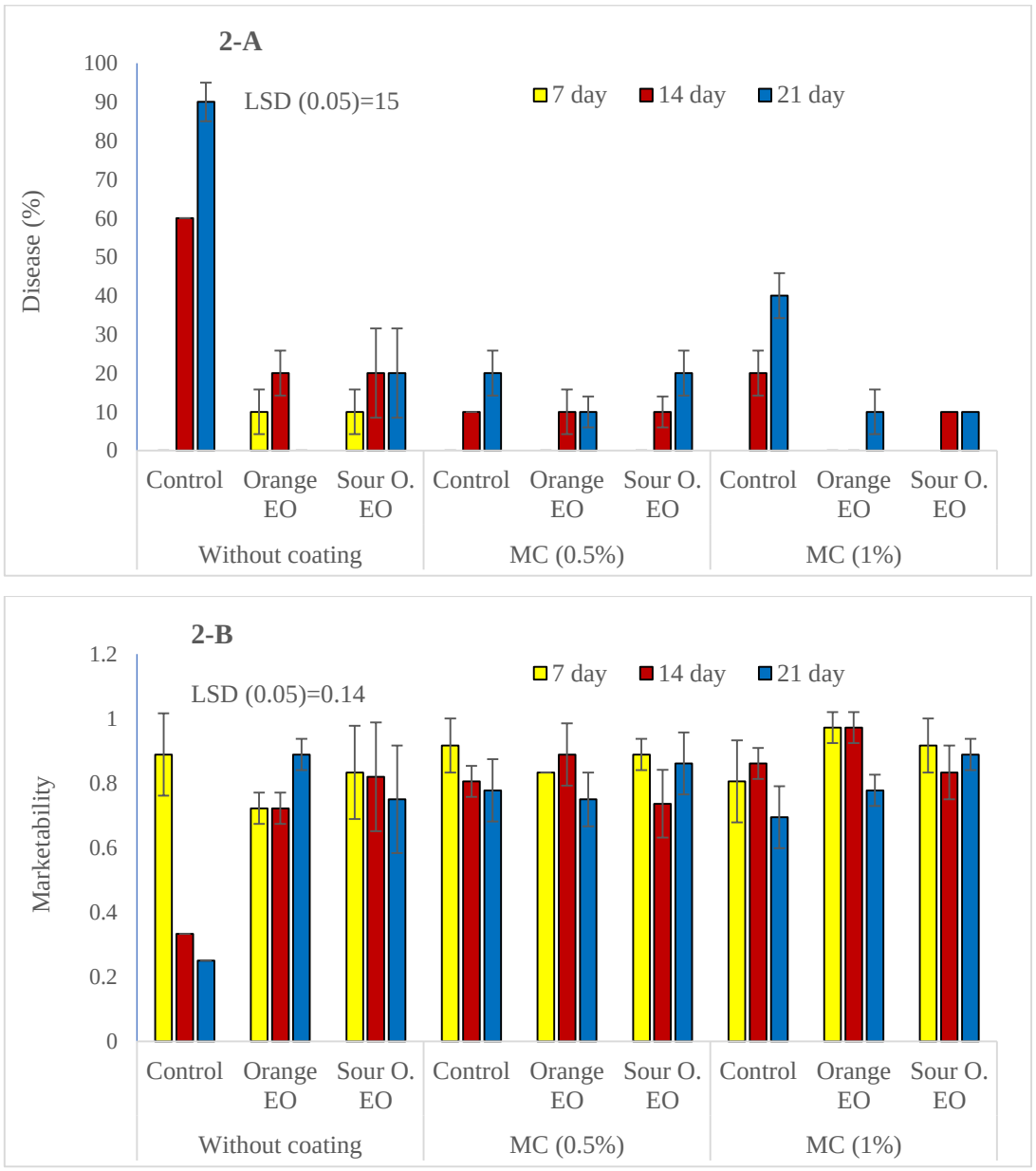

Fig. 2. The effect of MC edible coating and citrus EO on disease percentage (2-A) and marketability (2-B) of tomato fruit during storage at 10 °C with relative humidity ≥ 80%. Error bars represent the standard error of the mean of three replicates.

Marketability

The analysis of variance showed that the effects of ST, edible coating, and EO, as well as the effects of double and triple interactions between these factors on the marketability index of tomato fruit, were significant. The results of the means comparison also showed that the marketability of the fruits decreased over time in most of the treatments. The lowest degree of marketability among the samples was observed in fruits without MC edible coating and EO treatment (control). The application of MC edible coating treatments or EO, alone or in combination with each other resulted in proper preservation of the marketability of tomato fruit during this experiment. The highest degree of marketability was also observed in the MC 1% edible coating samples combined with orange EO (Fig. 2-B, Fig. 3).

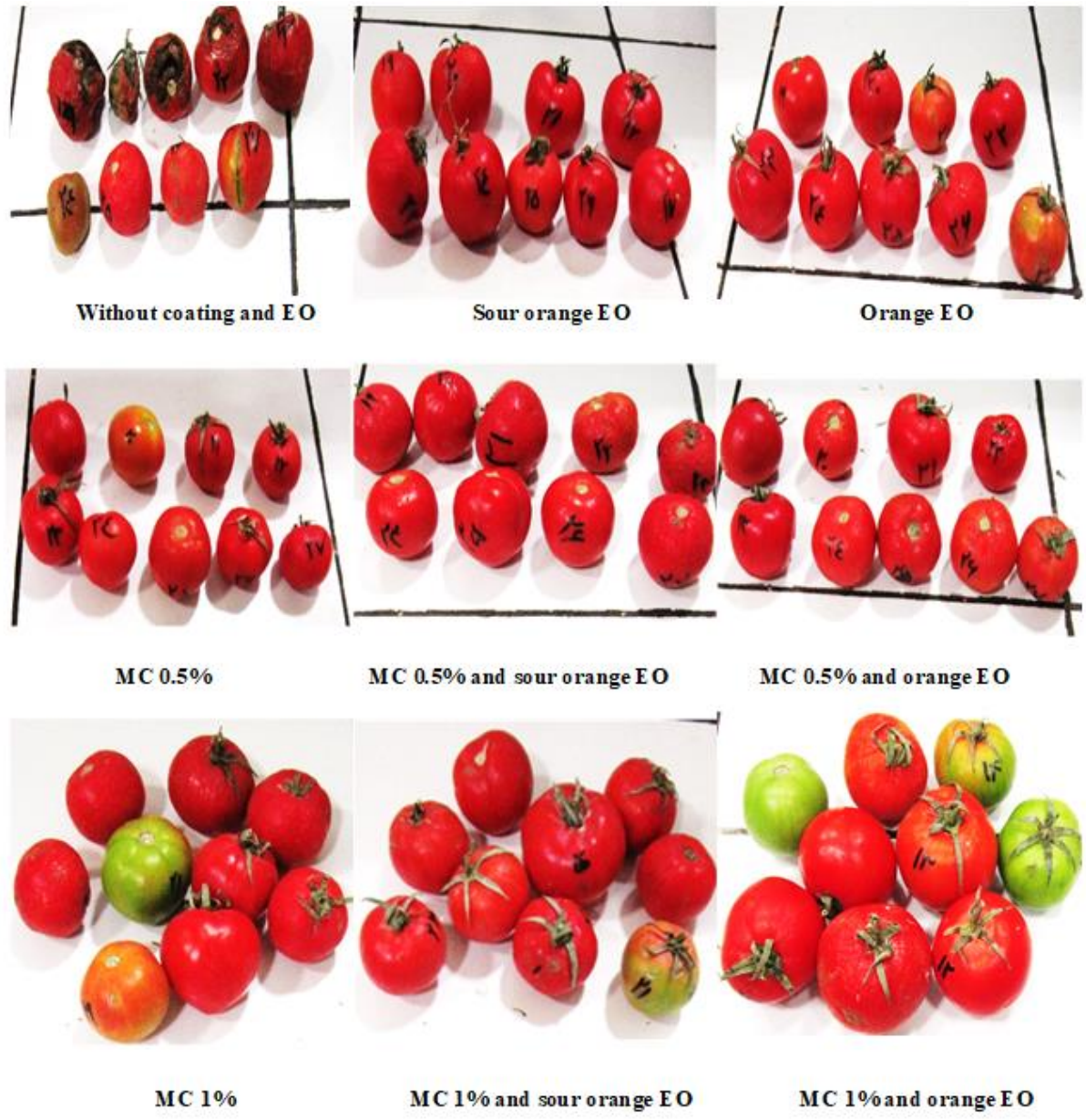

Fig. 3. The effect of MC edible coating and citrus EO in controlling the disease and maintaining tomato marketability for 14 days.

Using MC as a coating for strawberry fruit reduced the growth of microorganisms and subsequently significantly increased the fruit's shelf life during the storage period (Gol et al., 2013; Vu et al., 2011). Adding lemon EO to chitosan edible coating effectively improved the fresh-keeping performance of the film (Demircan & Özdestan-Ocak, 2021). In addition, Tragacanth gum coating has been shown to improve the quality of tomato fruits, including firmness and appearance. This can have a significant impact on the marketability and product sales of tomatoes (Jahanshahi et al., 2023). Moreover, pectin coating containing orange EO increased the shelf life of orange slices without any adverse effect on sensory characteristics (Radi et al., 2018).

Due to the presence of antimicrobial and polyphenol compounds in citrus EO, such as limonene, beta-pinene, gamma-terpinene, and linalool, it is expected that the use of citrus EO will reduce the number of microorganisms and infections induced by them (Lota et al., 2002), which showed in this experiment alone or combined with MC coating. The hydrophobic nature of EOs will cause them to penetrate the lipids of the cell membrane, release ions, and vital compounds, and eventually cause cell death. Toxic effects on membrane structure and function justify the antimicrobial action of plant EOs and their monoterpene compounds (Jugreet et al., 2020).

Fruit color

Based on the results of the analysis of variance (Table 2), the effect of ST ($P \leq 0.01$) and the effect of EO ($P \leq 0.05$) on the L* color index was significant. However, the effect of edible coating on the L* color index was insignificant. The double and triple interaction effects between the factors also significantly impacted the L* color index. The results showed that the highest L* index was observed in all treatments on day 7. As time passed, this index significantly decreased in all samples. On days 7 and 14, no significant difference between the treatments could be observed. Only on day 21 the 0.5% MC samples without EO (control) had a lower L* index than the other samples. At the same time, there was no significant difference between the other treatments regarding the L* color index (Fig. 4).

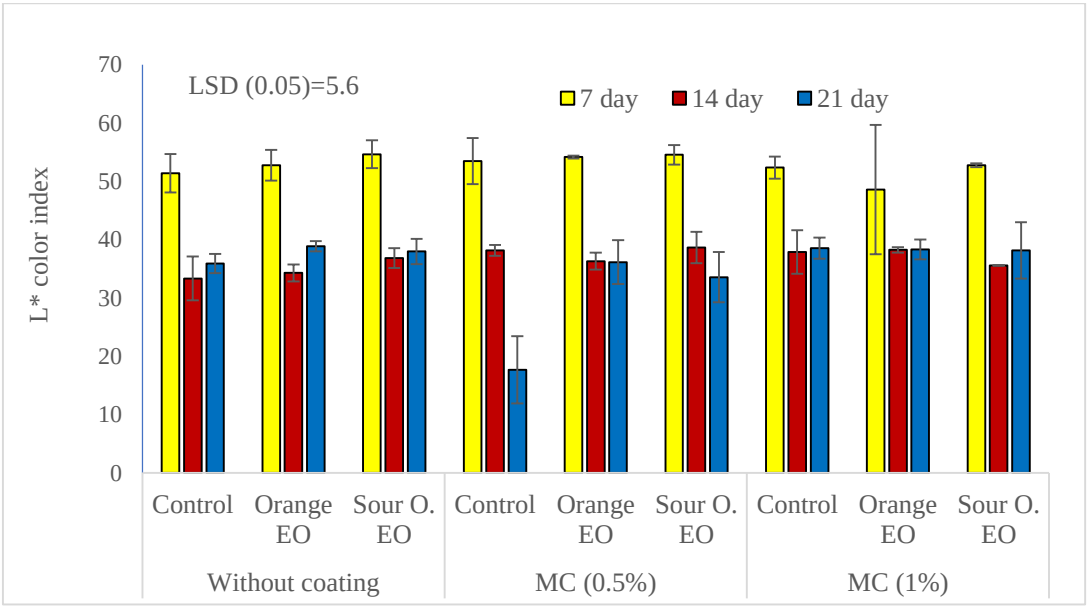

Fig. 4. The effect of MC edible coating and citrus EO on L* color index of tomato fruit during storage at 10 °C with relative humidity ≥ 80%. Error bars represent the standard error of the mean of three replicates.

Table 2 shows significant effects of ST, edible coating ($P \leq 0.01$), and EO ($P \leq 0.05$) on the hue angle. Interaction effects between ST and coating, and ST and EO ($P \leq 0.01$), were also significant, while interactions between coating and EO, and the three factors combined, were insignificant. The highest hue angle occurred on day 7, decreasing significantly by day 14, with no further change by day 21. ST and coating interaction revealed uncoated samples had lower hue angles than MC-coated ones on day 7, but no difference was observed by day 14. On day 21, samples with 0.5% MC had the lowest hue angle (Fig. 5-A). ST and EO interaction showed control samples had higher hue angles than orange and sour orange EO samples on day 7, with no differences between EO types. By days 14 and 21, no differences were observed among the treatments (Fig. 5-B).

The L* index indicates brightness and ranges from 0 to 100, with zero representing black and 100 representing white. An increase in color intensity in red fruits is associated with a decrease in the Hue angle. Additionally, a linear relationship between the reduction of chlorophyll content and the L* color index has been shown in various products (Wrolstad & Smith, 2017). In this experiment, tomato fruits were harvested at the mature green stage and then treated. As time passed, the color intensity of the fruits increased, leading to a decrease in both the Hue angle and the L* index. The results showed that the MC edible coating samples exhibited better color development than the other treatments, particularly the 0.5% concentration.

Weight loss

The analysis of variance revealed significant effects of ST, edible coating, EO, and double and triple interactions among these factors on weight loss ($P \leq 0.01$) (Table 2). Mean comparison results indicated that weight loss increased over time in all treatments, with the highest percentage observed in samples without edible coating or EO treatment. Applying the MC edible coating without EO or EO treatment without coating reduced weight loss. The lowest weight loss percentage was observed in samples treated with 1% MC coating and orange EO, and significant differences were observed between other treatments (Fig. 6-A).

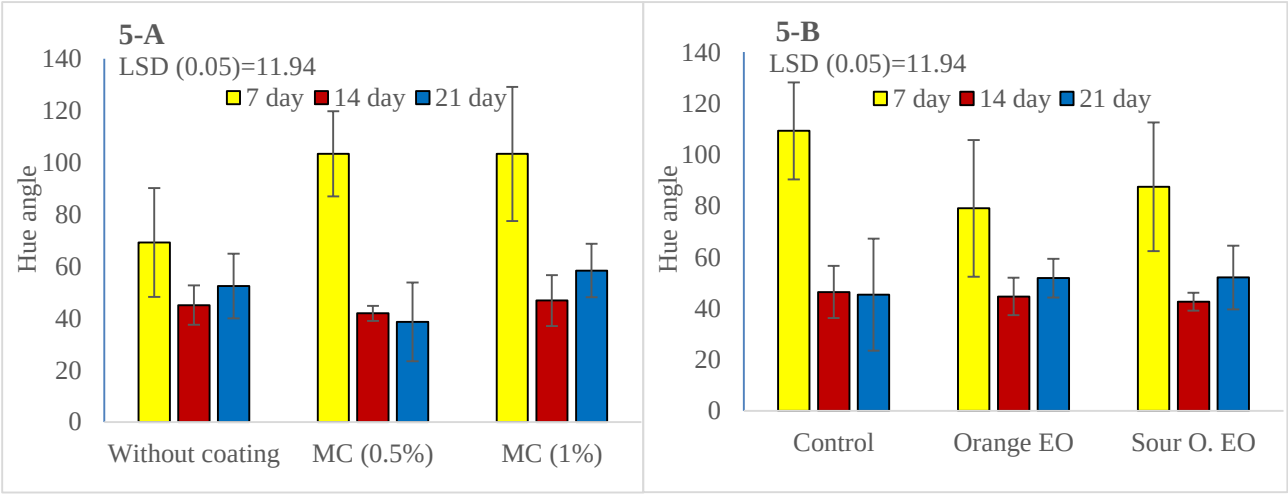

Fig. 5. The effect of MC edible coating (5-A), and citrus EO (5-B) on hue angle of tomato fruit during storage at 10 °C with relative humidity ≥ 80%. Error bars represent the standard error of the mean of three replicates.

After harvesting, fruits remain alive and experience weight loss due to respiratory processes, transpiration, and internal metabolic activities during the postharvest period (Davarynejad et al., 2015). Evaporation and transpiration occur due to differences in water vapor pressure between the intercellular spaces of the fruit tissues and the surrounding atmosphere, as well as increased respiratory conditions (Mostofi et al., 2010). Tomatoes lack thick cuticles and are particularly susceptible to water loss through transpiration, significantly reducing their storability (Khan et al., 2014). Water loss leads to significant changes in cell metabolism, and damage to the cell membrane contributes to weight loss (Mahmoudi et al., 2022).

Plant EOs indirectly control weight loss by delaying the aging process of the fruit (Perumal et al., 2022). Bacteria and fungi that grow on the fruit consume nutrients and accelerate their deterioration, resulting in increased metabolism and weight loss (Yao et al., 2023). In this experiment, EO treatments and MC coating reduced weight loss. Other studies have also reported similar results and the benefits of edible coatings for fruits, such as chitosan (Saleem et al., 2021), gum Arabic xanthan and carrageenan (Wani et al., 2021), pectin and carboxymethyl cellulose (Panahirad et al., 2021), alginate (Duong et al., 2022), and *Aloe vera* gel (Nia et al., 2021). These coatings act as barriers against the diffusion of water vapor and thus slow down weight loss.

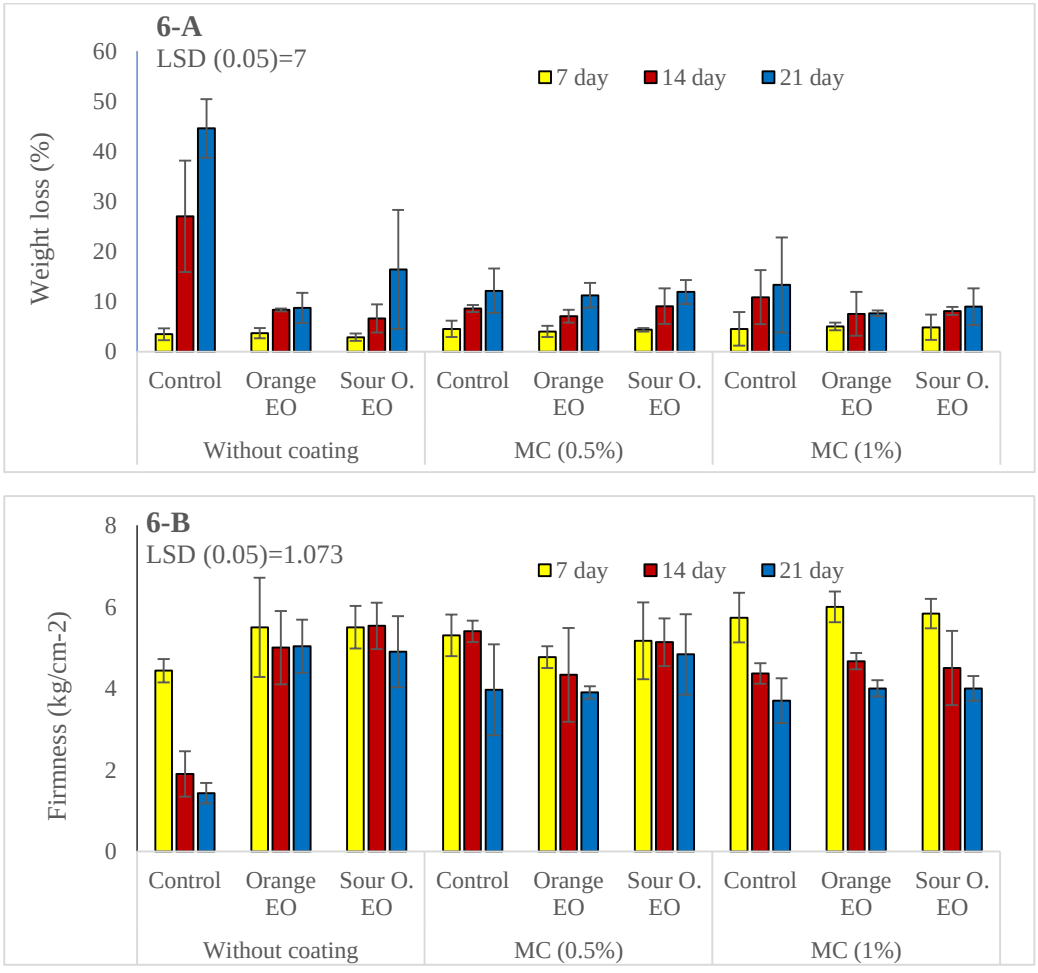

Fig. 6. The effect of MC edible coating and citrus EO on weight loss (6-A), and firmness value of tomato fruit (6-B) during storage at 10 °C with relative humidity ≥ 80%. Error bars represent the standard error of the mean of three replicates.

Tissue firmness

The results of the analysis of variance revealed that the effect of ST ($P \leq 0.01$), the effect of edible coating ($P \leq 0.05$), and the effect of EO ($P \leq 0.01$), as well as the interaction effects between ST and edible coating ($P \leq 0.01$), the interaction between edible coating and EO ($P \leq 0.01$), and the interaction between ST, edible coating, and EO ($P \leq 0.01$) on tissue firmness is significant. However, the interaction effect between ST and EO was non-significant (Table 2).

Based on the test results, the firmness of the tissue decreased significantly with the passage of the test time in all treatments, although the intensity of this decrease varied among the samples. The control samples had the lowest firmness during the study after seven days, indicating the lowest firmness during this test. In contrast, the highest firmness during the experiment was observed in EO-treated samples without coating. Therefore, the application of edible coating treatment (alone or in combination with EO) caused a decrease in firmness compared to the application of EO treatment alone. However, the samples coated with 0.5% MC with sour orange EO had higher firmness than the others (Fig. 6-B).

One of the most important characteristics used to determine the quality and storage life of fruits and vegetables is the degree of decrease in firmness during storage (Huang et al., 2018). The characteristics of fruit tissue depend on the cell mass, structure, and composition of cell wall polysaccharides (Moya-León et al., 2019). The softening of fruits is caused by the breakdown of cell wall compounds, especially pectin, by the activity of particular enzymes, including polygalacturonase (Wang et al., 2018). During storage, the increase in the production of free radicals, which is caused by the beginning of the aging process, leads to the destruction of the central vacuole breakdown of proteins, polysaccharides, cell wall structures, and middle membrane, resulting in the breakdown of wall polysaccharides, increased intercellular space, and reduced tissue firmness (Ghosh et al., 2021). On the other hand, EOs inhibited the activity of cell wall-decomposing enzymes to a large extent, reducing the rate of rotting and the activity of microorganisms (Fincheira et al., 2023). This delays the process of ripening and aging, preserving the firmness of the fruit during storage.

In this experiment, MC edible coating was also effective in maintaining tomatoes' proper firmness, but the effect of EO was more evident in this field. The positive impact of edible coatings of cellulose derivatives on the firmness of tomato tissue has been shown already (Das et al., 2022). MC coating had positive effects on the shelf life and firmness of the fruit tissue of avocados. It regulated water vapor, oxygen, and carbon dioxide transfer inside or outside the product and improved its quality and shelf life (Nadim & Ahmadi, 2016). Rajabi et al. (2022) showed that a carboxymethyl cellulose coating with walnut and lemon EO reduced firmness loss in mushrooms, consistent with our findings. Plant gum coatings with clove EO improved strawberry shelf life (Jodhani & Nataraj, 2019), and alginate with black cumin extract slowed weight loss and ripening in guavas (Hasan et al., 2022). In addition, chitosan oligosaccharides treated fruits exhibited significant delays of firmness and weight loss percentage compared to untreated fruits (Nitu et al., 2025).

Total soluble solids

The results showed that the effects of ST, edible coating, and EO, as well as the double and triple interactions between these factors on TSS, were significant ($P \leq 0.01$) (Table 2). The experiment results showed that the lowest amount of TSS was observed in the control samples without edible coating and EO treatment. In these samples, the amount of TSS decreased significantly over time, reaching about 5.0% at the end of the experiment. However, the application of edible coating or EO treatments, alone or in combination, maintained the amount of TSS compared to the control samples throughout the experiment. While

statistically significant differences in the amount of TSS were observed between the treatments, all treatments ultimately had higher TSS than the control samples (Fig. 7-A).

The increase in TSS during storage can be attributed to weight loss and decreased water content in the fruit tissue. As the fruit ripens and the respiration rate increases, polysaccharides are broken down and converted into simpler sugars, increasing TSS (Li et al., 2022). The increase in TSS in strawberry fruit during storage observed by (Saeed et al., 2021) aligns with the findings of this study. According to some studies, coating fruits with Arabic gum, which is a natural polysaccharide derived from the exudates of Acacia trees, increased in TSS during storage (Huang et al., 2021; Tiamiyu et al., 2023). Coatings play a crucial role in enhancing and stabilizing the TSS in fruits during storage by reducing moisture loss, slowing respiration, delaying ripening, maintaining acidity and ripening index, and modulating biochemical pathways. These combined effects help preserve the fruit's quality and ensure stable TSS levels over extended storage periods (Huang et al., 2021; Daraghmah & Qubbaj, 2021).

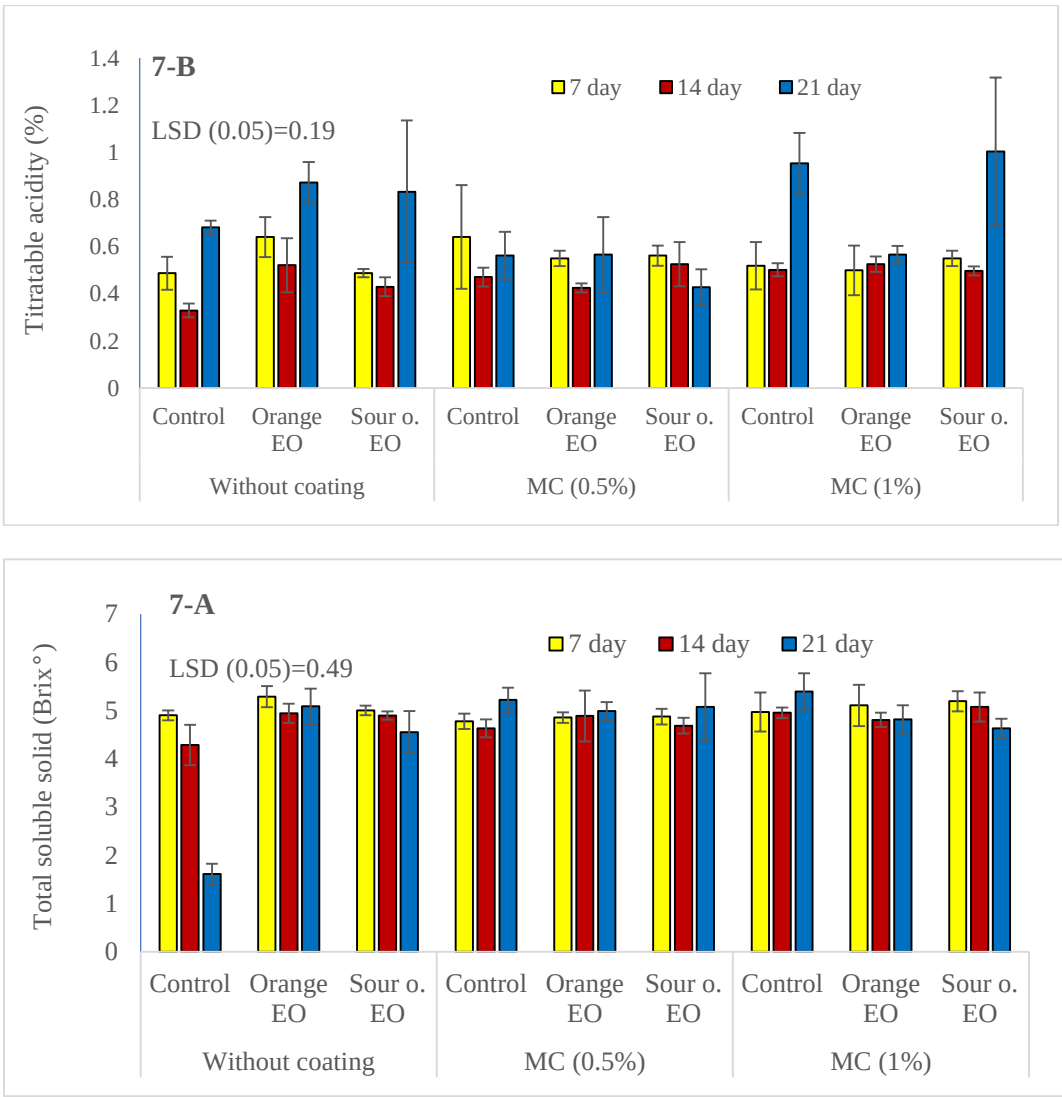

Fig. 7. The effect of MC edible coating and citrus EO on TSS (7-A), and TA percentage (7-B) of tomato fruit during storage at 10 °C with relative humidity ≥ 80%. Error bars represent the standard error of the mean of three replicates.

Titratable acidity

Based on the obtained results, the effect of ST ($P \leq 0.01$), the effect of edible coating ($P \leq 0.05$), the interaction effect between ST and edible coating ($P \leq 0.01$), the interaction between the edible coating and EO ($P \leq 0.01$), and the interaction between ST, edible coating, and EO ($P \leq 0.05$) were found to be significant on TA percentage. However, the effect of EO and the interaction between ST and EO were found to be insignificant (Table 2). The results of mean comparison showed that the trend of TA changes was different based on the type of treatment, but in general, TA in most treatments decreased on the 14th day of the study compared to the 7th day and increased again on the 21st day compared to the 14th day. In the treatment without coating (control, orange, and sour orange EOs) and MC 1% (combined with control and sour orange EO), the highest amount of TA was measured on the 21st day of the study, but in MC coating 0.5% (combined with control, orange, and sour orange EOs) and 1% MC (combined with orange EO) changes in TA were less compared to other treatments (Fig. 7-B).

TA provides valuable information about the taste and flavor of fruits and vegetables. The perceived acidity is a key factor influencing the overall sensory experience and consumer acceptance of the product (Xu et al., 2023). Fruits with an appropriate level of acidity often taste fresher and more flavorful. In general, organic acids act as an energy reserve for fruits during the ripening and aging process, and their consumption increases with metabolism, leading to a decrease in acidity (Rashmi & Negi, 2022). Some studies have reported a relationship between fruit acidity and enzyme activity. The decline in tomato acidity during storage is attributed to metabolic changes caused by consuming organic acids during cellular respiration (Zheng et al., 2022). In tomatoes, citric acid is the primary organic acid used in respiration, and organic acid utilization subsequently decreases TA (Oms-Oliu et al., 2011).

Edible coatings have been found to reduce the respiration rate and delay the reduction process of organic acids, thus maintaining the acidity in fruits (Ehteshami et al., 2022; Sousa et al., 2021). It was shown that gum Arabic coating, which has gas and water vapor barrier properties, resulted in higher TA corresponding with low pH while also maintaining quality, and extending the shelf life of mangoes compared to the control group (Daisy et al., 2020). Additionally, an edible coating made from cassava starch and vegetable oil was found to reduce TA during the storage of tomatoes (Adjouman et al., 2018).

Some research shows that during the ripening of tomato fruit, due to bulky size and the density of the tissues, a hypoxic state is created, which leads to a decrease in respiration and an increase in fermentation in the fruit tissues. In this case, the consumption of organic acids decreases, while some acids are also produced due to fermentation, which may be the reason for the increase in TA observed in this experiment at the end of the ripening stage. The greatest increase in acidity was observed in the control fruits and those treated with 1% methyl cellulose. Perhaps the thicker fruit coating in this case and the greater density of the control fruit tissue created more suitable conditions for the hypoxic phenomenon, which was followed by an increase in TA (Xiao et al., 2024).

CONCLUSION

In conclusion, this study demonstrated the significant potential of MC edible coating and citrus EO in enhancing disease control and preserving the postharvest quality of tomato fruit. Applying MC proved effective in reducing fruit weight loss, while both edible coating and EO treatments contributed to maintaining the overall quality of the fruit. Notably, the combination of these treatments outperformed individual applications. Highly perishable tomatoes face rapid deterioration postharvest. As a novel strategy, incorporating bioactive compounds such as EOs into edible coatings shows promise in extending shelf life by

addressing issues like moisture loss and metabolism and reducing microbial load. In addition, this research contributes valuable insights into postharvest management in the fresh-eating and processing industries, offering practical implications for sustainable and effective preservation methods for tomato fruit. This approach offers a sustainable and effective alternative to traditional chemical treatments while providing consumers with a healthier and more flavorful product. Further research and practical applications of these findings can contribute to developing sustainable and safe postharvest management practices in the tomato industry.

Conflict of interest

The authors state that they have no competing financial interests related to the publication of this work.

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Effects of PGPR inoculation on adventitious rooting and growth attributes in olive microcuttings of 'Mission' and 'Koroneiki' cultivars

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ABSTRACT

Purpose: This study aimed to investigate the synergistic effects of plant growth-promoting rhizobacteria (PGPR) and auxin on olive microcutting rooting, and assess how PGPR and arbuscular mycorrhizal fungi (AMF) inoculation affect the growth of rooted olive plantlets. **Research Method:** In the first experiment, native PGPR inoculation with indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) was tested to enhance rooting in olive microcuttings of 'Mission' cultivar. The second experiment evaluated six inoculation treatments (control, PGPR**,** *Funneliformis mosseae***,** *Claroideoglomus etunicatum*, PGPR + *F. mosseae*, and PGPR + *C. etunicatum*) for their impact on rooted plantlet growth of 'Mission' and 'Koroneiki' cultivars. **Findings:** The study showed that PGPR and IBA treatment for 12 weeks resulted in a higher rooting rate (63.33%) and more roots per cutting (4.5) compared to the control. Additionally, PGPR and IBA combination for 16 weeks produced the longest roots (59.03 mm), indicating PGPR's role in enhancing root initiation and growth through auxin modulation. The results also revealed that the 'Mission' cultivar had higher AMF colonization than the 'Koroneiki' cultivar. The inoculation with *F. mosseae* significantly increased the number of lateral shoots and leaves, stem diameter, and root length in 'Koroneiki', while PGPR + *F. mosseae* enhanced lateral shoots, leaf number, and stem diameter in 'Mission'. The 'Koroneiki' cultivar also exhibited greater growth responses in stem and root weights, and plant height to AMF and PGPR inoculation. **Research limitations:** No limitations were identified. **Originality/Value:** These findings underscore the importance of genetic background in biofertilization strategies for olive cultivation, demonstrating the synergistic potential of PGPR and auxin in rooting and the cultivar-specific benefits of combined PGPR and AMF inoculation.

INTRODUCTION

The olive tree (*Olea europaea* L.), native to the Mediterranean and part of the Oleaceae family, is valued for its edible fruit and oil, which are rich in monounsaturated fats and antioxidants (Visioli & Galli, 2002). However, the increasing global demand for olive products, coupled with climate challenges, is prompting changes in cultivation practices. These changes are altering traditional landscapes and potentially reshaping the structure and composition of microbial communities in orchards, which may, in turn, influence productivity and resilience to stress. The bacterial and fungal communities associated with plants are vital for plant growth and health, making them essential for sustainable agriculture (Dias et al., 2024). In particular, soil microbiota enhance plant growth, development, and overall fitness while improving soil health and fertility, which is crucial for advancing sustainable agricultural practices (Nadarajah & Abdul Rahman, 2023).

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that reside in the rhizosphere and enhance plant growth through various mechanisms. One of the primary benefits of PGPR is their ability to stimulate the production of plant hormones such as auxins, cytokinins, and gibberellins, which are pivotal for essential physiological processes, including root development and shoot elongation (Ajdig et al., 2024). Moreover, PGPR play a critical role in nutrient cycling and soil fertility by solubilizing and mobilizing essential nutrients like phosphorus and potassium, making them more accessible to plants (Maheshwari et al., 2019; Azarmi-Atajan & Sayyari-Zohan, 2020). Once established in the rhizosphere, PGPR can persist for extended periods, supported by a symbiotic relationship with the plant. Consequently, PGPR represent a valuable source of biofertilizers, biostimulants, and biocontrol agents, making them integral to organic olive production (Sallami et al., 2023).

Olive cutting propagation, a widely practiced technique, involves using stem cuttings to generate new clonal plants by relying on the innate capacity of olive tissue to form adventitious roots, often enhanced with synthetic auxins or other growth regulators (Hartmann et al., 2018). Microcutting, an alternative propagation method that uses small, excised shoot segments, offers advantages such as higher multiplication rates, better control over genetic fidelity, and the ability to propagate elite cultivars (Lambardi et al., 2023). Studies have demonstrated that PGPR can further enhance the rooting and establishment of microcuttings. For instance, Azospirillum species have been shown to stimulate root development, increase root biomass, and improve the survival rate of Prunus microcuttings (Russo et al., 2008). By leveraging the growth-promoting and stress-mitigating properties of PGPR, olive nurseries can optimize the efficiency and success of cutting propagation.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms that form mutualistic associations with the roots of many plant species, including olive trees (Palla et al., 2020). These fungi colonize the plant's root system, extending the root surface area and forming extraradical hyphae, which act as an extension of the root system (Gianinazzi et al., 2010). *Funneliformis mosseae* and *Claroideoglomus etunicatum* are two critical arbuscular mycorrhizal fungi that enhance plant growth, stress tolerance, and soil nutrient dynamics, showing promise for sustainable agriculture and ecological restoration (Berruti et al., 2016). The combined use of AMF and PGPR results in a more robust and productive plant system compared to the individual application of either microorganism (Vivas et al., 2003). The synergistic effects arise from the complementary roles of these microbes: AMF enhances nutrient and water acquisition, while PGPR stimulates plant growth and development through phytohormone production and stress alleviation. This integrated approach significantly improves olive growth (Bizos et al., 2020). Recent studies have revealed that dual inoculations of AMF and PGPR significantly improve survival, growth, physiology, and

biochemical traits of myrtle seedlings under drought stress by enhancing water and nutrient supply, stimulating antioxidant defense, and mitigating oxidative damage. This approach ultimately boosts drought tolerance and essential oil production (Azizi et al., 2021).

The aims of this study were to investigate the synergistic effects of PGPR and auxin treatments on the adventitious rooting in olive microcuttings of the 'Mission' cultivar and to examine the influence of PGPR and AMF inoculation on the growth attributes of rooted olive plantlets in the 'Mission' and 'Koroneiki' cultivars. The 'Mission' and 'Koroneiki' are two significant olive cultivars well-suited to the climatic conditions of the research area. Each possesses distinct characteristics and applications. The 'Mission' cultivar is versatile, suitable for both table olives and oil production, whereas the 'Koroneiki' is renowned for its robust, high-quality oil, characterized by a peppery and aromatic flavor.

MATERIALS AND METHODS

This research involved two experiments designed to investigate the effect of inoculation with native PGPR on the rooting of olive microcuttings and the growth of the rooted young plantlets. The experiments were conducted in 2020 in the greenhouse and laboratory of the Department of Horticultural Sciences at Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Isolation and preparation of PGPR inoculum

To isolate PGPR, four soil samples were collected from the rhizosphere of olive trees in a standard orchard, adhering to biological sampling principles. Ten grams of rhizosphere soil were mixed with 90 mL of physiological serum (0.9% sodium chloride) and shaken for 30 minutes at 150 rpm. Serial dilutions were prepared, and two replicates of samples from five lower dilutions (108, 107, 106, 105 and 104) were plated on nutrient agar medium. After bacterial growth, 24 bacterial isolates were selected based on colony shape, morphology, and color and combined to prepare the inoculum (Fig. 1A) (Marzban et al., 2019).

Experiment 1: The effect of PGPR inoculation on olive microcuttings rooting

This experiment followed a factorial design based on a completely randomized design with three replications, each comprising 10 microcuttings. The first factor was the rooting period, with two levels (12 and 16 weeks). The second factor included a non-inoculated control and PGPR inoculation, while the third factor consisted of treatments with indole-3-butyric acid (IBA) (3 g/L), naphthaleneacetic acid (NAA) (1.5 g/L), and controls. Microcuttings were prepared from healthy, mature 'Mission' cultivar trees, aged 8 years, grown in a standard orchard. Current-year shoots were harvested in the first week of October, and microcuttings (4-6 mm in diameter, 10-12 cm in length) were prepared, treated, and planted at a depth of 3-4 cm in rooting medium within greenhouse cultivation trays equipped with a misting system (Fig. 1B).

The culture medium consisted of perlite with a particle diameter of 1.5 to 2.5 mm, sterilized in an autoclave at 121°C and 15 psi for 15-20 minutes. Sterile perlite was used for the non-inoculated control, while perlite containing 107 CFU/mL bacteria was applied for PGPR inoculation (Liffourrena & Lucchesi, 2018). Microcuttings were treated with auxin for 10 seconds, while control microcuttings were treated with distilled water. After 12 and 16 weeks, the parameters measured included rooting percentage, number of roots, average root length, fresh root weight, and dry root weight.

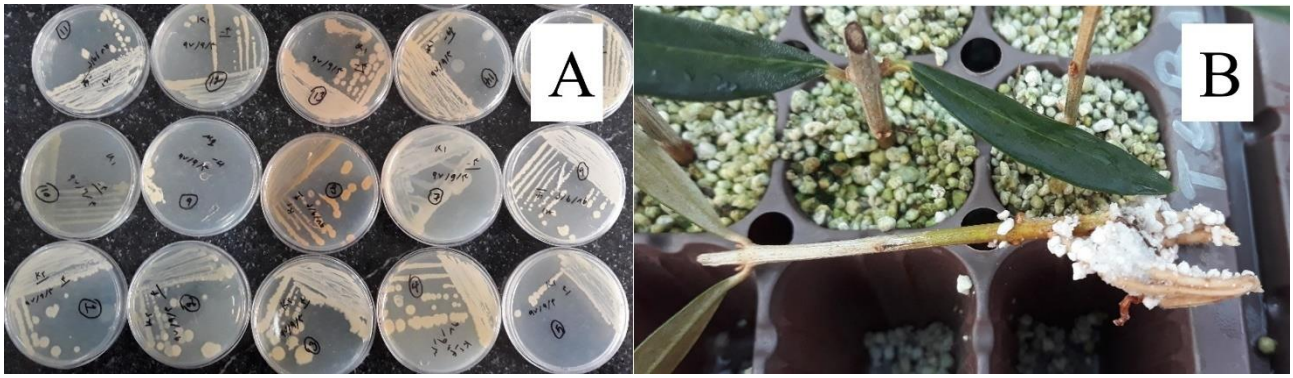

Fig. 1. A) Isolation and preparation of plant growth-promoting rhizobacteria; B) rooted microcuttings.

Experiment 2: The effect of PGPR and AMF inoculation on the growth of rooted olive plantlets

This experiment employed a factorial design based on a completely randomized design. The first factor was the cultivar, with two levels ('Koroneiki' and 'Mission'), and the second factor was inoculation with six treatments: non-inoculated control, PGPR, *F. mosseae*, *C. etunicatum*, PGPR + *F. mosseae*, and PGPR + *C. etunicatum*, with seven replications. Plantlets were planted in 2 L pots filled with a 1:1:1 mixture of agricultural soil, vermicompost, and sand. The potting mixture had the following composition: C (1.4%), N (0.10%), K (368 mg/kg), P (10.2 mg/kg), pH 7.7, and EC 1.5 dS/m. The mixture was sterilized at 121°C and 1.5 bars for one hour. Plantlets were grown in a controlled greenhouse environment with a relative humidity of 75 ± 10% and a daytime temperature of 25 ± 5°C.

For PGPR inoculum preparation, a bacterial population was added to NB medium at 2% volume and incubated for 48 hours on a shaker at 150 rpm. Plantlet roots were soaked in the PGPR suspension for 30 minutes prior to planting. AMF (*F. mosseae* and *C. etunicatum*) inoculum, obtained from Toran Biotechnology Company, consisted of rhizosphere soil, AMF spores (minimum 30 spores/g soil), hyphae, arbuscules, and root segments of *Trifolium repens* L. Each pot was inoculated with 40 g of fungi powder mixed into the potting medium. For combined PGPR and AMF treatments, roots were first soaked in the PGPR inoculum, followed by the application of AMF inoculum in the rhizosphere.

At the end of the seven-month growing period, the plant characteristics measured included the number of new lateral branches and leaves, leaf area per plant, internode length, plant height, stem diameter, and root length. Root colonization by symbiotic structures was determined 12 weeks post-inoculation via root staining and microscopic examination (Dalpe, 1993). Fresh weights of stems and roots were recorded, followed by dry weight determination after drying at 70°C for 48 hours (Ganjeali & Kafi, 2007). Total chlorophyll, chlorophyll a, and chlorophyll b content in leaves were measured using the DMSO method (Barnes et al., 1992), and total phenolic content was quantified using the Folin-Ciocalteu method and a spectrophotometer at 760 nm (Seifi & Bekran, 2024).

Data analysis

All data were analyzed using SAS software (version 9.3). Means were compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The effects of PGPR inoculation on adventitious rooting

The analysis of variance revealed a significant interaction effect between rooting period, PGPR inoculation, and auxin treatment on rooting percentage (P<0.001), root number (P=0.004), and root length (P<0.001) (Table 1). These findings align with previous studies that have demonstrated the synergistic effects of PGPR and auxin on root development in various plant species (Vacheron et al., 2013; Maniriho et al., 2021). Specifically, the data indicated that PGPR inoculation combined with IBA after 12 weeks resulted in a significantly higher rooting rate (63.33%) and more roots per cutting (4.5) compared to the control, which showed a rooting rate of only 3.33% and 0.33 roots per cutting. This supports the literature suggesting that PGPR enhances root initiation and growth by modulating auxin signaling and metabolism (Spaepen et al., 2007). Moreover, PGPR inoculation with IBA after 16 weeks produced the longest root length, reaching 59.03 mm. This result underscores the role of PGPR in promoting root elongation by solubilizing nutrients and making them available for plant growth and development (Russo et al., 2008; Vacheron et al., 2013).

Table 1. The interaction and independent effects of rooting period, PGPR inoculation, and auxin treatment on rooting percentage and root characteristics in olive cultivar 'Mission'.

Rooting period	PGPR	Auxin	Rooting (%)	Root (n)	Root length (mm)	Root fresh weight (g)	Root dry weight (g)
			P<0.001	P=0.004	P<0.001	P<0.001	P=0.223
12 wk	Non-inoculated	Control	3.33 f	0.33 f	14.33 g	0.03 e	0.006
		IBA	33.33 c	4.43 a	32.16 f	0.21 a	0.015
		NAA	16.63 e	3.16 c	44.83 b	0.20 a	0.025
	Inoculated	Control	0.00 f	0.00 f	0.00 i	0.00 g	0.000
		IBA	63.33 a	4.50 a	36.20 d	0.18 b	0.019
		NAA	20.00 d	2.60 d	33.93 e	0.11 d	0.026
16 wk	Non-inoculated	Control	20.00 d	0.83 e	4.61 h	0.02 ef	0.007
		IBA	60.00 b	2.29 d	45.27 b	0.13 c	0.021
		NAA	33.33 c	4.36 ab	36.20 d	0.13 c	0.020
	Inoculated	Control	20.00 d	1.21 e	13.90 g	0.01 fg	0.011
		IBA	60.00 b	4.36 ab	59.03 a	0.20 a	0.028
		NAA	33.33 c	4.00 b	40.52 c	0.20 a	0.027
Rooting period			P<0.001	P<0.001	P<0.001	P=0.816	P=0.659
12 wk			22.77 b	2.50 b	26.91 b	0.12	0.015
16 wk			37.77 a	2.84 a	33.25 a	0.11	0.019
PGPR			P<0.001	P=0.015	P<0.001	P=0.387	P=0.248
Non-inoculated			27.77 b	2.57 b	29.56 b	0.12	0.015
Inoculated			32.77 a	2.78 a	30.59 a	0.11	0.018
Auxin			P<0.001	P<0.001	P<0.001	P=0.035	P=0.820
Control			10.83 c	0.59 c	8.21 c	0.02 c	0.006
IBA			54.16 a	3.90 a	43.16 a	0.18 a	0.021
NAA			25.82 b	3.53 b	38.87 b	0.16 b	0.024

Different letters in each column represent significant differences at P=0.01, Duncan's multiple range test. The abbreviations are as following: IBA (indole-3-butyric acid), NAA (naphthaleneacetic acid), and PGPR (plant growth-promoting rhizobacteria).

The analysis of variance also showed a significant interaction effect of the three treatments on root fresh weight (P<0.001) but not on root dry weight (P=0.223) (Table 1). This suggests that the treatments primarily influenced water content and cellular expansion in the roots rather than overall biomass accumulation (Glick, 2012). The highest root fresh weight recorded was 0.21 g after treatment with IBA for 12 weeks without PGPR inoculation, indicating that IBA treatment alone had a more pronounced effect on root fresh weight. The lack of a significant effect on root dry weight suggests that none of the treatments directly contributed to substantial biomass accumulation, implying that water retention and expansion, rather than an increase in solid biomass, were the primary drivers of the observed treatment effects.

The independent effects of rooting period, PGPR inoculation, and auxin treatment on rooting percentage, root number, and root length were significant (Table 1). This highlights the critical role of these individual factors in promoting root development in the studied plant system (Hartmann et al., 2018). Notably, the data show that PGPR inoculation and auxin application, particularly IBA, significantly increased all three root traits. PGPR inoculation enhanced rooting significantly, achieving 32.77% compared to 22.77% in the non-inoculated control. Among auxin treatments, IBA induced the highest rooting rate at 54.16%, significantly outperforming the control (10.83%) and NAA (25.82%). A similar trend was observed for root length, with IBA-treated cuttings reaching 43.16 mm, compared to 38.87 mm for NAA and 8.21 mm for the control. These findings are consistent with previous research demonstrating the synergistic effects of PGPR and auxin on root growth and development (Vacheron et al., 2013). In contrast, the independent effects of the three treatments on root fresh weight and root dry weight were not significant, except for the effect of auxin on root fresh weight, which was significant (P=0.035). The superior efficacy of IBA over NAA in promoting root development aligns with prior studies highlighting IBA's greater effectiveness in stimulating root formation and growth (Pacurar et al., 2014). This difference may be attributed to the distinct mechanisms of action and transport dynamics of these two auxin compounds within plant tissues.

The effects of PGPR and AMF inoculation on growth attribute

The results of this study indicate that olive plantlets of the 'Mission' cultivar exhibited a higher percentage of colonization by AMF compared to the 'Koroneiki' cultivar (Fig. 2). In the 'Mission' cultivar, the highest colonization percentage was recorded at 89.19%, significantly higher than the 22.27% observed in the control treatment. This suggests that the 'Mission' cultivar is more responsive to AMF inoculation, potentially due to its genetic makeup or physiological characteristics. These findings align with the hypothesis proposed by Estaún et al. (2003), which suggests that different cultivars exhibit varying responses to the same fungal species. Similarly, Eftekhari et al. (2012) reported differences in root colonization percentages across four grape cultivars. Interestingly, within the 'Mission' cultivar, no significant differences were observed among the other main treatments of AMF or the combined treatments of PGPR with AMF. This indicates that combined inoculation did not have a synergistic effect on colonization percentage in this cultivar. The lack of a significant interaction between PGPR and AMF suggests that AMF inoculation alone may be sufficient to achieve optimal colonization levels in the 'Mission' cultivar. In contrast, the 'Koroneiki' cultivar exhibited significant differences in colonization percentages among the AMF treatments. The highest colonization was observed with *F. mosseae* at 76.5%, while the lowest was recorded with the PGPR + *C. etunicatum* treatment at 63.47%. These results suggest that cultivars may have varying sensitivities to different AMF species, a phenomenon also reported by Berruti et al. (2016). Additionally, findings by Seifi et al. (2014) reported

that *Glomus intraradices* achieved a higher colonization percentage (79.66%) in olive plantlets compared to *G. mosseae* (73.33%). The low levels of contamination by native fungi observed in the control treatments did not appear to affect the growth of the olive plantlets, consistent with results from other studies (Eftekhari et al., 2012; Ziatabar Ahmadi et al., 2024). This suggests that native fungal communities may not significantly impact the plant's performance, and the observed differences in colonization percentages can be attributed to the inoculated treatments.

The study's results indicate that inoculation with *F. mosseae* had the most significant positive effect on the number of new lateral shoots in the 'Koroneiki' olive cultivar, with the highest number recorded at 4 (Table 2). Conversely, the 'Mission' cultivar exhibited the highest number of new lateral shoots (4) when inoculated with PGPR + *C. etunicatum* (Fig. 3). This suggests that, for the 'Mission' cultivar, a synergistic interaction between PGPR and AMF may be more effective in stimulating lateral shoot production than either inoculant alone. These findings highlight the cultivar-specific nature of microbial interactions and the importance of tailoring inoculation strategies to the genetic and physiological characteristics of each olive cultivar. The lowest number of new lateral shoots (1.3) was observed in the control treatment of the 'Koroneiki' cultivar, suggesting that inoculation with beneficial microorganisms can enhance the production of new lateral shoots in olive plants (Hanane et al., 2020).

The highest number of new leaves (112) was recorded in the *F. mosseae* treatment (Table 2), demonstrating the positive impact of this AMF on leaf production (Russo et al., 2008; Meddich et al., 2015). Similarly, Chenchouni et al. (2020) reported that AMF application improved the number of leaves in olive plantlets. Conversely, the control treatments exhibited the fewest leaves, averaging just 10 and 18 in the 'Koroneiki' and 'Mission' cultivars, respectively. This highlights the importance of inoculating olive plantlets with beneficial soil microorganisms to promote vegetative growth.

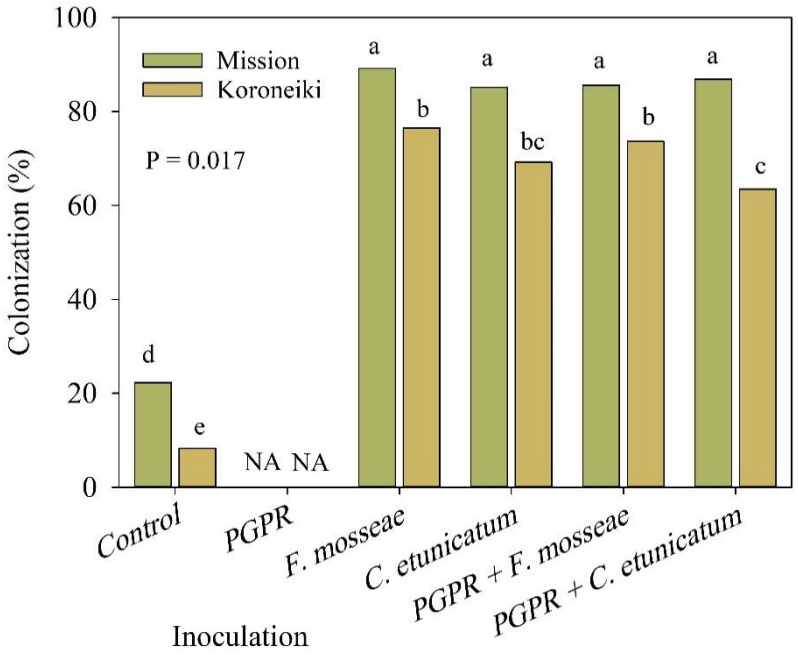

Fig. 2. The colonization percentage of different treatments in olive plantlets of cultivars 'Mission' and 'Koroneiki'. Different letters represent significant differences at P=0.05, Duncan's multiple range test. The abbreviations are as following: NA (not applicable), and PGPR (plant growth-promoting rhizobacteria).

Table 2. The interaction effects of PGPR and AMF inoculation on some growth parameters of olive plantlets cultivars 'Mission' and 'Koroneiki'.

Cultivar	Inoculation	New shoot (n)	New leaves (n)	Leaf area/ plant (cm^2)	Internode length (cm)	Plant height (cm)	Stem diameter (mm)	Root length (cm)
		P<0.001	P<0.001	P<0.001	P=0.002	P<0.001	P=0.031	P=0.022
Mission	Control	2.0 d	18.00 f	29.42 ef	0.86 f	13.5 e	2.74 d	25.66 efg
	PGPR	2.0 d	24.33 e	77.96 e	1.40 e	14.83 e	2.64 d	29.33 cde
	F. mosseae	3.0 bc	51.33 c	263.54 ab	2.33 abc	33.16 c	3.29 cd	26.56 d-g
	C. etunicatum	3.3 b	38.66 d	148.94 d	2.05 cd	24.26 d	2.72 d	25.26 fg
	PGPR + *F. mosseae*	4.0 a	69.00 b	157.57 cd	1.85 d	25.33 d	3.72 b	24.26 g
	PGPR + *C. etunicatum*	3.0 bc	47.66 c	168.62 cd	1.80 d	25.26 d	2.92 cd	24.16 g
Koroneiki	Control	1.3 e	10.33 g	14.71 f	0.46 g	5.66 f	2.71 d	32.66 bc
	PGPR	2.7 c	19.33 ef	38.58 ef	1.16 ef	10.93 e	2.91 cd	38.83 a
	F. mosseae	4.0 a	112.00 a	279.35 a	2.11 bcd	49.10 a	4.25 a	39.50 a
	C. etunicatum	2.0 d	64.33 b	213.19 bc	2.53 a	45.36 a	3.45 bc	35.03 b
	PGPR + *F. mosseae*	3.0 bc	65.33 b	319.57 a	1.96 cd	38.43 b	3.69 b	28.23 def
	PGPR + *C. etunicatum*	3.0 bc	40.66 d	184.48 cd	2.45 ab	32.90 c	3.64 b	29.50 cd

Different letters in each column represent significant differences at P=0.01, Duncan's multiple range test. The abbreviations are as following: AMF (arbuscular mycorrhizal fungi) and PGPR (plant growth-promoting rhizobacteria).

Fig. 3. The effects of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi inoculation on growth of olive plantlets cultivar 'Mission'. 1: Control, 2: PGPR, 3: *F. mosseae*, 4: *C. etunicatum*, 5: PGPR + *F. mosseae*, 6: PGPR + *C. etunicatum*.

The results also demonstrate that the highest leaf area per plant was observed in the 'Koroneiki' cultivar, with the *F. mosseae* and PGPR + *C. etunicatum* treatments recording 279.35 cm^2 and 319.57 cm^2, respectively (Table 2). In the 'Mission' cultivar, the highest leaf area per plant was recorded in the *F. mosseae* treatment at 263.54 cm^2. These findings align with those of Meddich et al. (2015), who reported significant improvements in leaf area in palm seedlings inoculated with *G. monosporus* and *G. clarum* compared to control plants. Similarly, Khalil and El-Ansary (2020) reported that 'Manzanillo' olive plantlets inoculated with AMF exhibited significantly higher leaf areas than non-inoculated control plantlets. Interestingly, the effect of the PGPR treatment on leaf area was more pronounced in the 'Mission' cultivar than in the 'Koroneiki' cultivar. This indicates cultivar-specific responses of

olive plantlets to beneficial microorganism inoculation, highlighting the importance of considering the genetic background and physiological characteristics of different plants when implementing biofertilization strategies (Berruti et al., 2016).

In the 'Koroneiki' cultivar, inoculation with the AMF species *C. etunicatum* resulted in the highest internode length of 2.53 cm. In contrast, the shortest internode lengths were observed in the control treatments, measuring 0.46 cm (Table 2). This finding is consistent with previous research demonstrating the ability of AMF to enhance nutrient uptake and improve plant growth characteristics, such as internode length (Seifi et al., 2014; Berruti et al., 2016). Additionally, PGPR application increased internode length in both cultivars compared to the control. PGPR is known to produce growth-promoting substances, such as auxins, cytokinins, and gibberellins, which stimulate cell elongation and division, thereby enhancing plant growth parameters (Glick, 2012).

The highest plant heights of 49.10 cm and 45.36 cm were achieved with *F. mosseae* and *C. etunicatum* inoculation, respectively, in the 'Koroneiki' cultivar (Table 2), further supporting the positive effects of AMF on plant growth (Berruti et al., 2016). Interestingly, no significant difference in plant height was observed between PGPR-inoculated (14.83 cm) and control plants (13.5 cm) in the 'Mission' cultivar. However, in the 'Koroneiki' cultivar, PGPR inoculation increased plant height to 10.93 cm compared to 5.66 cm in the control, indicating that the response to PGPR may be cultivar-dependent. Research has shown that AMF inoculation can significantly improve plant growth parameters. For instance, Chenchouni et al. (2020) reported that AMF inoculation increased plant heights in olive plantlets, with native AMF species particularly effective. They observed plant heights ranging from 77.2 cm in *Glomus* sp.2-inoculated plants to 145.6 cm in *Glomus* sp.1-inoculated plants, compared to an average height of 54.6 cm in control plants.

The highest root lengths were observed in the 'Koroneiki' cultivar, with *F. mosseae* and PGPR inoculation yielding root lengths of 39.50 cm and 38.83 cm, respectively (Table 2). The treatments' effect on increasing root length was more pronounced in the 'Koroneiki' cultivar than in the 'Mission' cultivar. The highest root length in 'Mission', measuring 29.33 cm, was observed with PGPR inoculation. This finding aligns with Hanane et al. (2020), who reported that olive young plants inoculated with the *Rhizolive consortium* or *G. irregulare* had greater root lengths compared to control plants. Similarly, Hadjouti et al. (2022) demonstrated that certain PGPR species significantly increased root length in zucchini compared to control.

The study also revealed that the highest stem fresh weight of 3.78 g was recorded in the 'Koroneiki' cultivar treated with *F. mosseae* (Table 3). Extensive research supports that AMF inoculation can significantly enhance plant growth and development across various species (Berruti et al., 2016; Chenchouni et al., 2020). Additionally, PGPR significantly increased stem fresh weight in both 'Koroneiki' and 'Mission' cultivars compared to the control. By producing growth-promoting substances like auxins, cytokinins, and gibberellins, PGPR stimulate cell growth and division, leading to improved plant biomass (Glick, 2012). The highest stem dry weight of 1.89 g was observed in the 'Koroneiki' cultivar treated with *F. mosseae*, over seven times higher than the control (0.25 g). This finding is consistent with previous research demonstrating AMF's ability to enhance nutrient uptake and biomass production (Rodrigues et al., 2021). Similarly, PGPR inoculation significantly increased stem dry weight in both cultivars compared to the control. These findings suggest that applying AMF and PGPR enhances both fresh and dry plant weights, reflecting overall improved growth and development. By producing growth-regulating substances such as auxins, cytokinins, and gibberellins, AMF and PGPR promote cellular growth and division, resulting in increased biomass accumulation.

Table 3. The interaction effects of PGPR and AMF inoculation on fresh and dry weight of stem and root in olive plantlets of cultivars 'Mission' and 'Koroneiki'.

Cultivar	Inoculation	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)	Root dry weight (g)
		P<0.001	P<0.001	P<0.001	P<0.001
Mission	Control	0.42 g	0.26 e	0.28 d	0.23 d
	PGPR	0.92 ef	0.58 d	0.34 d	0.24 d
	F. mosseae	1.93 bc	1.18 b	0.27 d	0.21 d
	C. etunicatum	1.04 e	0.65 d	0.41 cd	0.33 bcd
	PGPR + *F. mosseae*	1.18 de	0.68 d	0.34 d	0.26 d
	PGPR + *C. etunicatum*	1.60 cd	1.10 cd	0.26 d	0.21 d
Koroneiki	Control	0.50 fg	0.25 e	0.27 d	0.23 d
	PGPR	1.19 de	0.71 d	0.53 c	0.41 bc
	F. mosseae	3.78 a	1.89 a	1.27 a	0.82 a
	C. etunicatum	2.12 b	1.26 b	0.54 c	0.43 b
	PGPR + *F. mosseae*	2.13 b	1.12 bc	0.97 b	0.72 a
	PGPR + *C. etunicatum*	1.51 cd	0.85 cd	0.36 d	0.27 cd

Different letters in each column represent significant differences at P=0.01, Duncan's multiple range test. The abbreviations are as following: AMF (arbuscular mycorrhizal fungi) and PGPR (plant growth-promoting rhizobacteria).

This study also examined the effects of AMF and PGPR on root growth. The highest root fresh weight of 1.27 g was observed in the 'Koroneiki' cultivar after inoculation with *F. mosseae* (Table 3). In this cultivar, most treatments caused a significant increase in root fresh weight, whereas in the 'Mission' cultivar, all treatments had no significant effect on root fresh weight. Similar results were reported by Chenchouni et al. (2020), who found that native AMF species, particularly Glomus sp.1, produced the highest root fresh weight in olive plantlets compared to control plants. The highest root dry weight in the 'Koroneiki' cultivar was 0.82 g after inoculation with *F. mosseae* and 0.72 g after inoculation with PGPR + *F. mosseae*. In contrast, treatments had no significant effect on root dry weight in the 'Mission' cultivar. These findings suggest that the 'Koroneiki' cultivar may be more responsive to AMF and PGPR applications compared to the 'Mission' cultivar, highlighting the importance of considering cultivar-specific responses in olive cultivation. Mycorrhizal fungi, such as *F. mosseae*, enhance water and nutrient uptake through symbiotic relationships with plant roots, leading to improved biomass production (Rodrigues et al., 2021).

The study also revealed that the highest total chlorophyll content was observed in the control treatment and the PGPR + *C. etunicatum* treatment (1.86 and 1.73 mg/g FW, respectively), followed by the control and PGPR treatments of the 'Koroneiki' cultivar (Fig. 4A). Conversely, the lowest total chlorophyll content was observed with the inoculation of *C. etunicatum* in the 'Mission' cultivar (0.87 mg/g FW) and *F. mosseae* in the 'Koroneiki' cultivar (0.83 mg/g FW). This contrasts with findings by Esna-Ashari and Bahrami (2018), who reported increased chlorophyll a, b, and total chlorophyll content with AMF inoculation compared to controls. The discrepancy may arise from specific interactions between plant cultivars, microbial strains, and environmental conditions (Seifi et al., 2014). The highest chlorophyll b content was observed in the control and PGPR + *C. etunicatum* treatments of the 'Mission' cultivar (1.15 and 0.99 mg/g FW, respectively) (Fig. 4B). In contrast, the lowest chlorophyll b content was recorded in the *F. mosseae* treatment of the 'Mission' cultivar. This suggests that *F. mosseae* inoculation may negatively impact chlorophyll b content in the 'Mission' cultivar, potentially affecting photosynthetic performance. The analysis of variance revealed no significant differences in chlorophyll a content between the inoculation treatments or cultivars studied (Fig. 4C and D), suggesting that cultivar differences and microbial

inoculations had a more pronounced effect on chlorophyll b and total chlorophyll content rather than chlorophyll a content. These findings reveal that while AMF and PGPR applications significantly boosted olive plant growth, they were associated with a decrease in chlorophyll content. This could be due to resource allocation, as AMF and PGPR enhance nutrient uptake (Berruti et al., 2016). Additionally, this pattern may reflect a shift in physiological priorities, where the growth enhancement driven by AMF and PGPR leads to a dilution effect in chlorophyll concentration due to increased biomass production. These findings underscore the intricate relationship between microbial inoculations, photosynthetic pigment dynamics, and overall plant development, emphasizing the need for further research to elucidate the underlying mechanisms. This paradox underscores the complex interactions between plant growth, nutrient uptake, and physiological responses. Additionally, the lack of stress in the plants used in this experiment may explain the discrepancies. Ye et al. (2022) demonstrated that under water stress conditions, AMF inoculation significantly increased chlorophyll b and total chlorophyll content, whereas no such effect was observed under non-stress conditions.

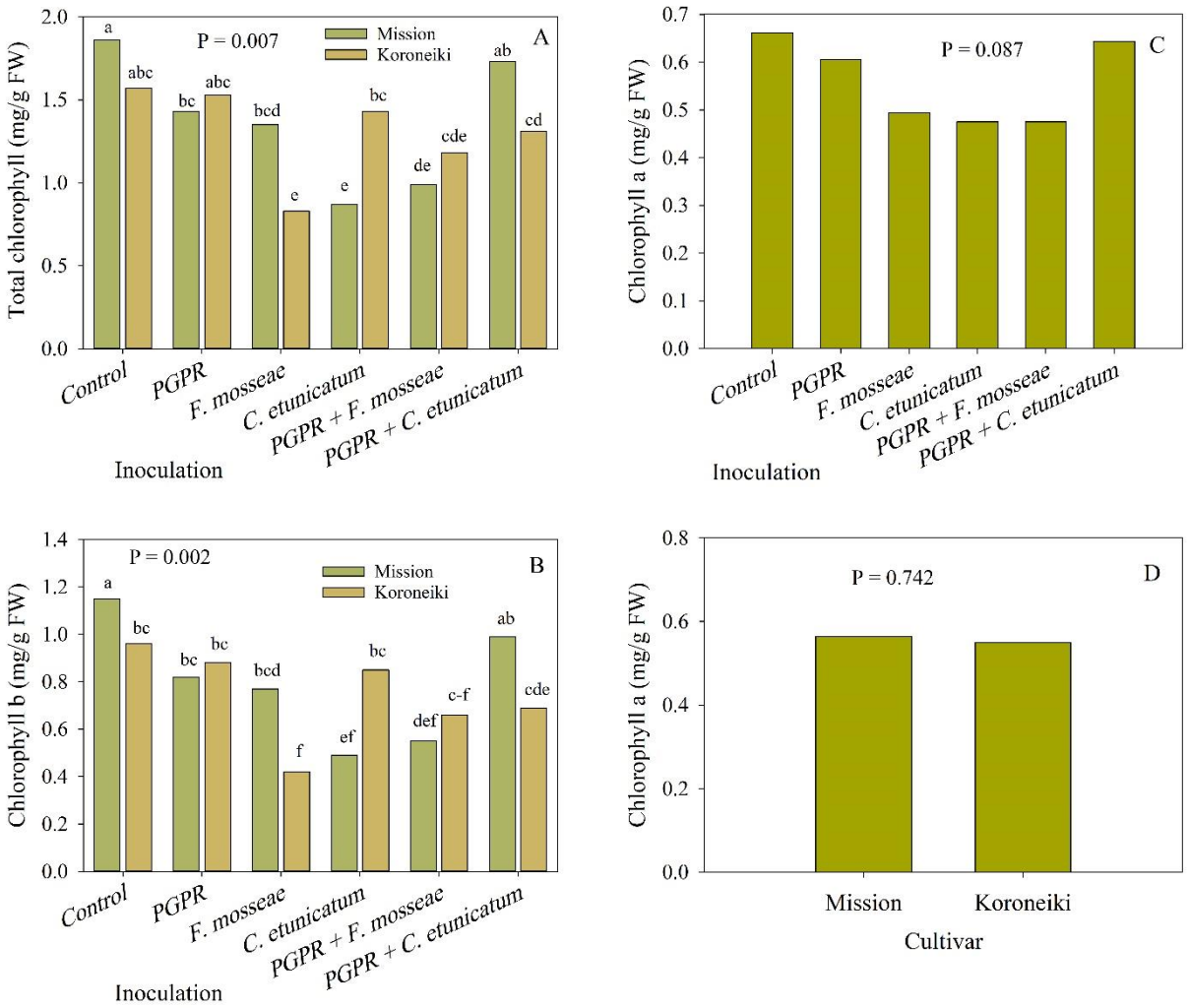

Fig. 4. The effects of inoculation treatments and cultivar on the content of total chlorophyll (A), chlorophyll b (B), and chlorophyll a (C and D) in olive plantlets. Different letters represent significant differences at P=0.01, Duncan's multiple range test. The abbreviation is as following: PGPR (plant growth-promoting rhizobacteria).

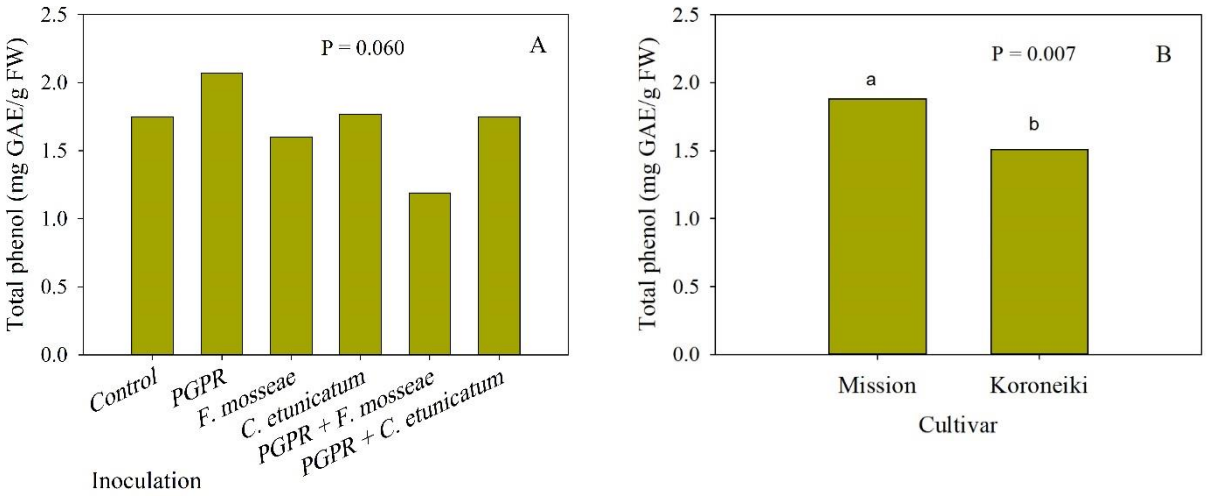

Fig. 5. The independent effects of inoculation treatments (A) and cultivar (B) on the total phenol content in olive plantlets. Different letters represent significant differences at P=0.01, Duncan's multiple range test. The abbreviation is as following: PGPR (plant growth-promoting rhizobacteria).

The analysis of variance showed that the interaction effect of cultivar and inoculation treatments on leaf total phenol was not significant. Additionally, no significant differences were observed in the amount of leaf total phenol in plants treated with AMF and PGPR (Fig. 5A). However, leaf total phenol content was higher in the 'Mission' cultivar (1.88 mg GAE/g FW) than in the 'Koroneiki' cultivar (1.51 mg GAE/g FW) (Fig. 5B). This suggests that the differences in leaf total phenol content were more influenced by the cultivar than by the inoculation treatments. Previous studies have reported conflicting results regarding the effects of AMF on leaf total phenol. Eftekhari et al. (2012) found that leaf total phenol in grapes increased after AMF inoculation compared to the control. In contrast, Ganz et al. (2002) reported no significant impact of AMF on leaf total phenol in olive. These inconsistencies suggest that the response of leaf total phenol to AMF may depend on factors such as plant species, cultivar, or environmental conditions (Hajiboland, 2013).

CONCLUSION

The combination of PGPR inoculation and IBA resulted in a significantly higher rooting rate and more roots per cutting. These findings suggest that PGPR can enhance root initiation and growth by modulating auxin signaling and metabolism, highlighting the synergistic potential of PGPR and auxin in promoting adventitious rooting. The second experiment demonstrated that the inoculation of *F. mosseae* had the most significant positive effect on the growth attributes of the 'Koroneiki' cultivar, while PGPR + *F. mosseae* led to enhanced growth in the 'Mission' cultivar. These findings emphasize the substantial benefits AMF and PGPR inoculation on the growth and development of olive plantlets, with cultivar-specific responses. The insights gained can inform the development of sustainable and tailored biofertilization approaches for olive production. The synergistic potential of PGPR and auxin in promoting adventitious rooting, as well as the cultivar-specific responses to combined inoculation of PGPR and AMF, provide valuable knowledge to optimize olive propagation and growth through the utilization of beneficial soil microorganisms. Further research is warranted to optimize the use of beneficial soil microorganisms for the successful propagation and establishment of olive plantlets.

Conflict of interest

The authors declare that there is no conflict of interest.

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Phytochemical screening and biological activity of *Centella asiatica* (L.) Urban extracts by different methods

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ABSTRACT

Purpose: Study on the effects of extraction methods on the biological activities of *Centella asiatica*, including antibacterial and antioxidant properties. **Research Method:** The main components of *C.asiatica* include triterpenoid saponins, polyphenols, flavonoids, and other health-beneficial compounds found through phytochemical screening. Ethanol extraction is performed using three extraction methods: immersion extraction, ultrasonic extraction, and reflux. *Centella asiatica* extract was tested for polyphenol content, total flavonoids, total triterpenoid saponins, and the ability to scavenge the free radicals DPPH and ABTS. **Findings:** The reflux extraction method was more effective than the other extraction methods in extracting chemical components, yielding relatively higher polyphenol, total flavonoid, and total triterpenoid saponins contents. All three types of extracts have the ability to fight oxidation, protecting cells from harmful free radicals. The IC_{50} value of pennywort extract in DPPH and ABTS free radical scavenging tests ranged from 455.52 and 270.05 µg/mL (soaked) < 333.63 and 206.56 µg/mL (ultrasound) < 239.75 and 199.75 µg/mL (reflux). The minimum inhibitory concentration (MIC) for the two bacterial strains *Staphylococcus aureus* and *Escherichia coli* was less than 2.188 mg/mL in all three methods. **Research limitations:** No limitations were encountered. **Originality/Value:** The development of extraction processes and evaluation of high quality extracts from gotu kola requires a combination of traditional methods and modern technology such as the use of advanced chemical and biological analytical techniques. This may create opportunities for the development of new technologies in the field of herbal extraction.

INTRODUCTION

Gotu Kola, whose scientific name is *Centella asiatica* (L.) Urb, is a small, herbaceous, perennial plant native to wetlands in Asia (Prakash et al., 2017). It is a member of the Apiaceae family and is known for its diverse application properties (Shakir Jamil et al., 2007). *Centella asiatica* thrives in tropical and subtropical regions, especially in moist, shady environments such as wetlands, riverbanks, and marshes. Its natural habitat ranges across the world in Southeast Asia, India, Sri Lanka, China, Indonesia, and South Africa (Jantwal et al., 2021; Loc & Nhat, 2013; Torbati et al., 2021). The plant has small, round, fan-shaped leaves with a smooth and palmate texture. The leaves are usually green to light green in color. The stems are slender, creeping, and spiny, allowing the plant to easily spread above ground. The flowers are arranged in clusters near the leaf nodes. The fruit is small, oblong, and ribbed, containing seeds.

The rich biological activities of gotu kola are attributed to constituents such as triterpenoid saponins (Asiaticoside, madecassoside, asiatic acid, and madecassic acid), flavonoids (quercetin, kaempferol, and catechins) that contribute to the total phenolic content, etc (Monton et al., 2019; Sen et al., 2019; Tsaltaki et al., 2019). These substances contribute to the antioxidant and anti-inflammatory activities of the plant (Hoang & Rehman, 2023; Shohel Hossain, 2018). Triterpenoids are components present in most *C. asiatica* species in different regions (James & Dubery, 2009). The presence and concentration of these compounds can vary depending on factors such as plant growth conditions, harvest time, and extraction method used. Some of the extraction methods used are maceration, reflux, microwave-assisted, ultrasound-assisted, enzymatic, soxhlet extraction, and supercritical extraction (Mohapatra et al., 2021). These techniques all have more or less the same goal in extracting biological activity from plants. However, new extraction techniques that are considered more effective and environmentally friendly are currently being widely used, on both laboratory and industrial scales, in nutraceuticals, food additives, pharmaceuticals, and many other fields.

Centella asiatica is famous for its antioxidant activities, mainly due to its rich content of bioactive compounds, including polyphenols, flavonoids, and triterpenoids. These compounds are known for their potent antioxidant activities, by eliminating free radicals and strengthening the body's antioxidant defense system. According to Zainol et al. (2003), the highest antioxidant activity is found in *C. asiatica* leaves compared to other parts and is also the part containing the highest phenolic content contributing to the antioxidant activity of *C. asiatica* (Zainol et al., 2003). Phenolic compounds and flavonoids were also found and demonstrated to contribute to antioxidant activity (Pittella et al., 2009). Other research in 2021 also demonstrated the anti-aging skin activity of *C. asiatica* extract in pharmaceutical and cosmetic products (Buranasudja et al., 2021).

C. asiatica exhibits remarkable antibacterial potential, contributing to the treatment of various infections and promoting wound healing. The antibacterial properties of *C. asiatica* are mainly attributed to its phytochemical components such as triterpenoids, flavonoids, polyphenols, and saponins. Here are some key points regarding its antibacterial activity. Ethanol extract of *C. asiatica* showed significant antibacterial activity against both Gram-negative and Gram-positive bacteria. Minimum inhibitory concentration (MIC) values were determined for different bacterial strains, indicating the effectiveness of the extract (Jagtap et al., 2009). The solvents (ethanol, chloroform, and hexane) used in gotu kola extract all have antibacterial activity against gram-positive and gram-negative strains at a concentration of 50 mg/mL (Rattanakom & Yasurin, 2015). Therefore, research on the extraction process and

evaluation of high-quality extract from *Centella asiatica* is necessary to ensure effectiveness and safety in use in medical and cosmetic practice.

MATERIALS AND METHODS

Raw material

C. asiatica was collected in Ben Tre (10° 14′ 25″ N, 106° 22′ 44″ E) and identified at the City Ginseng and Medicinal Center in Ho Chi Minh, Vietnam. The above ground parts of *C. asiatica* were collected and transported to the laboratory, washed, removed damaged parts and dried at 50ºC. The raw materials were ground and stored in glass zip bags as raw materials for extraction, qualitative and quantitative analysis of compounds in *C. asiatica*.

Chemicals

Analytical chemicals used in the study were purchased from Sigma-Aldrich, such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin & Ciocalteu's phenol reagent. Some other chemicals were purchased from China, such as ethanol, ascorbic acid, sodium carbonate (Na_2CO_3), gallic acid, hydrochloric acid (HCl), aluminum chloride ($AlCl_3.6H_2O$),... etc.

Plant extraction

Centella asiatica powder samples were extracted using three methods: soaking, reflux, and ultrasound using ethanol solvent (Mohapatra et al., 2021). The maceration process was carried out in a 100 mL conical flask containing 1 g of sample dissolved in 40 mL of ethanol solvent for 72 hours at room temperature. 1 g of raw material powder dissolved in 40 mL of ethanol solvent was placed in the flask, reflux extraction was carried out at 60 °C for 1 h. Ultrasonic extraction was carried out for 1 hour at 60°C with 1 g of powder using 40 ml of ethanol solvent in an ultrasonic apparatus. At the end of the process, the mixture was filtered to remove residue with a vacuum filter and Whatman filter paper in a round plate with a diameter of 90 mm. The extract was then evaporated by vacuum. The concentrated extract, about 5 ml, was collected in a small vial and allowed to dry by evaporation in a 50°C oven, finally obtaining the extract from all three methods.

Analytical methods

Qualitative Phytochemical Screening

Phytochemical analysis is the process of studying chemical compounds in plants. Bioactive compounds include phenolics, tannins, flavonoids, alkaloids, saponins, and many others. The presence of phytochemical components is noted by the sign (+), and vice versa, the absence is indicated by the sign (-) by identifying the phenomenon of chemical reactions.

Quantitative Phytochemical Screening

Total Polyphenol Content: Gallic acid is used as the standard in the Folin-Ciocalteu colorimetric method. The Folin-Ciocalteu reagent reduces polyphenol compounds to form a blue reaction product, whose absorbance is measured at 765 nm using a UV–Vis spectrophotometer (Segaran & Chua, 2021). Total polyphenol content is expressed as milligrams of gallic acid equivalents per 1 g of extract (mgQE/g DW).

Total Flavonoid Content: Flavonoid content was determined based on the color method with aluminum chloride at 415 nm, following the research by Mahboubi et al. (2013). Quercetin was used as a standard (Mahboubi et al., 2013). Total flavonoid content is expressed as milligrams of quercetin equivalents per 1 g of extract (mgQE/g DW).

Saponin Triterpenoid Content: Construction of a standard curve to determine saponin triterpenoid content was performed as described by Segaran and Chua et al. (2021). Oleanolic acid is used in a sugar formulation (0.1 mg/mL). The extracts were dissolved in ethanol, and 0.2 ml of the extract was used for the reaction.Add 0.2 ml of 5% (w/v) vanillin-acetic acid solution and 1.2 ml of perchloric acid, shake well and incubate at 70°C for 15 min. Cool and add ethyl acetate to a total volume of 5 ml. Determine the absorbance at 550 nm using a UV-Vis instrument (Agilent Cary 60).

Antioxidant Activity

Free Radical Scavenging Assay by DPPH Method: The antioxidant capacity of pennywort samples was determined using the modified DPPH free radical scavenging method (Brand-Williams *et al*., 1995). The reaction mixture included 1.5 mL of DPPH (6.10^{-4}M, mixed in ethanol, OD_{517} nm = 1.1 ± 0.02) into each test tube containing 0.5 mL of extracts with different concentrations. The control sample used ascorbic acid. Absorbance was analyzed on a UV-Vis instrument (Agilent Cary 60) at a wavelength of 517 nm. The antioxidant capacity of a sample is expressed through the IC_{50} value - the concentration of antioxidants at which 50% of DPPH free radicals can be inhibited (1).

$$\text{DPPH}\ (\%) = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100 \qquad (1)$$

Where Abs_C is the optical absorbance of the control sample, and Abs_T is the optical absorbance of the test sample.

Free Radical Scavenging Assay by ABTS Method: Prepare the ABTS stock solution by mixing 7.4 mM ABTS solution into 10 mL of 2.6 mM $K_2S_2O_8$ solution and incubate in the dark for 24 hours (OD_{734} nm = 1.1 ± 0.02) (Pham et al., 2017). The extracts were dissolved in ethanol and mixed into a series of different concentrations. 0.5 mL of the extract was mixed with 1.5 mL of clear $ABTS^+$ solution and incubated for 30 minutes in the dark at room temperature. Absorbance was determined at 734 nm. Ascorbic acid was used as a positive control. Each experiment was repeated 3 times, and the IC_{50} value was calculated similarly to the DPPH method mentioned above (2).

$$\text{ABTS}(\%) = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100 \qquad (2)$$

Where Abs_C is the optical absorbance of the control sample, and Abs_T is the optical absorbance of the test sample.

Antimicrobial Activity

The antibacterial activity of the extract was evaluated based on the agar disk diffusion method, with Gram-positive bacterial strains *Staphylococcus aureus* ATCC 6538 and Gram-negative *Escherichia coli* ATCC 8739 (Palaksha et al., 2010). Then, the bacterial density was determined in the range of 10^6-10^7 CFU/ml by measuring optical density at 660 nm wavelength. The antibacterial ability of the extract was tested by pipetting 50 µl of sample solutions of different concentrations in the sample diluent into wells on agar plates spread with test bacteria. Use chloramphenicol (1 mg/mL) as a positive control. Use solvent to dissolve the sample as a negative control. After 24 hours, results are recorded by image and diameter of the sterile zone. The experiment was performed 3 times. The minimum inhibitory concentration of the test sample was investigated using the dilution method on a 96-well microplate (Cockerill, 2010). Each well contains 150 µl of bacterial medium and 50 µl of

sample diluted in the medium. Samples were diluted according to different concentration series. Incubate at 37ºC for 24 hours. After 24 hours, 20 µL of 0.01% resazurin reagent was added to each well. Observe the color change and record the MIC value.

RESULTS

Physicochemical properties and effectiveness of *C. asiatica* extract

Issues related to the preservation of raw materials during the extraction process were identified. High humidity can reduce extraction efficiency due to incomplete dissolution of compounds. Additionally, *C. asiatica* extract has a moisture content of less than 5%, making it suitable for preservation and storage, meeting the moisture standards of dry extract (Table 1). The total ash and HCl insoluble content of pennywort ingredients were evaluated at 13.62 ± 0.094% and 1.994 ± 0.215%, respectively.

C. asiatica extract is obtained using different methods such as soaking, reflux, and ultrasound, which directly impact the performance and separation of biological activity in the plant. Table 2 illustrates that there is not a significant difference in performance among the three methods. Specifically, ultrasound and reflux extraction show higher efficiency (> 22%) compared to immersion extraction at 20.77%. Furthermore, the choice of extraction solvent significantly influences antioxidant activity, as different compounds with varying polarities and solubilities are present in the plant and are soluble in specific extraction solvents. The yield and composition of compounds from *C. asiatica* are regulated by factors such as the processing of raw materials post-harvest, extraction method, and plant parts used. For example, drying leaves with hot air and extracting with 50% ethanol yielded 37.3%, while freeze-drying the whole plant and extracting with water resulted in a yield of 23.3% (Shin et al., 2021).

Table 1. Humidity of *C. asiatica* extract.

Samples	Humidity (%)
C. asiatica powders	7.47 ± 0.123
Immersion extraction	0.63 ± 0.04
Reflux extraction	0.78 ± 0.03
Ultrasonic extraction	0.77 ± 0.04

Table 2. Efficiency of *C. asiatica* extraction process.

Samples	Yield (%)
Immersion extraction	20.462
Reflux extraction	22.909
Ultrasonic extraction	24.121

Table 3. Preliminary phytochemical composition of *C. asiatica* extract.

Parameters	Response	Results	Phenomenon
Alkaloid	Reaction with Mayer's reagent	+	Reddish-brown precipitate
Saponin	Foaming phenomenon	+	Durable foam
Flavonoid	$Pb(CH_3COO)_2$ (10%)	+	Yellow precipitate
Terpenoid	Chloroform and concentrated H_2SO_4	+	Brick-red color
Tannins	$FeCl_3$ 0.5% solution	-	Blue-black precipitate

Phytochemicals

Compounds that contribute to the biological activity of *C. asiatica* extract include alkaloids, flavonoids, terpenoids, and saponins, while tannins are not found in pennywort extract (Table 3). The medicinal potential is demonstrated by the presence of phytochemical components in *C. asiatica* extract. *C. asiatica* is rich in terpenoids, which contribute to antioxidants, wound healing, and the treatment of inflamed tissues (CU et al., 2020). Additionally, the presence of flavonoids and polyphenols in pennywort has important functions in antioxidant, anti-inflammatory, and antibacterial properties against bacteria such as *E. coli*, *Shigella flexneri*, and *S. aureus* (Utami et al., 2011). Tiwari et al. (2011) also pointed out that alkaloids are nitrogen-containing heterocyclic compounds that have analgesic and antibacterial effects. Differences between published studies may be due to growth environmental conditions or extraction methods used (Chaudhary et al., 2020). Specifically, secondary metabolites such as steroids, flavonoids, saponins, coumarins, etc., were also found in *C. asiatica* extracts from parts of India using solvents like methanol and petroleum ether (Shobana, 2014) and water, acetone, chloroform, and methanol (Saranya et al., 2017). Kavisa Ghosh et al. (2014) conducted the chemical analysis of ethanol extract of *Centella asiatica* leaves, which showed the presence of alkaloids, saponins, glycosides, triterpenoids, sterols and absence of tannins (Ghosh & Indra, 2014).

Total Polyphenol and Flavonoid Contents

Polyphenol and flavonoid compounds are commonly found in all *C. asiatica* extracts. The effect of the extraction method on total polyphenol content (TPC) and total flavonoid content (TFC) is shown in Figure 1. The reflux method showed high amounts of TPC and TFC with values of 168.97 ± 1.16 mg gallic acid (GAE)/g DW and 144.69 ± 1.61 mg (QE)/g DW, respectively. Similarly, TPC content with values of 147.96 ± 1.89 and 123.46 ± 0.83 mg gallic acid equivalent (GAE)/g DW and TFC are 140.29 ± 1.76 and 111.83 ± 2.24 mg (QE)/g DW for ultrasound and maceration methods. Using the reflux method to extract compounds from pennywort ensures the maximum yield of its beneficial components, such as flavonoids, triterpenoids, and polyphenols. The reflux system facilitates a consistent flow of the solvent, promoting a steady and efficient interaction between the solvent and the plant matrix. This results in increased dissolution and transfer of polyphenol and flavonoids from the plant material to the solvent. Ultrasound-assisted extraction (UAE) may extract flavonoids at a faster rate, but its lower temperature might not fully release all polyphenol and flavonoid glycosides or reach deep into the plant matrix. While extreme heat can cause flavonoids to degrade, the controlled temperatures in reflux extraction are typically gentle enough to prevent significant degradation, especially when ethanol is present as a stabilizing solvent.

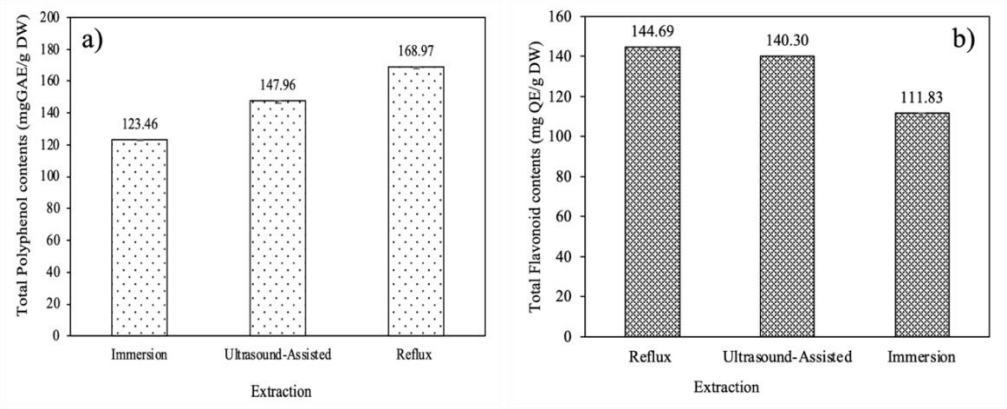

Fig. 1. Total polyphenol (a), and flavonoid (b) content in *C. asiatica* extract.

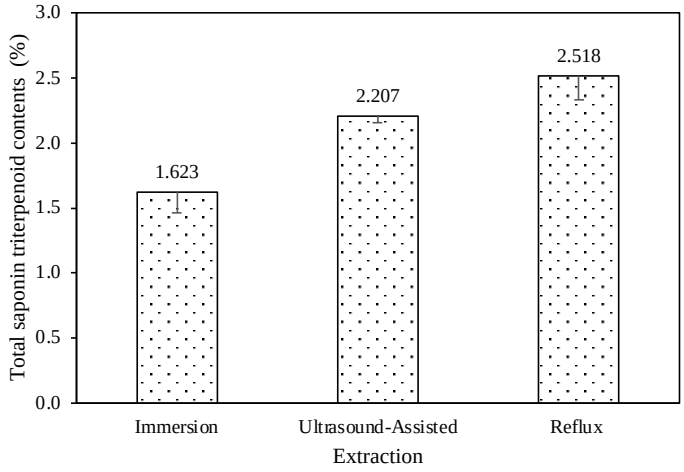

Fig. 2. Triterpenoid saponin content from *C. asiatica* extract by three different methods.

Triterpenoid saponins content

C. asiatica extract has a high triterpenoid saponin content, making it highly sought after in cosmetics, dietary supplements, and pharmaceutical products (AR, 2012; Huang et al., 2011). In this study, the triterpenoid saponin content obtained from immersion, ultrasound, and reflux extraction methods was 1.623%, 2.207%, and 2.518%, respectively (Fig. 2). The triterpenoid saponin content in *C. asiatica* extract may vary depending on factors such as the extraction method, the part of the plant used, and geographical differences. The elevated temperature during reflux extraction enhances the solubility of triterpenoid saponins in the solvent and disrupts cell walls, making the bioactive compounds more accessible. Ethanol, a polar solvent used in reflux extraction, is suitable for extracting triterpenoid saponins. While methods like ultrasound-assisted extraction (UAE) or enzyme-assisted extraction may offer advantages in speed or specificity, reflux extraction typically excels in maximizing compound yield due to prolonged exposure to optimal extraction conditions. This is particularly beneficial for triterpenoid saponins, which may require sustained high temperatures and sufficient solvent interaction for full extraction.

Antioxidant Activity Analysis

Extraction with ethanol solvent, which is related to the polarity of the solvent, can extract different fractions of polar/nonpolar components from plants. This finding was in good agreement with the total polyphenol and flavonoid content. The free radical scavenging activity of the compounds can be measured by the deuteration effect after trapping unpaired electrons by DPPH (Table 4). Experimental results show that *C. asiatica* extract has DPPH free radical scavenging activity with an IC_{50} value of 455.52 µg/mL (immersion) < 333.63 µg/mL (ultrasound) < 239.75 µg/mL (reflux), the inhibition rate is higher the higher the sample concentration. Similarly, the ABTS method is commonly used to measure the antioxidant capacity of compounds. In this assay, the ABTS radical cation ($ABTS^{\bullet+}$) is generated, which is blue-green in color. When an antioxidant is introduced, it donates electrons or hydrogen atoms to neutralize the ABTS radical cation, leading to decolorization. The degree of decolorization is measured spectrophotometrically at 734 nm, which corresponds to the absorbance of the ABTS radical. A greater reduction in absorbance indicates a stronger antioxidant capacity, as the compound stabilizes the free radicals. The obtained results also clearly showed that the tested extract had the ability to scavenge free radicals. As in the case of the described assay, the reflux extract showed the highest

antioxidant capacity, at the highest analytical concentration (1000 µg/mL) > 90% of ABTS radicals could be removed. Table 4 shows the analysis results from the ABTS method, *C. asiatica* extract has an IC_{50} value of 270.05 µg/mL (immersion) < 206.56 µg/mL (ultrasound) < 199.75 µg/mL (reflux).

Antimicrobial Activity

Antibacterial testing using the agar disk diffusion method of *C. asiatica* extract using ethanol solvent is presented in Table 5 and Figure 3. A test dose of 1 mg/well with a sterile ring diameter of 6 mm corresponds to no resistance. *C. asiatica* extract, obtained by all three methods (reflux, immersion, and ultrasound), showed inhibitory ability against the Gram-negative bacterium *E. coli*. In contrast, Gram-positive bacteria did not exhibit any zone of inhibition against *S. aureus*. The positive control Chloramphenicol had inhibition zones of 24.0 ± 0.2 mm (*E. coli*) and 24.5 ± 0.2 mm (*S. aureus*). However, *C. asiatica* extracts from the three methods showed MIC against both *E. coli* and *S. aureus* strains (Table 6 and Fig. 4). Specifically, the minimum inhibition value for the reflux method was 1.313 mg/mL, for immersion were 1.750 mg/mL and 2.188 mg/mL, and for ultrasound it was 1.131 mg/mL, corresponding to the two bacterial strains *E. coli* and *S. aureus*. The difference in results between the disk diffusion test and the 96-well test with resazurin reagent may be due to several factors. In the disk diffusion method, the ability of the antibacterial agent to diffuse through the agar medium may be limited, resulting in the antibacterial agent not having direct and consistent contact with *S. aureus* bacteria, which may reduce its effectiveness. The disk diffusion method uses a smaller extract concentration than the 96-well assay. In the 96-well test, the antibacterial agent is mixed directly with the bacteria in solution, ensuring direct and uniform contact.

Table 4. IC_{50} value of ethanol extract with *C. asiatica* extraction methods.

Methods	IC_{50} DPPH (µg/mL)	IC_{50} ABTS (µg/mL)
Immersion extraction	455.52	270.05
Reflux extraction	239.75	199.75
Ultrasonic extraction	333.63	206.56
Ascorbic aicd	4.47	18.88

Table 5. Antibacterial properties of three types of *C. asiatica* extracts.

Samples	Resistance index (sterile ring diameter – mm)	
	Escherichia coli ATCC 8739	*Staphylococcus aureus* ATCC 6538
Immersion extraction	6.3 ± 0.2	6.0 ± 0.0
Reflux extraction	6.5 ± 0.3	6.0 ± 0.0
Ultrasonic extraction	6.4 ± 0.2	6.0 ± 0.0
Chloramphenicol (20µg)	24.0 ± 0.2	24.5 ± 0.2

Table 6. Minimum inhibitory concentration (MIC) of bacterial strains by three *C. asiatica* extracts.

Samples	MIC (mg/ml)	
	Escherichia coli ATCC 8739	*Staphylococcus aureus* ATCC 6538
Immersion extraction	1.750	2.188
Reflux extraction	1.313	1.313
Ultrasonic extraction	1.313	1.313
Chloramphenicol (ppm)	0.2	0.2

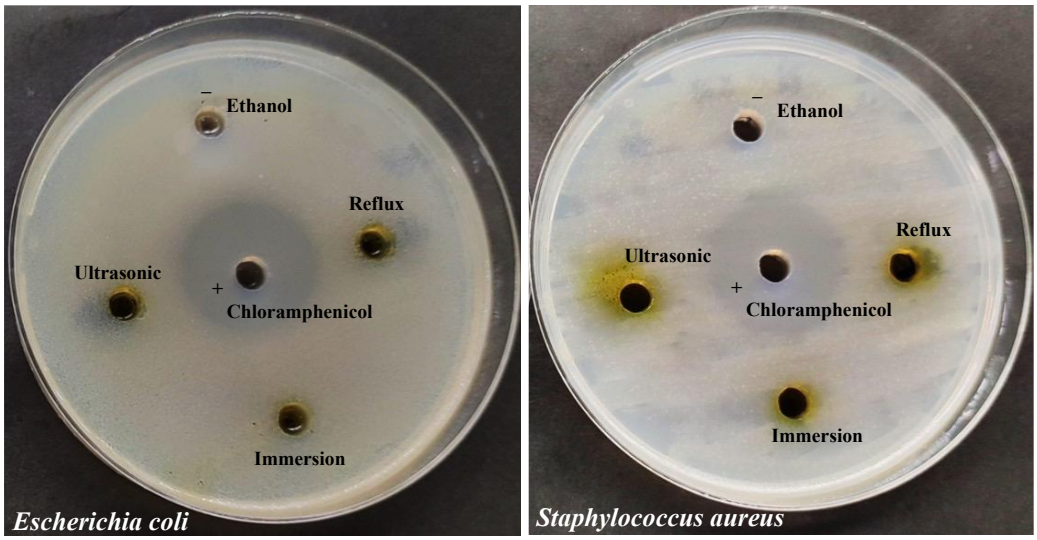

Fig. 3. Circles of inhibition of bacterial strains of three different methods: a) *E. coli* ATCC 8739 and b) *S. aureus* ATCC 6538

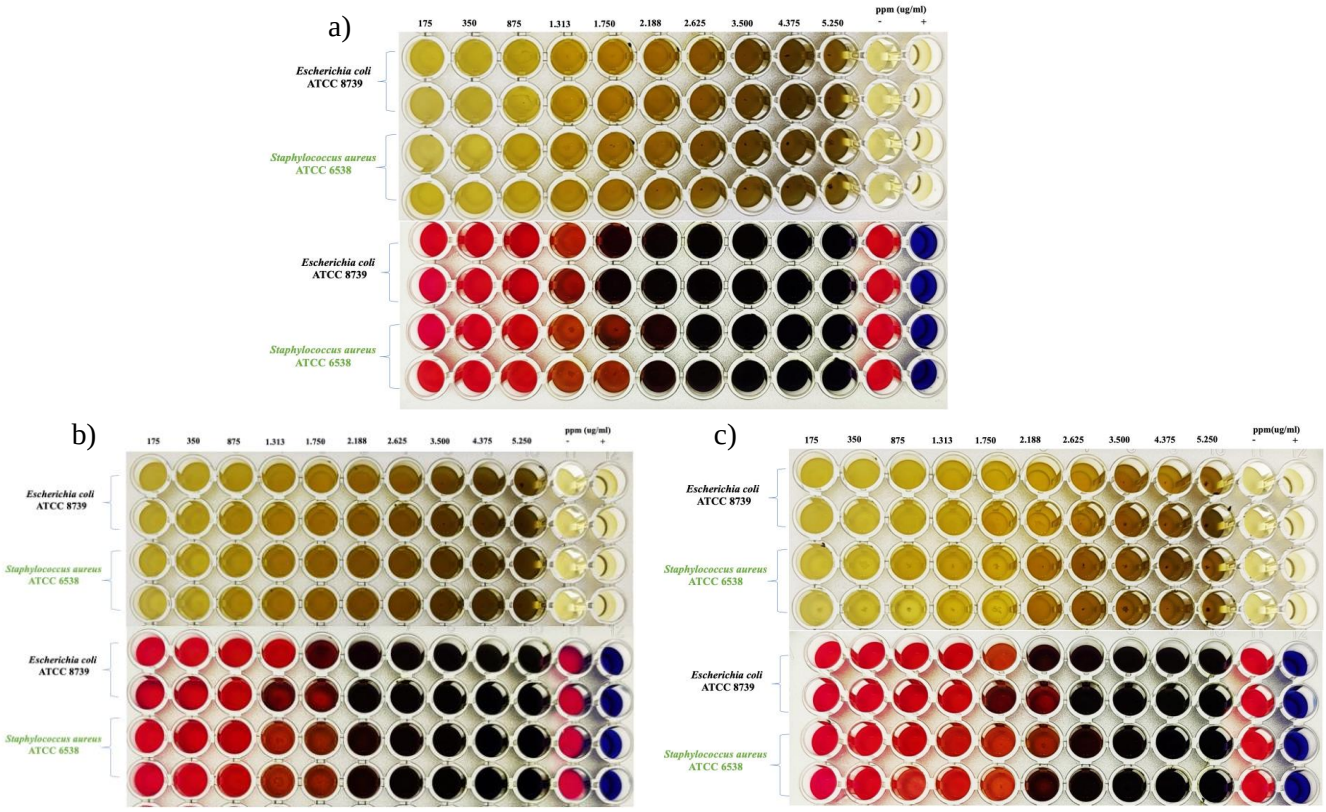

Fig. 4. Minimum inhibitory concentrations of three types of *C. asiatica* extracts on 96-well plates: a) Immersion, b) Ultrasonication, c) Reflux.

DISCUSSION

C. asiatica raw materials were evaluated for physicochemical parameters such as moisture, total ash, and ash insoluble in acid. The total ash content can reflect the purity level of the raw material. Raw materials with high ash content may contain many unwanted impurities or minerals. The determination of insoluble ash in HCl acid helps evaluate the content of insoluble minerals such as silica and other oxides, which are often related to the quality and purity of the raw material.

Each extraction method has its own advantages and disadvantages. The ultrasound method facilitates the separation of heat-sensitive compounds, reduces extraction time, and utilizes ultrasound waves to break down cell walls for quick release of compounds in the solvent. Reflux extraction is effective in recovering active ingredients and is highly efficient, but it requires careful attention to thermal degradation. Thermal degradation is minimized when using the soaking method, but this method is time-consuming and inefficient as solvent diffusion into the plant is static and slower compared to the other two methods.

Measuring polyphenol and flavonoid content is important to predict the antioxidant capacity of each extract. Similarly, the total phenolic content of *C. asiatica* in Thailand sample ranges from 98.5 mg GAE/g to 183.24 mg GAE/g (Chanwitheesuk et al., 2005). Polyphenol and flavonoid compounds are found in all *C. asiatica* extracts. Polyphenols and flavonoids, a diverse group of secondary metabolites known for their potent antioxidant properties. These compounds can neutralize free radicals, preventing oxidative stress and lipid peroxidation. Measuring polyphenol and flavonoid content is key to assessing the antioxidant potential of plant extracts, as higher levels generally correlate with better free radical scavenging ability. Sellathoroe et al. (2019) conducted an evaluation and comparison of biologically active ingredients in *C. asiatica* between different extraction methods, in which ultrasound was more effective than both immersion and soxhlet extraction with 0.47% saponin, 0.34% terpenoids, 0.11% flavonoids, and 0.03% alkaloids (Sellathoroe et al., 2019).

Table 7. Biological activities of *C. asiatica* analyzed by extraction methods.

Method	Conditions	Solvent	Compounds	References
Immersion	Dried plants, extraction time 110.5 min, and extraction temperature 70.20°C	Ethanol (37%)	Polyphenols 4.71 mg GAE/g DW	(Gunathilake et al., 2019)
Soxhlet	Dried plants, raw materil-solvent ratio 1:10 and extraction temperature 45°C.	Ethanol (50%)	β-carotene, Viatmin C, flavonoid và polyphenol total	(Rahman et al., 2013)
Microwave-based extraction	Dried plants, 40% microwave power, extraction time 6 min	Ethanol	Total phenolics and flavonoid, and total triterpenoids	(Sen et al., 2019)
Ultrasound-assisted extraction (UAE)	Dried plants, 1:10 raw materil-solvent ratio, 30 min	Water	Total phenolics 8.32 ± 0.105 mg GAE/g DW, DPPH antioxidant activity 86%	(Wan Zainal et al., 2019)
Solvent-Free Microwave-Assisted Extraction (SFME)	Dried plants, microwave power 450 W, extraction time 43.33 min		Total phenolics 2.39869 mg GAE/g	(Rahmawati et al., 2021)

The triterpenoid saponin content extracted from *C. asiatica* may vary depending on the solvent used due to the nature of triterpenoid saponins as amphipathic molecules. Some previous studies have shown that water is not effective as a solvent for extracting triterpenoid saponins from plants because water has poor solubility for many organic compounds, requiring longer time or higher temperature conditions to be effective (Zhao et al., 2010). Ethanol is generally considered a more effective solvent for triterpenoid saponin extraction due to its ability to solubilize a wide range of compounds, including nonpolar and semipolar components (Kim et al., 2009). The recovery efficiency of triterpenoid saponins from *C. asiatica* extract in Nakhon Pathom Province, Thailand reached 4.8% (dimethyl ether), 9.3% (ethanol), and 18.8% (dimethyl ether and ethanol mixture) (Pingyod et al., 2021).

Studies have demonstrated that *C. asiatica* extract exhibits significant DPPH radical scavenging activity, which in different ecotypes also has different IC_{50} values. This activity is due to the presence of various bioactive compounds, including triterpenoids (asiaticoside, madecassoside), flavonoids, and phenolic acids. The antioxidant activity of *C. asiatica* was determined to be higher when extracted with ethanol solvent than with water extraction, likely indicating high free radical scavenging activity due to efficient extraction of phenolics and flavonoids (Hamid et al., 2002).

Previously published studies showed the inhibitory effect on *S. aureus* strains with MIC values of 32 – 256 mg/mL by water extract and MIC values of 8 mg/mL by ethanol extract (Taemchuay et al., 2009). On the other hand, the water extract has the ability to inhibit *Helicobacter pylori* using the agar disk diffusion method, and the MIC value is 0.125 to 8 mg/mL (Zheng et al., 2016). In the study by Ferdous et al. (2017), positive effects were shown using ethanol as the extraction solvent. On the other hand, the extraction method soaked in *C. asiatica* with a methanol solvent could not inhibit *E. coli* strains (Gautam et al., 2007). These extracts can be prepared using different solvents, each of which affects the composition of the extract and the antibacterial efficacy. Ethanol is a solvent that has shown potential in the extraction of bioactive compounds from *C. asiatica* due to its effectiveness in solubilizing a variety of phytochemicals. In the present study, the antibacterial activity of *C. asiatica* may also be due to the similar effect of triterpenes, which are the active ingredients of *C. asiatica*. One of the important groups of compounds found in *C. asiatica* is flavonoids, which contribute to its wide range of biological activities, including antibacterial properties. Flavonoids can disrupt bacterial cell walls and membranes, leading to increased permeability and ultimately cell death.

CONCLUSION

In this study, three extraction methods: immersion, reflux, and ultrasound were used to recover extracts from *C. asiatica*. Identified natural compounds such as alkaloids, saponins, flavonoids, and terpenoids exist in *C. asiatica* extract. Ethanol extract from *C. asiatica* has been researched and proven to be effective in antioxidant and antibacterial properties. Ethanol extraction from *C. asiatica* by reflux method contains higher levels of polyphenols and flavonoids than other methods, which are known for their powerful antioxidant properties. These compounds have the ability to scavenge free radicals, help prevent cell damage due to oxidation, and have the best ability to inhibit the activity of free radicals with an IC_{50} value of 239.75 µg/mL (DPPH) and 199.75 µg/mL (ABTS) using reflux extraction. Triterpenoid saponins from *C. asiatica* also have antibacterial properties and can inhibit Gram-positive bacteria such as *S. aureus* and Gram-negative bacteria such as *E. coli*.

Conflict of interest

The Authors declare that there is no conflict of interest.

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Effect of paclobutrazol application and bunch covering on productivity and quality of banana

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ABSTRACT

Purpose: The experiment aimed to evaluate the effect and optimal dose of paclobutrazol, and appropriacy of bunch covering materials on productivity and quality of banana. **Research method**: This study was laid out following a randomized complete block design with four replications. The experiment consisted of two factors namely Factor A: Different doses of paclobutrazol i.e. no paclobutrazol, 1 g/L paclobutrazol and 2 g/L palobutrazol and Factor B: bunch covering i.e. control (no polythene covering), white polythene covering and blue polythene covering. Paclobutrazol was sprayed 2 times; firstly, at fruit set stage and secondly, one month after 1st spray. **Findings**: The treatments showed significant effect on the yield as well as qualitative traits of banana. Paclobutrazol sprayed plants performed better than non-paclobutrazol treated plants. Application of paclobutrazol (2 g/L) and blue bunch covering banana contained higher amount of sugar. However, blue and white polythene had no significant effect on color measurement of banana peel. Paclobutrazol (1g/L) and white polythene treated bunch produced the highest bunch weight (24.91 kg), pulp:peel ratio (3.57), fibre content (4.20%) while no polythene treated with no paclobutrazol produced the lowest bunch weight (16.77 kg), pulp:peel ratio (1.89), fibre content (2.60%) of banana. These results generally prove that paclobutrazol application could potentially be used to improve crop productivity and their quality value. **Research limitations**: There were no limitations identified. **Originality/Value:** Paclobutrazol (1mg/L) with white polythene as bunch covering resulted in the maximum banana production and quality when compared to other treatment combinations, without compromising of human health and environmental sustainability.

INTRODUCTION

Banana (*Musa paradisiaca* L.) is a prominent fruit crop that belongs to the Musaceae family. It is the world's most nutritious fruit crop, grown in over 130 nations in the tropical and subtropical areas and used as a staple food as well as dietary supplements (Al-Dairi et al., 2023). On 11590.30 ha of land, Bangladesh produces 183438.79 metric tons of bananas (BBS, 2022). It has ascorbic acid, riboflavin, niacin, protein, fat, ash, phosphorus, calcium, iron, β-carotene, and crude fiber (Sule et al., 2019). Majority of banana fruits are blemished by dust, bird droppings, leaf scars, and mechanical injuries sustained during postharvest processing.

To improve the growth, maturity, yield, and quality of banana fruits, bunch management techniques, pesticides, and plant growth regulators are applied. Paclobutrazol (PBZ) is a triazole derivative that is crucial for controlling excessive vegetative growth, encouraging flowering, causing early bearing, and boosting fruit crop productivity and quality (Gollagi et al., 2019). According to Carreno et al. (2007), fruit set was increased when paclobutrazol was applied prior to blooming. Increased dry matter partitioning to fruits may be the cause of the enhanced fruit set following paclobutrazol treatment. Because bananas are climacteric and highly perishable (Alhassan & Ndomakaah, 2024), pre-harvest chemical and growth regulator applications are required to increase shelf life while maintaining quality and minimizing post-harvest losses.

Bunch covering has also been linked to decreased fruit defects like sunburn and fruit splitting, as well as an increase in finger length and bunch weight and acceptable skin appearance and color (Rubel et al., 2019). According to Ali et al. (2021), bagging is a highly successful method for altering the fruit's microclimate, which prevents various stresses and preserves or enhances the fruit's overall quality. Covering the plantain or banana bunch soon after pollination can significantly reduce insect pest damage. Additionally, it has been demonstrated that fruit from sleeved bunches has a substantially lower incidence of postharvest anthracnose disease (Buthelezi et al., 2021). Using bunch cover enhances fruit quality and increases marketable product. Bunch coverings of various colors and conditions have been widely utilized in banana-growing countries for enhanced output and quality (Ali et al., 2018). Furthermore, bunches were protected from pests and diseases such as thrips, beetles, pitting, anthracnose, tip end rot, cigar end rot, brown spot, and diamond spot via bunch covering (Amani & Avagyan, 2014).

Knowledge on the effects of paclobutrazol treatment and bunch covering on productivity and quality of banana is scarce or scanty in the agricultural climate of Bangladesh. Therefore, the study aimed to evaluate the effect of paclobutrazol, and appropriate of bunch covering materials on productivity and quality of banana at the Horticulture Farm at Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh.

MATERIALS AND METHODS

Study area

The experiment took place from July 2022 to June 2023 on an open field at Sher-e-Bangla Agricultural University's Horticulture Farm in Bangladesh. The location was 24.09°N and 90.26°E longitude, with an elevation of 8.20 m from sea level. The soil was loamy, and the climate was divided into three seasons: winter (November to February), pre-monsoon (March to April), and monsoon (May to October).

Experimental design, plant materials and growing conditions

This study was laid out a randomized complete block design (RCBD) with four replications for each treatment and five plants in each replicate. Banana sucker (cv. BARI Banana 1) was obtained from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh and planted with spacing (1.5×1.5m). All plants were comparable in terms of age and growth, and they all received the recommended fertilization schedule, as well as all necessary cultural practices and plant protection measures.

Treatments and treatment combination

The experiment consisted of two factors as factor A: different doses of paclobutrazol (PBZ) i.e. No PBZ, 1 g/L PBZ and 2 g/L PBZ and Factor B: bunch covering i.e. control (no polythene), white polythene and blue polythene. There were 9 (3×3) treatments combinations such as: Control, No PBZ + White polythene, No PBZ + blue polythene, PBZ (1g/L) + No polythene, PBZ (1g/L) + white polythene, PBZ (1g/L) + blue polythene, PBZ (2g/L) + No polythene, PBZ (2g/L) + white polythene, PBZ (2g/L) + blue polythene. Afterwards, PBZ was sprayed 2 times, firstly during bunches emergence stage and secondly one month after 1st spray. Once the floral remnants were hardened and the bracts covering the hands and fingers were curling upward, bunches were covered. The bunch covers hung at least 15 cm below the banana's last hand and were left open at the bottom.

Sampling for experimentation

Three plants were chosen at random from each replication for analysis of fruit production, yield, and physiochemical characteristics.

Evaluation of the effect of paclobutrazol (PBZ) and bunch covering on pre-harvest and physical-harvest properties of banana

For all five of the chosen plants in each treatment, the number of days needed from bunch initiation to harvesting, bunch length, and bunch weight were noted. Fruits that attained harvestable ripeness were counted to determine the number of hands/bunch and fingers/hand. An electronic top pan balance was used to remove the pulp and peel from each chosen hand.

Evaluation of the effect of paclobutrazol (PBZ) and bunch covering on biochemical properties of banana *α-amylase activity assay*

The third hand of each bunch was used to conduct chemical analysis. The hand at the top of the bunch is the first to be initiated, and hands are generally numbered from this point down.

Total soluble solids content

A digital refractometer (MA871; Romania) was used to measure the TSS concentration of bananas. Using a dropper, a drop of banana juice was collected and put on the refractometer prism. The total soluble solids reading were displayed by the refractometer. Results were expressed as degree Brix (°Brix).

Titratable acidity (TA %)

In order to determine the TA, the 5g of fruit samples were macerated using a mortar and pestle. Following maceration, the samples were filtered and 100 milliliters of water were added. Two drops of phenolphthalein were then added to ten milliliters of stock solution in a conical flask. 1N NaOH was used to titrate the solution three times. Until the pink hue emerged, the titration was stopped.

Vitamin C determination

A 5 g sample of banana fruit was mixed, and filter paper (Whatman No. 1) was used to sieve the liquid. A solution of 5% oxalic acid was added to get the volume up to 100 milliliters. The dye solution 2, 6-dichlorophenol indophenol, was used for the titration. Using the L-ascorbic acid standard, the mean observations yielded the quantity of dye needed to oxidize a specific amount of L-ascorbic acid solution at an unknown concentration. Each time, a 5 ml solution was used for titration, and the final point of titration was identified by the pink color, which persisted for 10 seconds. Vitamin C contents in fruit samples were recorded as mg of Vitamin C per 100 gram of fruit pulp.

Total sugar

After chopping the fruit pulps into small bits, they were added to ethyl alcohol that was just starting to boil and left for ten minutes. For every gram of pulp, 10 milliliters of alcohol are needed. The extract was re-extracted for three minutes in 80% hot alcohols with two to three milliliters of alcohol per gram of tissue after being screened through two layers of cotton. The extract was cooled and then passed through two cotton layers. To purify both extracts, Whatman No. 41 filter paper was used. Before being cooled, the extract was evaporated over a steam bath to a quarter of its initial volume. After that, distilled water was added to a 100 ml volumetric flask until the appropriate amount was reached, and the concentrated extract was placed inside (1).

$$\text{Total sugar (mg/100g)} = \text{Amount of sugar obtained/ Weight of sample} \times 100 \quad (1)$$

Reducing sugar

The phenol-sulfuric acid method was used to determine the reducing sugars. The extract was filtered after 0.2 grams of fresh leaf were homogenized with deionized water. Two milliliters of the solution were mixed with 0.4 milliliters of 5% phenol. 2 cc of 98% sulfuric acid was then rapidly added to the liquid. To allow the color to develop, the test tubes were placed in a water bath that was set at thirty degrees Celsius for twenty minutes after being left at room temperature for ten minutes. The spectrophotometer was then used to detect the light absorption at 540 nm. The blank solution, which is distilled water, was made using the same procedure. In mg/100g FW, the reducing sugar content was reported.

Non-reducing sugar

The non-reducing sugar content of banana pulp was determined using the following formula (2):

$$\text{Non-reducing sugar (mg/100g)} = \text{Total sugar} - \text{reducing sugar} \quad (2)$$

Evaluation of the effect of paclobutrazol (PBZ) and bunch covering on proximate composition of banana

Ash content (%)

For calculating the ash content of banana, use the dry ash method, AOAC (1995) method no. 942.05. By precisely measuring one gram of each sample into a crucible, setting the crucible on a clay pipe triangle, heating the material over a low flame until it was completely charred, and then heating the material in a muffle furnace for roughly six hours at 600°C, it was possible to calculate the total amount of ash in banana. After cooling, it was weighed in

desiccators. To determine the total amount of ash, apply the equation below (3) (Raghuramulu et al., 2003).

Ash content (%) = sampled weight of banana divided by weight of ash (3)

Lipid content (%)

The AOAC (1995) technique was used to determine lipid. The mixture of 50 ml of chloroform, methanol (2:1 v/v), and five grams of ground banana was carefully mixed before being allowed to stand for three days. A table centrifuge was used to filter the solution and centrifuge it at 1000g. The Pasteur pipette was used to remove the top layer of the methanol, and heating caused the chloroform to evaporate, thus crude lipid is remained.

Fiber content (%)

The AOAC method no. 962.03 was used to determine the banana fiber content. 200 ml of boiling 0.255 N H_2SO_4 was added to a beaker containing five grams of moisture- and fat-free substance. Five grams of the sample, which was free of moisture and fat, were put in a beaker, and 200 ml of boiling 0.255 N H_2SO_4 were added. The volume was maintained constant throughout the 30-minute boiling process by adding water at regular intervals. After filtering the mixture through a muslin cloth, the remaining material was thoroughly washed with hot water to remove any remaining acids. 200 ml of boiling 0.313 N NaOH was then added to the mixture in the same beaker. After boiling for 30 minutes (at the same volume), the mixture was filtered through a muslin cloth. The remaining substance was then washed in ether and alcohol before being rinsed with hot water to remove any remaining alkali. After spending the night in a crucible drying at 80 to 100 °C, it was then weighed using an electric balance. The crucible was heated in a muffle furnace at 600°C for 5–6 hours before being cooled, weighed, and calculated (Raghuramulu et al., 2003)

Moisture content (%)

The moisture content of banana powder was determined using an electric moisture meter and expressed as a percentage.

Color measurement

The colors of the banana peel were measured nondestructively using a Minolta Chroma meter (Model CR400) that was configured with a D65 illuminant and a 10° observer angle. The color values were represented by the letters L*, a*, b*, and C*. For every fruit, the reading was set to require an average of six random points. The colorimeter must be completely in contact with the fruits to stop light from leaking from it.

Statistical analyses

The Statistical Analysis System (SAS), version 9.4 (SAS Institute, Cary, NC, USA), was used for statistical analyses. Statistical significance was reached when the mean value of the treatments was $p = 0.05$.

RESULTS AND DISCUSSION

Time (days) to harvest from fruit set

Days to harvest from set were significantly influenced by paclobutrazol and bagging materials. The lowest number of days (110) required for harvesting was observed from the treatment combination of paclobutrazol (1g/L) and white polythene and the highest days (151

days) required for harvesting was recorded from control which was significantly highest from other treatment combinations (Table 1). It was observed that exogenous application of paclobutrazol at 1g/L and bunch covering with white polythene influenced some physiological attributes of banana and this treatment took the lowest number of days to harvesting. Singh and Ranganath (2006) observed that paclobutrazol exhibited earlier harvest than that of the control. Fruits harvesting occurred 18 days earlier in plants treated with paclobutrazol due to early fruit maturity (Rahman et al., 2023). Ruiz et al. (2005) stated that Paclobutrazol reduces days to harvesting from flowering by accelerating the ovule maturity in flower. Santosh et al. (2017) observed the maintenance of fruit quality with bunch covering. Rajan et al. (2020) proved that the white perforated bags create a microclimate inside the bunch which helps the bunch in early maturity by increasing the temperature around the developing bunch.

Bunch weight (kg)

The maximum bunch weight (24.91 kg) was observed when Paclobutrazol (1 g/L) was applied and bunch was covered with white polythene which was statistically similar to the treatment combination of same rate of paclobutrazol application with blue bunch covering (24.36 kg). The minimum bunch weight (16.77 kg) was recorded in untreated plants (Table 1). According to Ashraf and Ashraf (2020), paclobutrazol increases bunch weight by reallocating carbohydrates from the sources to the sinks as it were in the case of transport of photosynthates to developing fruits. Maximum fruit weight was obtained from bagging with white polythene and might be due to the protection of fruit from ultra violet rays which helps in increasing cell division and proper availability of photosynthates in the growing regions e.g. fruits of the plant (Rahman et al., 2017).

Number of hands bunch^{-1}

The highest number of hands bunch^{-1} (9.33) was observed from the treatment combination of paclobutrazol (1 g/L) and white polythene. The lowest number of hands bunch^{-1} (7.66) was recorded from the control which was statistically similar to the treatment combination of no paclobutrazol with white polythene and blue polythene respectively (Table 1). The application of paclobutrazol resulted in a larger number of hands per bunch, but bagging did not significantly alter the number of hands per bunch. Amani and Avagyan (2014) reported that there was no significant difference on number of hand with the treatment of bagging material and control. Paclobutrazol inhibits gibberellin, which lowers the level of vegetative promoter and raises the level of florigenic promoter, which may be responsible for the induction of the number of hands per bunch (Desta et al., 2021).

Number of fingers bunch^{-1}

The highest number of fingers bunch^{-1} (151.00) was observed from the treatment combination of Paclobutrazol (1 g/L) and bunch covering with white polythene. The result was statistically similar for the same rate of paclobutrazol application with blue bunch covering (152.33). The lowest result (111.67) was found from control (Table 1). Paclobutrazol improves flowering by modifying assimilate partitioning and nutrient delivery patterns for new growth, which supports increasing the number of flushes per plant (Meena et al., 2014).

Pulp-peel ratio

The maximum pulp-peel ratio (3.57) was observed when the foliar application of paclobutrazol (1 g/L) with white bunch covering was executed and was statistically similar to results was found from the treatment combination of palclobutrazol (1 g/L) with blue

polythene (3.17). The minimum pulp-peel ratio (1.89) was recorded from the plants without paclobutrazol and bagging treatments (Table 1). Increase in pulp weight in paclobutrazol treated fruits may be due to increase in sugars all the sinks (Prasana et al., 2018). Lobo et al. (2020) observed that paclobutrazol creates a strong correlation in pulp to peel ratio where pulp increases and peel decreases. According to Rubel et al. (2019) bunch covering of banana facilitates higher pulp thickness than that of the control treatment. This decrease in peel thickness may be due to the movement of moisture from the peel to the pulp during ripening and as well contributes to the ease with which the peel can be separated from the pulp. This is because as bananas and plantains ripen the ratio of the pulp mass: peel mass increases and the peel becomes progressively easier to separate (Rubel et al., 2019).

Total soluble solids (°Brix)

The maximum amount of Total soluble solid (TSS) (24.63 °Brix) was observed from the treatment combination of paclobutrazol (1 g/L) and white polythene which was statistically similar to the same rate of paclobutrazol with blue polythene (21.23 °Brix). The minimum TSS (17.03 °Brix) was recorded from the control (Table 2). The amount of TSS was not influenced largely with bunch covering but paclobutrazol has a great impact. Pre-harvest bagging can reduce titratable acids due to rapid maturity and ripening (Sharma et al., 2014) and increase respiration and ethylene production rates, which can greatly increase the concentration of total soluble solids (Islam et al., 2017). According to Khodair et al. (2018) and Reddy et al. (2013), TSS (°Brix) is increased due to spray of paclobutrazol which is capable of inducing ripening, however through ethylene synthesis than that of control.

Table 1. Effect of paclobutrazol (PBZ) and bunch covering on pre-harvest and physical harvest properties of banana.

Treatments	Time (days) from fruit set to harvest	Bunch weight (kg)	Number of hands/bunch	Number of fingers/bunch	Pulp: Peel ratio
Control	150.33a	16.77d	7.66d	111.67e	1.89e
No PBZ + White polythene	127.00de	19.36c	7.66d	120.00d	2.36cd
No PBZ + blue polythene	139.33b	18.11cd	7.66d	119.00d	2.27de
PBZ (1g/L) + No polythene	122.33e	22.29b	8.33cd	144.33b	3.16ab
PBZ l(1g/L) + white polythene	110f	24.91a	9.33a	152.33a	3.57a
PBZ (1g/L) + blue polythene	122.67e	24.36a	8.89b	151.00a	3.17ab
PBZ (2g/L) + No polythene	134.00bc	22.29b	8.66bc	138.67c	2.55cd
PBZ (2g/L) + white polythene	129.33cd	21.44b	8.66bc	136.33c	2.79bc
PBZ (2g/L) + blue polythene	131.67cd	21.18b	8.66bc	140.33bc	2.76bc
CV%	2.82	5.31	6.28	2.34	9.80
$LSD_{0.05}$	6.325	1.95	0.94	5.46	0.46

Means within each column with different letters are different significantly ($p<0.05$).

Table 2. Effect of paclobutrazol and bunch covering on biochemical properties of banana.

Treatments	TSS (°Brix)	Vitamin C (mg/100g)	TA (%)	Reducing sugar (mg/100g)	Non reducing sugar (mg/100g)	Total sugar (mg/100g)
Control	17.03c	3.84d	0.57a	4.12g	0.48d	4.60i
No PBZ + White polythene	18.66abc	5.76abc	0.17cd	4.13g	0.50d	4.63h
No PBZ + blue polythene	17.80bc	5.12bcd	0.23b	4.41f	1.09b	5.50g
PBZ (1g/L) + No polythene	19.43abc	5.76abc	0.19bc	4.69e	1.11b	5.80f
PBZ l(1g/L) + white polythene	24.63a	7.04a	0.06f	4.73d	1.13b	5.86e
PBZ (1g/L) + blue polythene	24.50ab	6.40ab	0.12de	4.86c	1.13b	5.99d
PBZ (2g/L) + No polythene	18.66abc	4.48bcd	0.10ef	5.11b	1.14b	6.25b
PBZ (2g/L) + white polythene	21.23abc	5.76abc	0.06f	5.20a	1.29a	6.51a
PBZ (2g/L) + blue polythene	21.23abc	6.40ab	0.08ef	5.10b	0.90c	6.02c
CV%	19.48	15.43	16.99	0.01	7.48	0.18
$LSD_{0.05}$	6.75	1.49	0.05	0.27	0.07	0.01

Means within each column with different letters are different significantly ($p<0.05$).

Ascorbic acid (mg/100g FW)

The maximum Vit C Content (7.04 mg/100g) was observed from the treatment combination of Paclobutrazol (1 g/L) and white polythene and minimum Vit C Content (3.84) was recorded from the control (Table 2). Ascorbic acid content was higher under this paclobutrazol application with white bunch covering. Jungklang et al. (2017) stated that PBZ treated plants showed the highest levels of ascorbic acid compared to other treatments. This might be due to paclobutrazol having the mechanism to protect the plant from oxidative damage which was ensured by the high level of endogenous ascorbic acid. This finding is consistent with Jangid et al. (2021), who observed that bunch covering enhanced vitamin C content. This could be due to the bags' selective solar permeability and the microenvironment (temperature, humidity, and moisture) around the fruit (Srivasta et al., 2023).

Titratable acidity (%)

Titratable acidity (TA) content of banana was varied significantly due to combined effect of paclobutrazol and bagging materials (Table 2). The maximum TA (0.57%) was observed from the treatment combination of no paclobutrazol and no bunch covering and the minimum TA (%) was recorded from the treatment combination of paclobutrazol (1 g/L) with white covering which was statistically similar with paclobutrazol (2 g/L) with blue bagging, both had (0.06%) (Table 2). Titratable acidity (TA) represents the organic acids that greatly affect overall eating quality and flavor of fruit. Moscoso-RamÃrez and Peña-Peña (2020) observed less TA percentage was present in fruit under bagging treatment than that of control. According to Trad et al. (2013), titratable acids can be decreased by pre-harvest bagging since it raises the interior temperature.

Reducing sugar, non-reducing sugar and total sugar (mg/100g)

The maximum reducing sugar (5.20), non-reducing sugar (1.29) and total sugar (6.51) was recorded in paclobutrazol (2 g/L) with white polythene and the minimum reducing sugar (4.12), non-reducing sugar (0.48) and total sugar (4.60) was recorded in no paclobutrazol and

no polythene (Table 2). Paclobutrazol application (2 g/L) with white bunch covering showed higher amount of sugar content compared to other treatment. Suman et al. (2017) found that the highest value of the reducing sugar was exhibited when paclobutrazol was applied to the plant whereas the lowest value appeared in the control. Sarker et al. (2018) found similar result that paclobutrazol helps to increase total sugar. Jaya et al. (2016) reported that this rapid increase in sugar content under covering materials is believed to occur due to hydrolysis of starch into simple sugars and also due to the polymerization of tannins (perhaps the most important phenolic from the point of view of fruit utilization) to insoluble compounds resulting in a reduction of astringency (sharp/acidic taste) during ripening.

Moisture content (%)

The maximum moisture content (26.29%) was observed from the treatment combination of paclobutrazol (1 g/L) and white polythene cover which was statistically similar to the treatment combination of paclobutrazol (1 g/L) with blue polythene cover (25.98%). The minimum moisture content (14.62%) was recorded from control (Table 3). Application of Paclobutrazol exhibited maximum dry matter by accumulation of biomass which leads to minimum moisture content compared to control. Campbell et al. (2018) concluded that the microclimate under the polythene resulted higher relative humidity that facilitate higher moisture content on ripened fruit.

Ash, lipid and fibre content (%)

The maximum ash content (2.96%) was noticed from the treatment combination of paclobutrazol (1 g/L) and white polythene cover. The minimum ash content (1.72%) was recorded from the control which was statistically similar (1.77%) to that under blue bunch covering without paclobutrazol application (Table 3). Applying paclobutrazol to the fruit crops increases its ash content by absorbing mineral elements (Wang et al., 2019). Haryanto et al. (2021) carried out an experiment and observed that ash percentage increases by the influence of the covering material.

The maximum Lipid (5.33%) was observed from the treatment combination of paclobutrazol (1 g/L) and white polythene and the minimum Lipid (2.40%) was recorded from the control which was significantly different from other treatment combinations (Table 3). It has been demonstrated that hydrogen peroxide, which is produced as a result of the oxidative damage caused by paclobutrazol, promotes the accumulation of neutral lipids (Yang et al., 2023).

The maximum fiber content (4.20) was observed from the treatment combination of paclobutrazol (1 g/L) and white polythene which was statistically similar to the treatment combination of paclobutrazol (1 g/L) with blue polythene (4.00). The minimum fibre content (2.60%) was recorded in the untreated plant which was similar to the application of paclobutrazol (1 g/L) with no bagging (2.70) (Table 3). According to Kumar et al. (2021) fruit bagging has been shown to improve the quality attributes hence, the amount of fibre may increase under bagging conditions.

Table 3. Effect of paclobutrazol (PBZ) and bunch covering on proximate composition of banana.

Treatments	Moisture content (%)	Ash (%)	Lipid (%)	Fiber (%)	L*	a*	b*
Control	14.62d	1.72g	2.40e	2.60f	53.00e	3.84c	11.97c
No PBZ + White polythene	23.47bc	1.91f	3.33d	2.70ef	66.33cd	3.84c	25.14b
No PBZ + blue polythene	21.20c	1.77g	3.40cd	2.90de	61.41d	6.16ab	35.50a
PBZ (1g/L) + No polythene	24.04ab	2.30d	4.30b	3.80b	54.64e	6.66a	25.14b
PBZ l(1g/L) + white polythene	26.29a	2.96a	5.33a	4.20a	80.20a	6.40ab	41.70a
PBZ (1g/L) + blue polythene	25.98ab	2.76b	4.60b	4.00ab	76.10ab	5.77a	37.11a
PBZ (2g/L) + No polythene	23.45bc	2.02e	3.80c	3.30c	72.87bc	5.54ab	33.68ab
PBZ (2g/L) + white polythene	24.96ab	2.81b	4.33b	3.50c	76.96ab	5.45ab	33.22ab
PBZ (2g/L) + blue polythene	25.11ab	2.66c	4.23b	3.00d	71.84bc	5.40b	33.15ab
CV%	3.00	1.89	5.97	5.15	5.60	12.67	17.63
$LSD_{0.05}$	2.80	0.07	0.41	0.29	6.87	1.22	9.38

Means within each column with different letters are different significantly ($p<0.05$).

Color measurement

Significant changes in color values (L* and b*) during ripening was found in paclobutrazol and bagging materials treatments. The highest lightness value (L* value) (80.20) and the highest yellowness (b* value) (41.70) is found in paclobutrazol (1 g/L) with white bagging treatment whereas the lowest lightness value (L* value) (80.20) and the lowest yellowness (b* value) (53.00) was found in control (Table 3). It can be observed from table 3, that the lightness value (L* value) and yellowness (b* value) increased during ripening and the value was more in fruits treated with paclobutrazol and banana bunch covered with polythene. This could be due to the degradation of chlorophyll, which makes the yellow carotenoid pigments visible (Choudhury et al., 2023). The lower values of L and b may be the result of brown patches and specks developing on the banana's peel as it ripened (Escalante-Minakata et al., 2018).

CONCLUSION

The postharvest treatment of paclobutrazol is the best suited and beneficial for the yield and quality of banana. Fruits grown under bagging condition had substantially more total soluble solids, vitamin C and sugar content. Furthermore, in white polythene bagging, the fruit peel color, yield and all the quality contributing parameters of the banana were higher except sugars. The sugar content was higher in blue polythene covering fruits. From the present investigation, it is concluded that banana can be grown successfully using paclobutrazol (1g/L) and white polythene covering for improved productivity and quality banana produce.

Hence, we recommend this concentration as the optimal choice for achieving the desired improvements in banana productivity and quality.

Conflict of interest

The authors declare that they have no conflict of interest.

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Optimizing bio-chemical fertilizer treatments for quantitative and qualitative traits of *Artemisia annua* L. using graphical analysis

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ABSTRACT

Purpose: *Artemisia annua* L., a medicinal herb of significant importance due to its high artemisinin content, a potent antimalarial compound. This study aimed to investigate the influence of various bio-chemical fertilizer combinations on the growth characteristics and artemisinin content of *Artemisia annua* L. Graphical analysis techniques were employed to visualize and optimize fertilizer treatments for achieving the desired balance between quantitative (biomass) and qualitative (artemisinin content) traits. **Research Method:** This experiment was conducted as a factorial experiment with a basic randomized complete block design (RCBD) with four replications in a research greenhouse. The main factors in this experiment included the application of bio-fertilizers (control, nitroxin, bio-phosphorus, and vermicompost), and the sub-factor consisted of four levels of chemical phosphorus (P) and nitrogen (N) fertilizers (0, N40 P40, N80 P40, and N80 P80). **Findings:** The results of the combined analysis for all traits revealed significant effects of bio-fertilizer and chemical fertilizer at the 5% and 1% probability levels, respectively. The interaction of treatments also exhibited significant differences for most traits. Mean comparison using LSD showed that vermicompost and $N_{80}P_{80}$ treatments were superior to other treatments. Correlation analysis revealed a positive and significant correlation between the traits, as evidenced by the correlation coefficients and correlation charts. Graphical analysis identified treatments Vermicompost + $N_{80}P_{80}$ and Nitroxin + $N_{80}P_{80}$ as optimal based on trait desirability. The results of the focused scatter plot analysis further confirmed Vermicompost + $N_{80}P_{80}$ as the most favorable treatment. The findings revealed a strong correlation among the evaluated traits. **Research limitations:** There was no limitation. **Originality/Value:** Treatments Vermicompost + $N_{80}P_{80}$ and Nitroxin + $N_{80}P_{80}$ emerged as the most favorable options based on the assessed traits, demonstrating remarkable efficacy in augmenting artemisinin levels.

INTRODUCTION

Malaria, a devastating disease that continues to claim lives, resulted in nearly 429,000 fatalities worldwide in 2015. Predominantly prevalent in African countries (accounting for approximately 90% of global cases), malaria poses a significant barrier to social and economic progress in these regions.

One of the most lethal malaria parasites has developed resistance to commonly used antimalarial drugs such as quinine, chloroquine, mefloquine, and sulfadoxine-pyrimethamine in Asia and Africa. This resistance poses a major challenge to malaria control and treatment (Brisibe et al., 2012; Kumar & Rathinam, 2013).

Numerous studies conducted by the World Health Organization (WHO) since 2001 have demonstrated the efficacy of artemisinin-based combination therapy (ACT) in combating malaria (World Health Organization, 2016). Artemisinin, a molecule extracted from the Chinese annual herb *Artemisia annua* L., holds immense promise in the battle against this devastating disease (Aftab et al., 2014).

Artemisia annua L., an ancient herb belonging to the Asteraceae family, has been utilized in traditional Chinese medicine for centuries to treat fevers. The discovery of the antimalarial properties of its extracts in the 1970s marked a turning point in the fight against malaria. The plant's active compound, artemisinin, emerged as a new generation of antimalarial drugs, and in combination with other medications, significantly improved the treatment of uncomplicated malaria cases (Shahrajabian et al., 2020; Siddiqui et al., 2018).

Artemisinin has not only proven its effectiveness but also exhibits minimal side effects. Unlike conventional chemical drugs, this plant-derived compound exhibits low toxicity and has demonstrated superior efficacy in malaria treatment (Aftab et al., 2014).

Despite these advantages, the WHO does not recommend direct consumption of *Artemisia annua* L. for malaria treatment (World Health Organization, 2016). This stems from the insufficient production of artemisinin to meet the growing global demand and its high price in China, rendering this treatment inaccessible to low-income populations. To address these limitations, production chains for this compound were established in the early 2000s, initially in Vietnam and later in East Africa (Konaré et al., 2023).

In the world, the use of chemical fertilizers has been well-documented as a key factor in the remarkable increase in crop yields. This holds true for a wide range of crops cultivated under diverse climatic and soil conditions. Evidence from numerous field trials suggests that in many soils, nutrient deficiencies, particularly nitrogen, pose a major constraint to plant growth, and chemical fertilization can significantly alleviate this limitation (Nyoni et al., 2020).

Nitrogen, an essential element for plant growth, plays a crucial role in the synthesis of proteins and nucleic acids. Studies by Singh (2000) have demonstrated that the application of varying levels of nitrogen fertilizer positively impacts artemisinin content and essential oil in *Artemisia annua* L. plants. This is due to the direct role of nitrogen in the structure of the chlorophyll molecule; a strong positive correlation exists between leaf nitrogen content and plant chlorophyll levels (Jia et al., 2021).

In today's agricultural landscape, there is a growing demand for sustainable and environmentally friendly solutions to enhance crop production. In this context, biological fertilizers have emerged as a new generation of fertilizers that utilize beneficial microbes instead of chemicals to promote plant growth. These fertilizers employ various mechanisms, including nitrogen fixation from the air, enhancing nutrient solubility and uptake from the soil, producing plant hormones, and diversifying soil microbial communities, which stimulate plant growth directly and indirectly (Kumar, 2004; Raimi et al., 2021).

Unlike chemical fertilizers, which are not only expensive but also pose environmental risks, biological fertilizers are cost-effective, eco-friendly, and sustainable, contributing to long-term soil health (Raimi et al., 2021).

Artemisia annua L., a remarkable medicinal herb, has gained significant attention for its potent antimalarial compound, artemisinin. While chemical fertilizers have played a crucial role in boosting crop yields, their environmental impact and potential drawbacks have prompted a shift towards sustainable alternatives. In this context, biological fertilizers, enriched with beneficial microbes, have emerged as a promising approach for enhancing *Artemisia annua* L. cultivation and artemisinin production (Bijeh Keshavarzi & Omidi, 2025).

A diverse array of microorganisms, particularly fungi and certain bacteria, have demonstrated the potential to augment the production of bioactive compounds in *Artemisia annua* L. Among the common bacteria employed in *Artemisia annua* L. biofertilizers are *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, Streptomyces, and Rhizobium (Aidah et al., 2023).

The utilization of biological fertilizers in *Artemisia annua* L. cultivation offers not only a means to increase the yield of this valuable medicinal herb but also presents a sustainable and environmentally friendly approach to promote soil health and foster sustainable agricultural practices. Yazdani et al. (2009) demonstrated that the application of phosphate-solubilizing microorganisms and plant growth-promoting rhizobacteria (PGPR) in conjunction with chemical fertilizers (nitrogen, phosphorus, and potassium) in maize led to significant improvements in cob weight, number of rows per cob, and number of grains per row. Galindo et al. (2022) investigated the effects of nitrogen fertilizer, *Azospirillum* inoculation, and their combined application on cowpea growth. Their findings demonstrated that the combined application of nitrogen fertilizer and *Azospirillum* inoculation significantly increased both cowpea nitrogen and grain yield compared to the control and other treatments. When *Azospirillum* co-inoculation is employed in cowpea cultivation, nitrogen supplementation via mineral fertilizers becomes unnecessary.

The low artemisinin content in *Artemisia annua* L. and the economic challenges associated with large-scale production prompted the researchers to undertake this study. The objective was to investigate the influence of biofertilizers, chemical fertilizers, and their combinations on artemisinin content.

MATERIALS AND METHODS

This experiment was conducted as a factorial experiment with a basic randomized complete block design (RCBD) with four replications in a research greenhouse located in Tehran province during the summer of 2011. The main factors in this experiment included the application of bio-fertilizers, and the sub-factor consisted of four levels of chemical phosphorus and nitrogen fertilizers. Nitrogen fertilizer was applied in the form of urea, and phosphorus fertilizer was applied as triple superphosphate. All related laboratory analyses were performed at the Biotechnology Laboratory of Hamdard University, India.

Experimental factors

Experimental factors included bio-fertilizer at 4 levels: control (without fertilizer), Nitroxin (Containing Azotobacter and Azospirillum Bacteria), Biophosphorus (Containing Bacillus and Pseudomonas Bacteria), Vermicompost (10 tons per hectare) and N and P chemical fertilizer at 4 levels: Control (No Fertilizer), Nitrogen 40 and Phosphorus 40 kg/ha ($N_{40}P_{40}$), Nitrogen 80 and Phosphorus 40 kg/ha ($N_{80}P_{40}$), Nitrogen 80 and Phosphorus 80 kg/ha ($N_{80}P_{80}$).

Table 1. Treatment characteristics, names, and measured traits.

Treatment code	Treatment characteristics	Traits code	Traits
tr_1	Control	C_1	Artemisinin – Pre flowering
tr_2	$N_{40}P_{40}$	C_2	Artemisinin – Post flowering
tr_3	$N_{80}P_{40}$	C_3	Leaf Water Content – Pre flowering
tr_4	$N_{80}P_{80}$	C_4	Leaf Water Content – Post flowering
tr_5	Nitroxin	C_5	Leaf Number – Pre flowering
tr_6	Nitroxin + $N_{40}P_{40}$	C_6	Leaf Number – Post flowering
tr_7	Nitroxin + $N_{80}P_{40}$	C_7	Fresh Leaf Weight – Pre flowering
tr_8	Nitroxin + $N_{80}P_{80}$	C_8	Fresh Leaf Weight – Post flowering
tr_9	Biophosphorus	C_9	Dry Leaf Weight – Pre flowering
tr_{10}	Biophosphorus + $N_{40}P_{40}$	C_{10}	Dry Leaf Weight – Post flowering
tr_{11}	Biophosphorus + $N_{80}P_{40}$	C_{11}	Protein
tr_{12}	Biophosphorus + $N_{80}P_{80}$	C_{12}	Chlorophyll a
tr_{13}	Vermicompost	C_{13}	Chlorophyll b
tr_{14}	Vermicompost + $N_{40}P_{40}$	C_{14}	Total Chlorophyll
tr_{15}	Vermicompost + $N_{80}P_{40}$	C_{15}	Stem Height – Pre flowering
tr_{16}	Vermicompost + $N_{80}P_{80}$	C_{16}	Stem Height – Post flowering
		C_{17}	Number of Lateral Stems - Pre flowering
		C_{18}	Number of Lateral Stems - Post flowering
		C_{19}	Stem Water Content – Pre flowering
		C_{20}	Stem Water Content – Post flowering
		C_{21}	Fresh Stem Weight – Pre flowering
		C_{22}	Fresh Stem Weight – Post flowering
		C_{23}	Dry Stem Weight – Pre flowering
		C_{24}	Dry Stem Weight – Pre flowering

Planting

For seed mixing and inoculation, the desired *Artemisia annua* L. seeds were first spread on a clean, wide plastic sheet. The inoculant was then sprinkled on the seeds and the seeds were mixed to ensure even inoculation. The inoculated seeds were then placed in the shade for 1 hour to allow the inoculant to dry, and were then ready for planting (Shakouri & Keshavarzi, 2020).

Nitrogen fertilizer was applied from urea (with 48% nitrogen) and phosphorus fertilizer from triple superphosphate (with 46% phosphorus). All phosphorus fertilizer was applied before planting, and nitrogen fertilizer was applied in 3 split applications based on soil testing. Table 1 shows the characteristics and names of the treatments and the traits measured in the experiment.

Planting was conducted on the first of September 2011. Prior to planting, a plastic bag (with drainage holes) was placed in each of the pre-prepared plastic pots (20 cm diameter and 25 cm height) to prevent fertilizers from leaching out of the bottom of the pots. The desired soil was then filled into the pots. After the potential risk of pests was eliminated, thinning was performed by retaining three healthy plants and removing the remaining plants from the pots. The soil used had a sandy loam texture with a pH of 7. The plant's light requirement was met artificially for 16 hours per day. The set temperature was 18°C for the day and 22°C for the night. The relative air humidity was also set at 65% (Zhang et al., 2023).

Trait measurement

To measure stem height, the plants were cut at ground level and the exact height of each plant was individually measured using a ruler. This was done both before and after flowering.

For measuring the fresh and dry weight of the entire aboveground biomass, the plants were cut at ground level. Then, using a digital scale, the fresh weight of each plant organ (leaf, stem) was individually measured and recorded. The separated organs were then placed in separate bags and dried in an oven at 70°C for 24 hours. The dry weight of each organ was then individually measured and recorded using a digital scale.

To measure the water content of each plant organ (leaf, stem), the dry weight was subtracted from the fresh weight to determine the water content (in milligrams). Chlorophyll content was measured using the method of Hiscox and Israelstam (1979), and protein content was measured using the Bradford method (1976).

Chlorophyll content

100 mg of fresh Artemisia leaves were placed in a test tube, and 10 mL of Dimethyl Sulfoxide (DMSO) was added. The mixture was then incubated at 65°C for 1 hour to ensure complete extraction of chlorophylls and to achieve complete bleaching of the leaves. Subsequently, aliquots of the samples were transferred to spectrophotometer cuvettes, and absorbance was measured separately at wavelengths of 663 nm for chlorophyll a and 645 nm for chlorophyll b. The recorded absorbance values were then used in the following formulas (1, 2 & 3) to calculate the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll.

$$\text{Chlorophyll a (mg/g)} = 12.7\,(OD663) - 2.69(OD645) \times (V/(1000 \times wt)) \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = 22.9\,(OD645) - 4.68(OD663) \times (V/(1000 \times wt)) \quad (2)$$

$$\text{Total chlorophyll (mg/g)} = 20.2\,(OD645) + 8.02(OD663) \times (V/(1000 \times wt)) \quad (3)$$

Where V represents the volume of the filtered solution, wt represents the fresh weight of the sample used, and OD represents the optical density (absorbance) at wavelengths of 663 nm and 645 nm (Hiscox & Israelstam, 1979).

Protein content

0.5 g of fresh plant sample was added to 5 mL of Buffer Solution No. 1 and thoroughly ground until the sample was completely dissolved in the buffer. The resulting solution was centrifuged at 12,000 rpm for 20 minutes. 1 mL of the supernatant was carefully collected using a micropipette and transferred to a separate Eppendorf tube. Then, 1 mL of 20% Trichloroacetic Acid was added, and the mixture was incubated at 20°C for 2 hours. Subsequently, the mixture was centrifuged again at 10,000 rpm for 10 minutes. After centrifugation, 1 mL of 0.1 M NaOH was added to the pellet and dissolved. 100 µL of the resulting solution was then added to 5 mL of Buffer Solution No. 2 (Bradford reagent). By adding Milli-Q water (double-distilled water), the final volume was adjusted to 6 mL. Finally, the sample was placed in a spectrophotometer at a wavelength of 595 nm, and the obtained absorbance was recorded.

Artemisinin content was determined in the samples using the Gupta et al. (1996) method and HPLC. 1 g of dried material was finely ground and 20 ml of petroleum ether was added. The samples were shaken at 37°C for 12 hours, and the resulting solution was collected. This process was repeated three times. The collected material was placed on a heater to evaporate the solvent and dry the sample. Four ml of ethanol was added to the sample and after filtration; the residue was washed with 2 ml of ethanol. The entire filtered solution was made up to 10 ml with ethanol. One ml of the final solution was diluted with 4 ml of 2% NaOH and heated in a water bath at 50°C for 30 minutes. After cooling, 1 ml of ethanol was added and the volume was made up to 10 ml with 2 N acetic acid.

Statistical analysis

To gain deeper insights from the composite analysis, we employed various techniques, which were included comparing treatment means using the LSD method, evaluating the interaction effects of treatments on averages, performing correlation analysis, and conducting graphical analysis. The graphical analysis utilized multivariate diagrams and correlation plots to identify

the optimal treatment based on an ideal standard. Additionally, concentrated scatter plots visualized the relationships between desired treatments and traits. Software like Excel, SAS, V9, and Genstat V. 12.1 were used for the data analysis.

RESULTS AND DISCUSSION

The results of the analysis of variance revealed significant effects of replication on all traits except artemisinin (pre-flowering), artemisinin (post-flowering), chlorophyll a, chlorophyll b and total chlorophyll at the 0.05 and 0.01 probability levels. Bio-fertilizer and chemical fertilizer effects were also significantly different for all traits. The interaction effect of bio-fertilizer and chemical fertilizer was also significantly different for all traits except Leaf Water Content (post-flowering), leaf number (pre-flowering), leaf number (post-flowering), fresh leaf weight (pre-flowering), dry leaf weight (pre-flowering), chlorophyll b, and number of lateral stems (pre-flowering). The highest coefficient of variation percentage was observed for trait number of lateral stems (pre-flowering) (17.20%), while the lowest was for trait fresh stem weight (pre-flowering) (8.30%). Additionally, the highest R-squared value was for trait artemisinin (post-flowering) (99.0%) and the lowest was for trait c13 (71.0%) (Table 2).

Mean comparison using the LSD method was employed to select the most suitable treatment based on trait means. The results indicated that the vermicompost and N80P80 treatments exhibited greater desirability compared to the other treatments under investigation for all traits. Furthermore, the nitroxin treatment demonstrated superior performance for traits such as leaf water content (before flowering), leaf fresh weight (after flowering), leaf dry weight (before flowering), protein content, number of lateral branches (before flowering), stem water content (before flowering), stem water content (after flowering), and stem dry weight (after flowering) (Table 3). In a study aimed at selecting iron and zinc nanofertilizers for morphological and biological traits of rice plants under drought stress conditions, LSD mean comparison was utilized to evaluate the treatments, and the most desirable treatments for various traits were selected based on this analysis (Jafarsalehi et al., 2024).

Table 2. Analysis of variance of treatments for evaluated traits in the experiment.

S.O.V	df	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf Water Content – Post flowering	Leaf Number – Pre flowering	Leaf Number – Post flowering
Repetition (R)	3	0.0001^{ns}	0.0001^{ns}	1.12^{**}	1.69^{**}	348^{**}	504.08^{**}
Bio-Fertilizer (A)	3	0.02^{**}	0.026^{**}	4.66^{**}	11.1^{**}	1286.8^{**}	1762.04^{**}
Error1	9	0.0002	0.0009	1.18	0.67	19.6	26.4
Chemical Fertilizer (B)	3	0.04^{**}	0.1^{**}	8.43^{**}	12.1^{**}	1246.37^{**}	1698^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.0008^{**}	0.0019^{**}	0.12^{**}	0.38^{ns}	36.2^{ns}	53.82^{ns}
Error 2	36	0.0001	0.00005	0.22	0.21	25.01	34.5
CV%	-	5.58	3.69	18	14.2	8.28	8.3
R-Square	-	0.96	0.99	0.86	0.91	0.91	0.91

Table 2. *Continued.*

S.O.V	df	Fresh Leaf Weight – Pre flowering	Fresh Leaf Weight – Post flowering	Dry Leaf Weight – Pre flowering	Dry Leaf Weight – Post flowering	Protein	Chlorophyll a	Chlorophyll b
Repetition (R)	3	0.06^{**}	0.31^{**}	0.75^{*}	1.91^{**}	2.34^{**}	0.002^{ns}	0.014^{ns}
Bio-Fertilizer (A)	3	0.047^{**}	1.5^{**}	8.06^{**}	20.78^{**}	22.3^{**}	0.48^{**}	0.36^{**}
Error1	9	0.07	0.13	1.44	0.48	1.6	0.005	0.009^{*}
Chemical Fertilizer (B)	3	0.57^{**}	1.06^{**}	13.4^{**}	20.3^{**}	19.29^{**}	0.7^{**}	0.21^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.018^{ns}	0.09^{**}	1.47^{ns}	0.67^{*}	1.02^{**}	0.02^{**}	0.02^{ns}
Error 2	36	0.011	0.017	0.22	0.23	0.1	0.005	0.023
CV%	-	12.39	12.58	13.37	11.33	5.4	3.43	17.05
R-Square	-	0.91	0.94	0.91	0.94	0.97	0.95	0.71

Table 2. *Continued.*

S.O.V	df	Total Chlorophyll	Stem Height – Pre flowering	Stem Height – Post flowering	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering
Repetition (R)	3	0.01^{ns}	12.22^{**}	236.1^{**}	5.93^{*}	68.59^{*}
Bio-Fertilizer (A)	3	1.66^{**}	156.9^{**}	266.16^{**}	67.55^{**}	631.8^{**}
Error1	9	0.01	1.65	2.9	4.14	3.36
Chemical Fertilizer (B)	3	2.11^{**}	255.9^{**}	458.2^{**}	76.76^{**}	571.18^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.08^{**}	6.3^{**}	5.6^{**}	2.19^{ns}	30.11^{*}
Error 2	36	0.01	0.9	1.8	1.72	15.78
CV%	-	3.7	3.03	3.42	20.17	13.64
R-Square	-	0.96	0.97	0.97	0.89	0.87

Table 2. *Continued.*

S.O.V	df	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Repetition (R)	3	0.012^{**}	0.014^{**}	0.024^{**}	0.02^{**}	0.001^{**}	0.001^{**}
Bio-Fertilizer (A)	3	0.12^{**}	0.16^{**}	0.32^{**}	0.24^{**}	0.026^{**}	0.017^{**}
Error1	9	0.007	0.009	0.01	0.009	0.0004	0.0002
Chemical Fertilizer (B)	3	0.32^{**}	0.38^{**}	0.66^{**}	0.58^{**}	0.04^{**}	0.035^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.006^{**}	0.009^{**}	0.012^{**}	0.008^{**}	0.0007^{**}	0.0004^{*}
Error 2	36	0.001	0.003	0.003	0.002	0.0001	0.0001
CV%	-	12.67	14.5	0.83	10.69	8.11	10.6
R-Square	-	0.96	0.94	0.96	0.97	0.97	0.96

Table 3. Mean comparison of treatments for evaluated traits in the experiment.

Bio-Fertilizer (A)	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf Water Content – Post flowering	Leaf Number – Pre flowering	Leaf Number – Post flowering
Control (A_1)	0.2^{d}	0.14^{d}	2.08^{b}	2.4^{c}	50.12^{d}	58.75^{d}
Nitroxin (A_2)	0.26^{b}	0.16^{b}	2.7^{ab}	3.47^{b}	65.2^{b}	76.25^{b}
Bio-Phosphorus (A_3)	0.22^{b}	0.18^{c}	2.43^{b}	2.82^{bc}	56.06^{c}	65.43^{c}
Vermicompost (V_4)	0.28^{a}	0.24^{a}	3.36^{a}	4.31^{a}	70.06^{a}	82.06^{a}
LSD	0.01	0.007	0.86	0.65	3.54	4.1
Chemical Fertilizer (B)	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf Water Content – Post flowering	Leaf Number – Pre flowering	Leaf Number – Post flowering
Control (B_1)	0.18^{d}	0.086^{d}	1.8^{d}	2.34^{c}	49.75^{c}	58.12^{c}
$N_{40}P_{40}$ (B_2)	0.22^{c}	0.18^{c}	2.38^{c}	2.8^{c}	57.93^{b}	67.87^{b}
$N_{80}P_{40}$ (B_3)	0.27^{b}	0.22^{b}	2.92^{b}	3.55^{b}	63.06^{b}	73.87^{b}
$N_{80}P_{80}$ (B_4)	0.29^{a}	0.27^{a}	3.48^{a}	4.31^{a}	70.75^{a}	82.66^{a}
LSD	0.01	0.003	0.43	0.49	5.65	6.46

Table 3. *Continued.*

Bio-Fertilizer (A)	Fresh Leaf Weight – Pre flowering	Fresh Leaf Weight – Post flowering	Dry Leaf Weight – Pre flowering	Dry Leaf Weight – Post flowering	Protein	Chlorophyll a
Control (A_1)	0.65^{c}	0.73^{c}	2.74^{b}	3.14^{d}	4.71^{c}	1.89^{d}
Nitroxin (A_2)	0.92^{b}	1.15^{ab}	3.63^{ab}	4.62^{b}	6.49^{ab}	2.2^{b}
Bio-Phosphorus (A_3)	0.79^{bc}	0.91^{bc}	3.22^{b}	3.73^{c}	5.5^{bc}	2.02^{c}
Vermicompost (V_4)	1.05^{a}	1.44^{a}	4.42^{a}	5.75^{a}	7.43^{a}	2.27^{a}
LSD	0.65	0.73	2.74	3.14	1.01	0.05
Chemical Fertilizer (B)	Fresh Leaf Weight – Pre flowering	Fresh Leaf Weight – Post flowering	Dry Leaf Weight – Pre flowering	Dry Leaf Weight – Post flowering	Protein	Chlorophyll a
Control (B_1)	0.63^{d}	0.77^{d}	2.43^{d}	3.11^{d}	4.8^{d}	1.86^{d}
$N_{40}P_{40}$ (B_2)	0.78^{c}	0.93^{c}	3.16^{c}	3.72^{c}	5.52^{c}	1.99^{c}
$N_{80}P_{40}$ (B_3)	0.94^{b}	1.19^{b}	3.87^{b}	4.75^{b}	6.5^{b}	2.2^{b}
$N_{80}P_{80}$ (B_4)	1.06^{a}	1.34^{a}	4.55^{a}	5.66^{a}	7.31^{a}	2.33^{a}
LSD	0.1	0.12	0.36	0.46	0.23	0.04

Table 3. *Continued.*

Bio-Fertilizer (A)	Chlorophyll b	Total Chlorophyll	Stem Height – Pre flowering	Stem Height – Post flowering	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering
Control (A_1)	0.744^{d}	2.64^{d}	28.29^{c}	35.05^{d}	3.93^{c}	22.43^{d}
Nitroxin (A_2)	0.919^{b}	3.13^{b}	32.55^{b}	4.97^{b}	7.5^{ab}	30^{b}
Bio-Phosphorus (A_3)	0.838^{c}	2.86^{c}	29^{c}	36.96^{c}	5.93^{b}	26.68^{c}
Vermicompost (V_4)	1.1^{a}	3.38^{a}	34.98^{a}	44.15^{a}	8.68^{a}	37.31^{a}
LSD	0.07	0.08	1.02	1.36	1.62	1.46
Chemical Fertilizer (B)	Chlorophyll b	Total Chlorophyll	Stem Height – Pre flowering	Stem Height – Post flowering	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering
Control (B_1)	0.74^{b}	2.58^{d}	26.59^{d}	32.95^{d}	3.87^{a}	22.5^{d}
$N_{40}P_{40}$ (B_2)	0.89^{b}	2.84^{c}	29.45^{c}	37.26^{c}	5.75^{c}	26.06^{c}
$N_{80}P_{40}$ (B_3)	1.003^{a}	3.17^{b}	33.1^{b}	41.6^{b}	7.5^{b}	32^{b}
$N_{80}P_{80}$ (B_4)	0.96^{a}	3.41^{a}	35.68^{a}	45.31^{a}	8.93^{a}	35.87^{a}
LSD	0.17	0.1	0.68	1.46	0.92	1.25

Table 3. *Continued.*

Bio-Fertilizer (A)	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Control (A_1)	0.22^{b}	0.25^{c}	0.36^{d}	0.3^{b}	0.1^{d}	0.077^{d}
Nitroxin (A_2)	0.36^{a}	0.42^{a}	0.58^{b}	0.49^{a}	0.15^{b}	0.128^{b}
Bio-Phosphorus (A_3)	0.25^{b}	0.33^{b}	0.47^{c}	0.35^{b}	0.13^{c}	0.1^{c}
Vermicompost (V_4)	0.41^{aa}	0.49^{aa}	0.69^{a}	0.57^{aa}	0.19^{a}	0.154^{a}
LSD	0.068	0.078	0.08	0.079	0.016	0.013
Chemical Fertilizer (B)	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Control (B_1)	0.15^{d}	0.199^{d}	0.28^{d}	0.21^{d}	0.08^{d}	0.06^{d}
$N_{40}P_{40}$ (B_2)	0.24^{c}	0.31^{c}	0.44^{c}	0.34^{c}	0.136^{c}	0.09^{c}
$N_{80}P_{40}$ (B_3)	0.38^{b}	0.46^{b}	0.62^{b}	0.52^{b}	0.167^{b}	0.14^{b}
$N_{80}P_{80}$ (B_4)	0.47^{a}	0.54^{a}	0.74^{a}	0.64^{a}	0.202^{a}	0.16^{a}
LSD	0.038	0.051	0.053	0.045	0.009	0.009

Considering the significance and widespread application of traits artemisinin (pre-flowering) and artemisinin (post-flowering) in this experiment, and since identifying suitable treatments based on these traits is a primary objective of this research, mean comparison plots were employed to visualize the interaction effect of bio-fertilizer and chemical fertilizer on these two traits. According to the plotted graph for trait artemisinin (pre-flowering), treatments tr16, tr8, and tr15 were identified as desirable treatments. Conversely, treatments tr1, tr9, and tr2 were categorized as undesirable treatments (Fig. 1a). Similarly, based on the graph for trait artemisinin (post-flowering), treatments tr16 and tr8 were identified as desirable treatments, while treatments tr1, tr9, and tr5 were classified as undesirable treatments for this trait (Fig. 1b). Several researchers have utilized this type of graph to evaluate their treatments (Khatibi et al., 2023; Omrani et al., 2022; Shojaei et al., 2022).

The results obtained from the mean comparison chart of the interaction between bio-fertilizer and chemical fertilizer showed similar results compared to those obtained from the mean comparison analysis using the LSD method (Table 4). These results indicated that the application of vermicompost + N80P80 treatment can have a positive effect on increasing the performance of traits in growth and enhancing the content of the active ingredient in this plant.

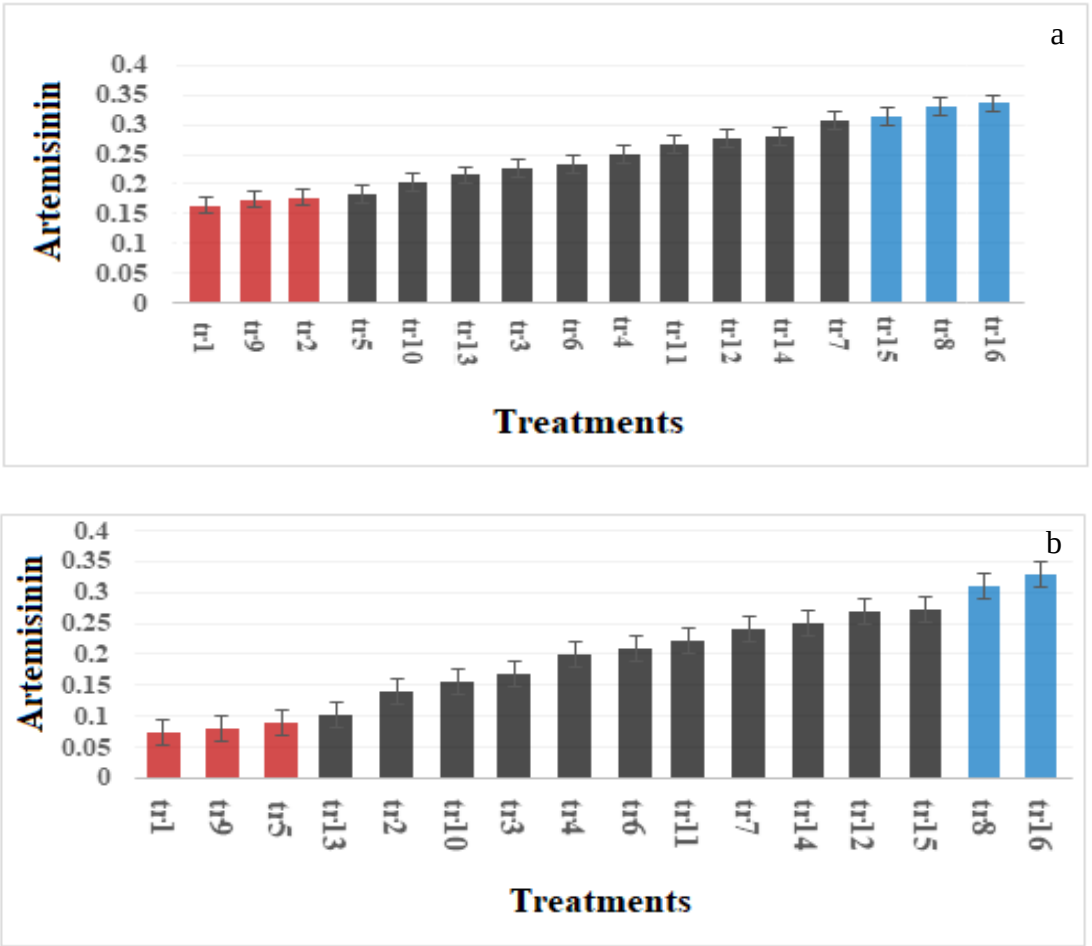


Fig. 1. Mean comparison plots of the interaction effect of bio-fertilizer and chemical fertilizer, a: Based on Trait c1 (Artemisinin (pre-flowering)), b: Based on Trait c2 (Artemisinin (post-flowering)).
(tr_1: Control; tr_2: $N_{40}P_{40}$; tr_3: $N_{80}P_{40}$; tr_4: $N_{80}P_{80}$; tr_5: Nitroxin; tr_6: Nitroxin + $N_{40}P_{40}$; tr_7: Nitroxin + $N_{80}P_{40}$; tr_8: Nitroxin + $N_{80}P_{80}$; tr_9: Biophosphorus; tr_{10}: Biophosphorus + $N_{40}P_{40}$; tr_{11}: Biophosphorus + $N_{80}P_{40}$; tr_{12}: Biophosphorus + $N_{80}P_{80}$; tr_{13}: Vermicompost; tr_{14}: Vermicompost + $N_{40}P_{40}$; tr_{15}: Vermicompost + $N_{80}P_{40}$; tr_{16}: Vermicompost + $N_{80}P_{80}$)

Table 4. Means comparison of interaction effects of treatments for evaluated traits in the experiment.

	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf water content – Post flowering	Leaf number – Pre flowering	Leaf number – Post flowering	Fresh leaf weight – Pre flowering	Fresh leaf weight – Post flowering
Control	0.16^{j}	0.074^{l}	1.28^{g}	1.78^{h}	42.5^{i}	49.5^{i}	0.5^{h}	0.55^{i}
$N_{40}P_{40}$	0.17^{j}	0.13^{i}	1.75^{gf}	1.79^{h}	44.75^{hi}	53^{hi}	0.59^{gh}	0.68^{hi}
$N_{80}P_{40}$	0.22^{g}	0.16^{g}	2.53^{def}	2.37^{ghf}	5.075^{gh}	59.5^{gh}	0.58^{fgh}	0.79^{fghi}
$N_{80}P_{80}$	0.25^{ef}	0.19^{f}	2.78^{cde}	3.65^{cd}	62.25^{def}	73^{def}	0.83^{def}	0.9^{efgh}
Nitroxin	0.18^{ij}	0.088^{k}	1.7^{gf}	2.37^{fgh}	5.075^{gh}	58.5^{gh}	0.72^{gf}	0.83^{fghi}
Nitroxin + $N_{40}P_{40}$	0.23^{gh}	0.2^{f}	2.29^{ef}	2.84^{efg}	65.25^{cd}	76.5^{cd}	0.81^{ef}	0.99^{efg}
Nitroxin + $N_{80}P_{40}$	0.3^{c}	0.24^{d}	2.99^{bcde}	4.04^{bc}	69.75^{bc}	84.75^{bc}	1.03^{bcd}	1.31^{cd}
Nitroxin + $N_{80}P_{80}$	0.331^{ab}	0.308^{b}	3.81^{ab}	4.6^{b}	75.25^{ab}	88.25^{ab}	1.14^{abc}	1.47^{bc}
Biophosphorus	0.17^{j}	0.081^{kl}	1.75^{gh}	2.2^{gh}	47.5^{hi}	55.75^{hi}	0.59^{gh}	0.73^{ghi}
Biophosphorus + $N_{40}P_{40}$	0.2^{hi}	0.15^{h}	2.25^{ef}	2.47^{fgh}	56^{fg}	65.5^{fg}	0.76^{efg}	0.87^{fgh}
Biophosphorus + $N_{80}P_{40}$	0.26^{de}	0.22^{e}	2.61^{def}	3.07^{def}	56.5^{fg}	66.25^{efg}	0.85^{def}	0.96^{efgh}
Biophosphorus + $N_{80}P_{80}$	0.27^{d}	0.26^{c}	3.1^{bcde}	3.52^{cde}	64.25^{cde}	74.25^{cde}	0.95^{cde}	1.07^{de}
Vermicompost	0.21^{gh}	0.1^{j}	2.43^{def}	2.99^{defg}	58.25^{ef}	68.75^{def}	0.71^{ghf}	0.95^{efgh}
Vermicompost + $N_{40}P_{40}$	0.27^{d}	0.25^{d}	3.22^{bcd}	4.05^{bc}	65.75^{cd}	76.5^{cd}	0.96^{cde}	1.17^{ef}
Vermicompost + $N_{80}P_{40}$	0.31^{bc}	0.27^{c}	3.54^{abc}	4.71^{ab}	75.25^{ab}	88^{ab}	1.21^{ab}	1.72^{ab}
Vermicompost + $N_{80}P_{80}$	0.335^{a}	0.328^{a}	4.25^{a}	5.49^{a}	81^{a}	95^{a}	1.34^{a}	1.92^{a}

Table 4. *Continued.*

	Dry leaf weight – Pre flowering	Dry leaf weight – Post flowering	Protein	Chlorophyll a	Chlorophyll b	Total chlorophyll	Stem height – Pre flowering	Stem height – Post flowering
Control	1.79^{g}	2.34^{l}	4.08^{l}	1.74^{b}	0.67^{f}	2.4^{j}	26.62^{jk}	29.27^{j}
$N_{40}P_{40}$	2.34^{fg}	2.47^{kl}	4.42^{kl}	1.84^{gf}	0.73^{ef}	2.56^{ij}	26.42^{ij}	33.35^{hi}
$N_{80}P_{40}$	3.22^{cdef}	3.17^{ijk}	4.93^{ijk}	1.94^{ef}	0.81^{def}	2.73^{gh}	28.97^{h}	36.5^{fg}
$N_{80}P_{80}$	3.62^{cde}	4.55^{ef}	5.43^{ghij}	2.02^{de}	0.75^{ef}	285^{fg}	32.15^{f}	41.07^{e}
Nitroxin	2.43^{fg}	3.21^{hijk}	5.02^{hij}	1.88^{fg}	0.74^{ef}	2.63^{hi}	26.72^{ij}	33.67^{hi}
Nitroxin + $N_{40}P_{40}$	3.1^{def}	3.84^{fghi}	5.99^{efg}	2.15^{c}	0.87^{cdef}	2.96^{ef}	30.55^{g}	38.32^{f}
Nitroxin + $N_{80}P_{40}$	4.13^{bcd}	5.36^{cd}	7.04^{cd}	2.31^{b}	1.01^{bcd}	3.25^{cd}	35.95^{cd}	45.31^{bc}
Nitroxin + $N_{80}P_{80}$	4.95^{ab}	6.08^{bc}	7.92^{bc}	2.45^{a}	1.05^{bc}	3.66^{b}	37^{bc}	46.57^{b}
Biophosphorus	2.34^{fg}	3.17^{ijk}	4.56^{ijkl}	1.78^{gh}	0.74^{ef}	2.48^{ij}	24.65^{k}	31.75^{i}
Biophosphorus + $N_{40}P_{40}$	3.01^{ef}	3.35^{ghij}	5.14^{ghijk}	1.87^{fg}	0.85^{cdef}	2.72^{gh}	27.17^{i}	34.95^{gh}
Biophosphorus + $N_{80}P_{40}$	3.46^{cde}	4.04^{fg}	5.86^{efgh}	2.09^{cd}	0.86^{cdef}	2.96^{ef}	29.67^{gh}	37.5^{f}
Biophosphorus + $N_{80}P_{80}$	4.06^{bcd}	4.59^{def}	6.43^{def}	2.34^{b}	0.89^{cde}	3.28^{c}	34.5^{de}	43.65^{cd}
Vermicompost	3.14^{def}	3.94^{fgh}	5.56^{fghi}	2.04^{de}	0.81^{def}	2.82^{fg}	29.37^{gh}	37.12^{f}
Vermicompost + $N_{40}P_{40}$	4.18^{bc}	5.22^{de}	6.53^{de}	2.09^{cd}	1.12^{ab}	3.11^{de}	33.67^{e}	42.42^{de}
Vermicompost + $N_{80}P_{40}$	4.75^{ab}	6.43^{b}	8.16^{b}	2.45^{a}	1.32^{a}	3.75^{ab}	37.77^{ab}	47.1^{b}
Vermicompost + $N_{80}P_{80}$	5.59^{a}	7.41^{a}	9.45^{a}	2.5^{a}	1.16^{ab}	3.84^{a}	39.1^{a}	49.97^{a}

Table 4. *Continued.*

	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Control	1.75^{i}	19^{i}	0.11^{i}	0.13^{k}	0.18^{j}	0.14^{j}	0.05^{j}	0.034^{k}
$N_{40}P_{40}$	3.25^{hi}	18.25^{i}	0.15^{hi}	0.18^{ijk}	0.28^{ij}	0.22^{hij}	0.092^{h}	0.06^{j}
$N_{80}P_{40}$	5^{gh}	25.5^{gh}	0.26^{gh}	0.34^{fgh}	0.42^{fg}	0.36^{fg}	0.12^{fg}	0.09^{gh}
$N_{80}P_{80}$	5.75^{efg}	27^{efgh}	0.37^{de}	0.41^{def}	0.55^{de}	0.49^{d}	0.14^{e}	0.11^{ef}
Nitroxin	5^{gh}	25.75^{gh}	0.17^{hi}	0.21^{ijk}	0.3^{hi}	0.24^{hi}	0.095^{h}	0.066^{ij}
Nitroxin + $N_{40}P_{40}$	6.25^{defg}	25.75^{gh}	0.27^{f}	0.33^{fgh}	0.47^{ef}	0.38^{ef}	0.13^{ef}	0.1^{fg}
Nitroxin + $N_{80}P_{40}$	8^{cd}	31^{def}	0.44^{cd}	0.54^{bc}	0.72^{c}	0.6^{c}	0.18^{d}	0.16^{c}
Nitroxin + $N_{80}P_{80}$	10.75^{ab}	37.5^{bc}	0.56^{ab}	0.62^{ab}	0.82^{b}	0.75^{ab}	0.2^{bc}	0.18^{b}
Biophosphorus	3.5^{hi}	19.25^{i}	0.13^{hi}	0.21^{ijk}	0.28^{ij}	0.18^{ij}	0.07^{i}	0.05^{j}
Biophosphorus + $N_{40}P_{40}$	5.75^{efg}	25.25^{h}	0.17^{hi}	0.27^{hij}	0.39^{fgh}	0.25^{hi}	0.12^{fg}	0.08^{hi}
Biophosphorus + $N_{80}P_{40}$	7.25^{def}	30.5^{defg}	0.33^{de}	0.38^{efg}	0.53^{e}	0.45^{de}	0.15^{e}	0.12^{de}
Biophosphorus + $N_{80}P_{80}$	7.25^{def}	31.75^{de}	0.37^{de}	0.48^{cd}	0.66^{c}	0.52^{cd}	0.18^{d}	0.14^{cd}
Vermicompost	5.25^{fgh}	27^{efgh}	0.19^{gh}	0.24^{hij}	0.35^{ghi}	0.28^{gh}	0.11^{gh}	0.08^{hi}
Vermicompost + $N_{40}P_{40}$	7.75^{cde}	35^{cd}	0.37^{de}	0.44^{de}	0.64^{cd}	0.51^{d}	0.19^{cd}	0.13^{de}
Vermicompost + $N_{80}P_{40}$	9.75^{bc}	41^{b}	0.49^{bc}	0.61^{ab}	0.83^{b}	0.68^{b}	0.21^{b}	0.19^{b}
Vermicompost + $N_{80}P_{80}$	12^{a}	47.25^{a}	0.59^{a}	0.66^{a}	0.94^{a}	0.8^{a}	0.27^{a}	0.21^{a}

Trait Correlation

As all traits exhibited significant positive correlations with each other, the magnitude of the correlation coefficient between two traits reflected the strength of the association between them (Table 5).

Artemisinin (pre-flowering) demonstrated the strongest correlations with artemisinin (post-flowering), stem height (pre-flowering), and stem dry weight (post-flowering). Artemisinin (post-flowering) exhibited the highest correlations with chlorophyll b, stem fresh weight (pre-flowering), stem fresh weight (post-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering). Leaf water content (pre-flowering) displayed the strongest positive correlation with leaf dry weight (pre-flowering), while leaf water content (post-flowering) exhibited the highest correlation with leaf dry weight (post-flowering) compared to other traits (Table 5).

Number of leaves (pre-flowering) showed the strongest correlation with number of leaves (post-flowering); number of leaves (post-flowering) exhibited the highest correlation with stem height (post-flowering); leaf fresh weight (pre-flowering) demonstrated the strongest correlation with leaf dry weight (post-flowering); leaf fresh weight (post-flowering) exhibited the highest correlations with protein, stem fresh weight (post-flowering), stem dry weight (pre-flowering), stem dry weight (post-flowering), leaf dry weight (pre-flowering) with leaf dry weight (post-flowering), protein, number of lateral branches (pre-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering); and leaf dry weight (post-flowering) exhibited the strongest correlation with stem dry weight (pre-flowering) (Table 5).

In the analysis of protein correlations, protein displayed the strongest correlation with chlorophyll a. Chlorophyll a and chlorophyll b exhibited the strongest correlations with chlorophyll a; chlorophyll a with stem height (pre-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering); stem height (pre-flowering) with stem fresh weight (pre-flowering), stem fresh weight (post-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering); stem height (post-flowering) with stem dry weight (post-flowering); number of lateral branches (pre-flowering) with number of lateral branches (post-flowering); number of lateral branches (post-flowering) with stem dry weight (post-flowering); stem water content (pre-flowering) with stem fresh weight (post-flowering); stem water content (post-flowering) with stem fresh weight (pre-flowering); leaf fresh weight (pre-flowering) with leaf fresh weight (post-flowering) and stem dry weight (post-flowering); and leaf fresh weight (post-flowering) with stem dry weight (post-flowering) (Table 5).

Table 5. Correlation analysis of evaluated traits in the experiment.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
C1	1											
C2	0.931**	1										
C3	0.712**	0.737**	1									
C4	0.839**	0.792**	0.724**	1								
C5	0.801**	0.791**	0.741**	0.814**	1							
C6	0.799**	0.788**	0.731**	0.809**	0.997**	1						
C7	0.819**	0.793**	0.647**	0.844**	0.701**	0.692**	1					
C8	0.75**	0.731**	0.734**	0.691**	0.774**	0.774**	0.712**	1				
C9	0.782**	0.798**	0.984**	0.798**	0.78**	0.77**	0.769**	0.777**	1			
C10	0.869**	0.827**	0.774**	0.979**	0.856**	0.852**	0.863**	0.821**	0.848**	1		
C11	0.835**	0.806**	0.803**	0.834**	0.825**	0.821**	0.795**	0.848**	0.853**	0.892**	1	
C12	0.906**	0.888**	0.708**	0.786**	0.782**	0.778**	0.796**	0.797**	0.774**	084**	0.856**	1

Table 5. *Continued.*

	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
C13	1											
C14	0.741**	1										
C15	0.688**	0.924**	1									
C16	0.659**	0.831**	0.841**	1								
C17	0.566**	0.819**	0.827**	0.767**	1							
C18	0.657**	0.868**	0.838**	0.798**	0.853**	1						
C19	0.623**	0.878**	0.898**	0.844**	0.784**	0.81**	1					
C20	0.622**	0.876**	0.898**	0.832**	0.809**	0.819**	0.96**	1				
C21	0.654**	0.903**	0.92**	0.858**	0.834**	0.851**	0.965**	0.993	1			
C22	0.64**	0.897**	0.914**	0.856**	0.805**	0.83**	0.997**	0.968**	0.976**	1		
C23	0.697**	0.914**	0.913**	0.871**	0.843**	0.881**	0.904**	0.897**	0.941**	0.924**	1	
C24	0.669**	0.928**	0.93**	0.864**	0.839**	0.863**	0.954**	0.959**	0.977**	0.974**	0.953**	1

(C1:, C2:, C3:, C4: Leaf Water Content – Post flowering, C5: Leaf Number – Pre flowering, C6: Leaf Number – Post flowering, C7: Fresh Leaf Weight – Pre flowering, C8: Fresh Leaf Weight – Post flowering, C9: Dry Leaf Weight – Pre flowering, C10: Dry Leaf Weight – Post flowering, C11: Protein, C12: Chlorophyll a, C13: Chlorophyll b, C14: Total Chlorophyll, C15: Stem Height – Pre flowering, C16: Stem Height – Post flowering, C17: Number of Lateral Stems - Pre flowering, C18: Number of Lateral Stems - Post flowering, C19: Stem Water Content – Pre flowering, C20: Stem Water Content – Post flowering, C21: Fresh Stem Weight – Pre flowering, C22: Fresh Stem Weight – Post flowering, C23: Dry Stem Weight – Pre flowering, C24: Dry Stem Weight – Post flowering)

Correlation plots were employed to further investigate the relationships between traits. In these plots, the smaller the angle between traits vectors, the stronger the correlation between the corresponding traits. The cosine of the angle between vectors represents the correlation coefficient. A positive correlation is indicated by an angle between vectors smaller than 90 degrees. A 90-degree angle between vectors implies no correlation between genotypes, signifying their independence. Conversely, an angle larger than 90 degrees between vectors indicates a negative correlation between the corresponding genotypes (Ghasemi et al., 2021; Khatamain et al., 2011).

The correlation plot derived from the experimental data also revealed positive correlations among all traits. The results obtained from the correlation plot were consistent with those presented in the correlation coefficient table. According to the correlation plot, the correlation between the traits "number of leaves (post-flowering)" and "number of leaves (pre-flowering)" with the trait "number of lateral branches (post-flowering)" was relatively weaker compared to other trait pairs (Fig. 2).

Graphical Analysis

Biplot polygons are a crucial feature of the biplot method for identifying the best treatment for evaluated traits. The polygon observed in this plot is formed by connecting the treatments that are farthest from the origin, such that all other treatments lie within this polygon. Perpendicular vectors are then drawn from the origin to the edges of this polygon, representing the interactions between treatments and traits (Yan et al., 2000).

The first principal component accounted for over 97% of the data variance, according to the constructed biplot polygon, while the second principal component explained nearly 2%. Based on this plot, treatments tr16, tr8, tr7, tr6, tr9, tr2, tr5, and tr11 exhibited superior performance compared to the other treatments.

Within each section, treatment tr16 demonstrated the highest desirability for the trait "number of lateral branches (post-flowering)," while treatment tr8 exhibited the highest desirability for the traits "stem height (post-flowering)," "number of leaf (pre-flowering)," and "number of leaves (post-flowering)" compared to the other treatments. Treatments that fell on the origin of the plot, such as treatment tr12, displayed no significant response to changes in performance (Fig. 3a).

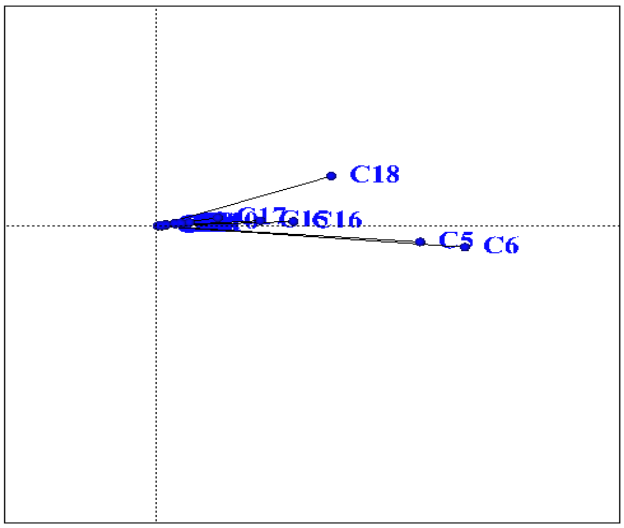


Fig. 2. Correlation plot of evaluated traits in the experiment.

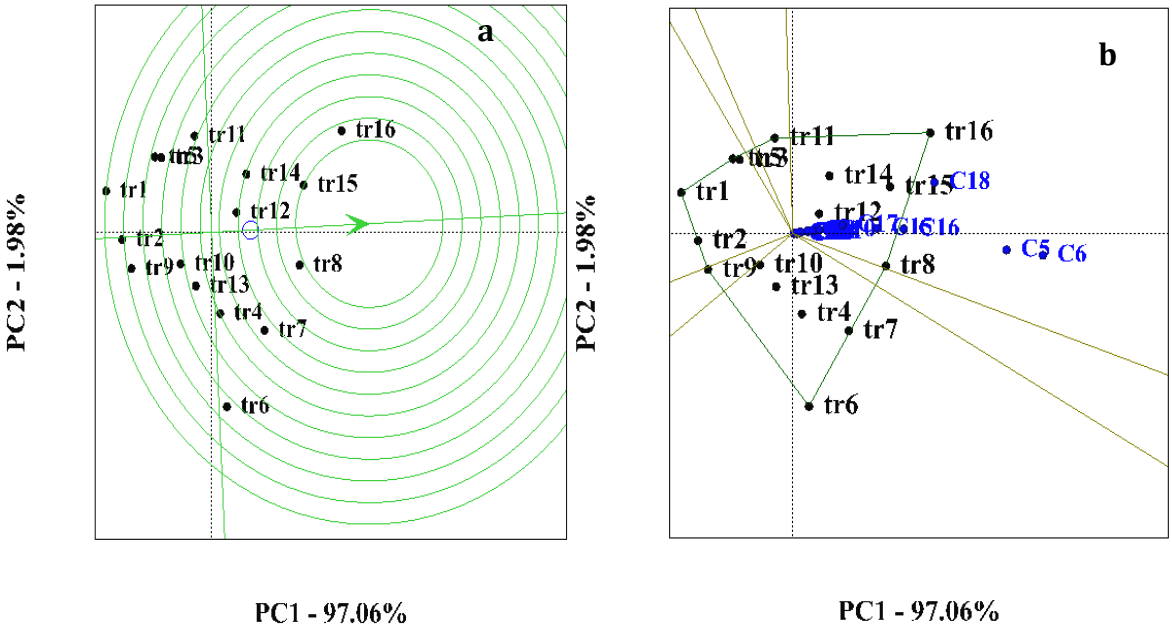


Fig. 3. Graphical analysis for selecting the most suitable treatment based on evaluated traits in the experiment: a) Biplot Polygon, b) Ideal Treatment Selection Plot.
(C1: Artemisinin – pre flowering, C2: Artemisinin – post flowering, C3: Leaf water content - before flowering, C4: Leaf Water Content – Post flowering, C5: Leaf Number – Pre flowering, C6: Leaf Number – Post flowering, C7: Fresh Leaf Weight – Pre flowering, C8: Fresh Leaf Weight – Post flowering, C9: Dry Leaf Weight – Pre flowering, C10: Dry Leaf Weight – Post flowering, C11: Protein, C12: Chlorophyll a, C13: Chlorophyll b, C14: Total Chlorophyll, C15: Stem Height – Pre flowering, C16: Stem Height – Post flowering, C17: Number of Lateral Stems - Pre flowering, C18: Number of Lateral Stems - Post flowering, C19: Stem Water Content – Pre flowering, C20: Stem Water Content – Post flowering, C21: Fresh Stem Weight – Pre flowering, C22: Fresh Stem Weight – Post flowering, C23: Dry Stem Weight – Pre flowering, C24: Dry Stem Weight – Post flowering.

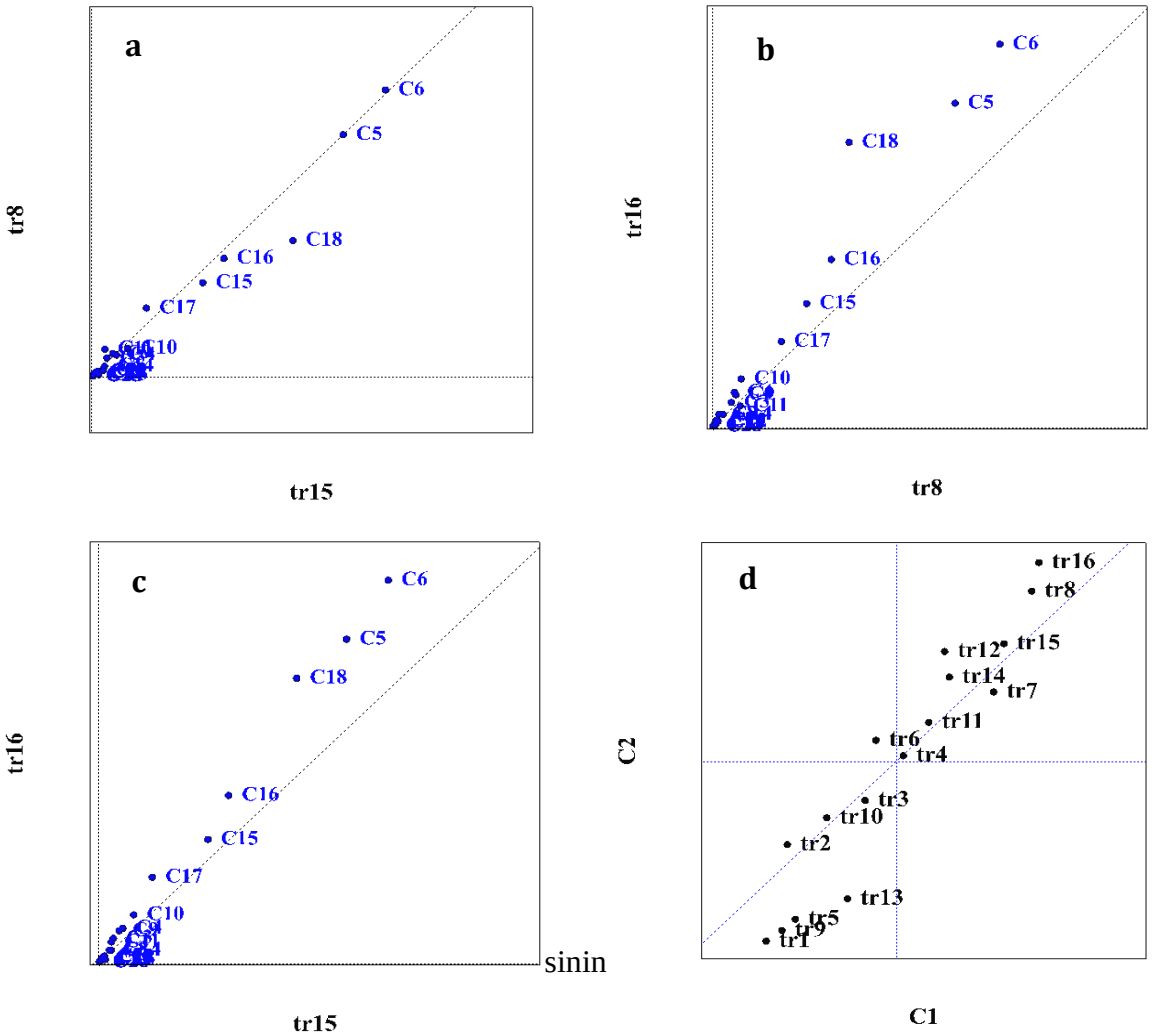
Based on the ideal treatment method the ranking plot connects a line from the origin of the plot to the mean point and extends it in both directions. The best treatment is the one that tends towards the positive end and has a smaller vertical distance from this line. In this figure, the best point is the center of the concentric circles, marked with an arrow, and the other treatments are ranked based on this point (Shojaei et al., 2022). According to this plot, the most desirable treatments based on the ideal treatment were tr15, tr8, tr16, tr14, and tr12 compared to the other treatments.

Treatments tr1, tr2, and tr9 were identified as undesirable treatments. The order of treatments from desirable to undesirable is as follows (Fig. 3b):

$tr_{15} > tr_8 > tr_{16} > tr_{14} > tr_{12} > tr_7 > tr_4 > tr_{13} > tr_{10} > tr_{11} > tr_{15} > tr_3 > tr_5 > tr_6 > tr_9 > tr_2 > tr_1$.

Based on the graphical analysis (Fig. 3), treatments tr15, tr8, and tr16 were identified as desirable treatments. Regarding the results obtained from this analysis, it showed complete agreement with the interaction effect chart (Fig. 1a and 1b), and in terms of the tr16 treatment, a high degree of similarity was also observed with the interaction effect mean comparison analysis (Table 4).

Various researchers have used treatment stability charts and the assessment of treatments based on the ideal treatment to investigate the stability of their genotypes and treatments. This includes research conducted on wheat (Omrani et al., 2024), maize (Khatibi et al., 2023; Shojaei et al., 2023a), and oilseed plants like canola (Shojaei et al., 2023b).



sinin

Fig. 4. Scatter Plot Analysis of Superior Treatments and Traits. a: Comparison of Treatments tr15 and tr8, b: Comparison of Treatments tr8 and tr16. c: Comparison of Treatments tr15 and tr16, d: Comparison of Traits C1 (Artemisinin (pre-flowering)) and. C2 (Artemisinin (post-flowering)). (tr_1: Control; tr_2: $N_{40}P_{40}$; tr_3: $N_{80}P_{40}$; tr_4: $N_{80}P_{80}$; tr_5: Nitroxin; tr_6: Nitroxin + $N_{40}P_{40}$; tr_7: Nitroxin + $N_{80}P_{40}$; tr_8: Nitroxin + $N_{80}P_{80}$; tr_9: Biophosphorus; tr_{10}: Biophosphorus + $N_{40}P_{40}$; tr_{11}: Biophosphorus + $N_{80}P_{40}$; tr_{12}: Biophosphorus + $N_{80}P_{80}$; tr_{13}: Vermicompost; tr_{14}: Vermicompost + $N_{40}P_{40}$; tr_{15}: Vermicompost + $N_{80}P_{40}$; tr_{16}: Vermicompost + $N_{80}P_{80}$)

Additionally, considering traits Artemisinin (pre-flowering) and Artemisinin (post-flowering) as the most critical traits in this experiment, with their enhancement being of paramount importance, scatter plot analysis was employed to investigate the separation of traits based on superior treatments and the separation of treatments based on key traits (Fig. 4).

According to the separation of traits based on treatments tr15 and tr8, traits stem height (pre-flowering), stem height (post-flowering), and number of lateral stems (post flowering) exhibited superior performance in treatment tr15, while the remaining traits demonstrated higher performance in treatment tr8 (Fig. 4a).

In the comparison of treatments tr8 and tr16, traits protein and dry stem weight (pre-flowering) showed superior performance in treatment tr8, while the remaining traits displayed higher performance in treatment tr16 (Fig. 4b). Furthermore, when comparing treatments tr16 and tr15, approximately 99% of the traits exhibited higher performance and desirability in treatment tr16 (Fig. 4c).

Upon examining the scatter plot comparing the two key traits artemisinin (pre-flowering) and artemisinin (post-flowering), treatments tr16, tr8, tr15, tr12, tr11, tr6, tr10, and tr2 exhibited superior desirability for trait artemisinin (post-flowering), while the remaining treatments demonstrated high performance and desirability for trait artemisinin (pre-flowering). Treatment tr4, located on the average axis of the plot, was used as the intermediate treatment (Fig. 4d).

Based on the evaluation of the treatments, it can be concluded that the application of N40P40 and vermicompost can significantly enhance trait artemisinin (post-flowering) performance, while the effects of nitroxin can be highly effective in increasing trait Artemisinin (pre-flowering) performance.

In the comparison between tr16 and tr15 treatments, it is noteworthy that 99% of the traits exhibit suitable performance efficiency in the tr16 treatment, which indicates the superiority of the tr16 treatment.

CONCLUSION

Artemisinin, a vital metabolite of *Artemisia annua* L., plays a crucial role in malaria treatment. Consequently, enhancing both the quantity and quality of this compound through optimized agricultural practices is of paramount importance for cultivating this valuable medicinal plant. This study aimed to investigate the effects of biological and chemical fertilizers on the growth and artemisinin content of *Artemisia annua* L. The results demonstrated that the interaction between different fertilizer treatments significantly affected most of the evaluated traits. Mean comparison analysis of the interaction effects using the LSD test identified treatment tr16 as the superior treatment. This treatment, along with tr8, emerged as the most favorable options for increasing artemisinin levels, exhibiting a strong correlation among the assessed traits. Notably, a comparison between treatments tr16 and tr15 revealed that 99% of the traits in tr16 exhibited higher performance efficiency, clearly confirming the superiority of tr16. Therefore, it can be concluded that treatment tr16, as the most effective treatment for enhancing both growth and artemisinin content in *Artemisia annua* L., can be recommended for implementation in cultivation programs of this medicinal plant.

Conflict of interest

The authors declare no competing interests.

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Physio-biochemical and antioxidative enzymatic changes in ambient stored 'Misribhog' mango in response to chitosan and *aloe vera* gel coatings

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ABSTRACT

Purpose: Mango shelf life has significance for both market availability and long-distance transportation. So, effective treatments of postharvest are vital for maintaining the climacteric character of mangoes by limiting postharvest losses during storage. **Research method:** A total of 96 physiologically mature mango fruits (8 fruits in each replication) were taken. This study assessed the effect of *Aloe vera* gel (1:1 AVG), chitosan (1.5% CTS), and combinations (CTS+AVG) on mango shelf life and postharvest features following 16 days at ambient storage (28±3°C and 80±5% RH). The experiment was conducted using completely randomized design. **Findings:** The results demonstrated that either CTS or AVG had a positive effect compared to control on different parameters but their combinations was considerably superior treatment equated to the control in terms of weight loss (13.09, 20.03%), reduced respiration rate (11.22, 19.89 mlCO_2/kg/h), ethylene production (0.50, 0.56 µl/kg/h), total soluble solids (17.33, 22.23 °Brix), pH (5.86, 7.40) and decay percentage (13.14, 27.64%). Fruit quality metrics were all higher when CTS+AVG was used than the control, such as titratable acidity (0.66, 0.61), fruit firmness (28.61, 21.95 N/m^2), ascorbic acid (14.52, 10.84 mg/100g), total phenolic content (112.99, 80.02 mg GAE/100g) and antioxidant activity (274.86, 196.65 µmol/g). Coated fruits exhibited a considerable reduction in polyphenol oxidase (PPO) (5.49, 7.87 U/mg FW), while higher levels of catalase (CAT) (0.54, 0.45 U/mg FW) and peroxidase (POD) (0.75, 0.70 U/mg FW) enzyme activity. During storage, coated fruit peels exhibited notably less discoloration than control fruits. **Research limitations:** In future, mechanism of CTS and AVG for prolonging shelf life of mangoes will be revealed using molecular approach. **Originality/Value:** These results suggest that chitosan (CTS) and *Aloe vera* gel (AVG) coatings combined can preserve 'Mishribhog' mango shelf life and postharvest quality for 16 days during ambient storage.

INTRODUCTION

In Bangladesh, mangoes (*Mangifera indica* L.) are popular and widely consumed fruit for their excellent eating properties, flavor, and distinctive color. Bangladesh ranks seventh in mango production worldwide. At present, the area under mango production is about 123997.70 hectares, with a production of over 1482937.04 MT (BBS, 2024). Mishribhog is a fibreless, sweet, and delicious variety with yellow color and attractive aroma. Because of mangoes climacteric fruit position, ripen promptly, turning them mushy and enabling a variety of microbes to grow in them during storage. Due to this behavior of less storage life and postharvest loss, a considerable amount of mango has become spoiled in several mango-producing countries around the world (Parvin et al., 2023). Mango fruits' postharvest life is prolonged by synthetic substances that are harmful to both the environment and human health. So, the applications of natural eatable coverings are very appealing, as they are incredibly fruitful for providing significant protection to the produce (Liu et al., 2020). Rajinith et al. (2022) reported that peptide-based edible coatings are used to reduce postharvest contaminations that impede anthocyanin synthesis and slow ripening in mangos. Chitosan applied exogenously lowers the transpiration rate, preserves firmness, boosts antioxidant activity, and promotes overall fruit quality (Wang et al., 2021). Because it is a naturally occurring chemical with antimicrobial properties, it effectively delays the deterioration of fruit by increasing mangos' durability and reducing the possibility of microbial attacks (Parvin et al., 2023; Nitu et al., 2025). The application of chitosan successfully extended the storage period of mango fruit (Cosme Silva et al., 2017).

Likewise, a naturally occurring material that's used as a fruit coating is *Aloe vera*. Its antibacterial and anti-oxidant properties, which come from the presence of many bioactive components, shelf life lengthen of various fruits (Jati et al., 2022; Alhassan & Ndomakaah, 2024). It also contains several complex polysaccharides, including glucomannans, creating a barrier against gaseous exchange. As a result, ripening and senescence are slowed down, and quality traits are improved (Aboryia et al., 2022). However, *Aloe vera* coating, useful alone or combined with other constituents, prolongs length of storage of mango (Amin et al., 2021), table grapes (Ayyub et al., 2024), apples (Kaur et al., 2024), apricots (Farooq et al., 2023), and guava (Supa et al., 2024) by slowing down respiration, cell wall softening, weight loss, and fruit decay, while also preserving other quality characteristics.

The research conducted to evaluates the length of application of chitosan and *Aloe vera* for postharvest treatment due to their biological and ecological benefits. Furthermore, fruits were kept at room temperature to test the potential of incorporating chitosan and *Aloe vera* to facilitate commercial exportation and long-term preservation. As a result, the current study was aimed toward exploring the possibility of chitosan and *Aloe vera* as an environmentally friendly preservation technique for reducing fruit softening, maintaining postharvest mango quality and extending storage time for commercial use.

MATERIALS AND METHODS

Mangoes were taken from a garden near Hajee Mohammad Danesh Science and Technology University, Bangladesh-5200 (Lat. 25°38'11.6664" N and Long. 88°38'10.9592" E). Mangoes were harvested when their skins (peels) were yellow at the bottom and green at the top. The normal harvest stages for local producers range from 75 to 82 days (Goutom et al., 2010). A total of 96 physiologically mature and healthy ripe mango fruits (8 fruits in each replication) were taken and submerged for three minutes in 1% sodium hypochlorite. Four treatments were control, 1.5% Chitosan (CTS; w/v), 1:1 Aloe vera (AVG; v/v), and CTS+AVG.

Mangoes were coated for 5 minutes and then air dried at room temperature for 2 hours. Fruit samples were analyzed every four days for 16 days. Mangoes were held at ambient condition (28±3°C and 80±5% RH) in this experiment.

Preparation of chitosan (CTS), *Aloe vera* gel (AVG) and chitosan-*Aloe vera* gel (CTS +AVG) coating

According to the method of Rahim et al. (1998) the chitosan solution was obtained. 1.5 g CTS powder was mixed with 1% lactic acid solution and 1ml glycerin. A magnetic stirrer homogenized the solution for 4 hours at 28°C. *Aloe vera* leaves were collected and separated the parenchyma layers and homogenized (Sing et al., 2013). CTS-AVG (1:1, v/v) was mixed for two hours at room temperature using a magnetic stirrer. Finally, four layers of muslin cloths filter the mixture to eliminate fibrous materials for the mucilaginous gel (Vieira et al., 2016).

Loss of weight (LW)

The loss of weight was obtained using this established method (1):

$$\text{LW (\%)} = \frac{\text{Weight of initial fruit (g)} - \text{Observation day fruit weight (g)}}{\text{Weight of initial fruit (g)}} \times 100 \qquad (1)$$

Respiration rate and ethylene production

The respiration rate and ethylene biosynthesis was measured according to the method described by Pristijono et al. (2019) with minor modification. The experiment involved storing fruits in sealed containers with septa at 28±3°C for two hours. A gas light hypodermic needle collected 1 ml of gas from the container's headspace and measured respiration rate with a CO_2/O_2 Gas Analyzer (FELIX Three, F-950, USA). The volume of CO_2 in the container was measured by inserting gas analyzer syringe through septa into container head space to measure ethylene. Lastly, fruit volume, weight of fruit, gas volume of container, and incubation time estimated respiration (2) and ethylene (3).

$$Respiration\ rate\ (ml\ CO_2 Kg^{-1}h^{-1}) = \frac{CO_2\ \%\times volume\ of\ container\ (ml)}{The\ sample\ weight\ (kg)\times 100\times Incubation\ time(h)} \qquad (2)$$

$$Ethylene\ production\ (\mu l\ C_2H_4 kg^{-1}h^{-1}) = \frac{C_2H_4\ \mu l\times volume\ of\ continer\ (ml)}{The\ sample\ weight\ (kg)\times Incubation\ time(h)} \qquad (3)$$

Fruit firmness

Fruit firmness was measured using an HP200 Force gauge (Handpi, China). After peeling, a 2 mm round stainless-steel probe was used to sample the fruit. Values were expressed in Newton (N).

Fruit decay

For fruit decay determination following equation (4) was used:

$$Decay\ (\%) = \frac{Number\ of\ decayed\ fruits}{Number\ of\ initial\ fruits} \times 100 \qquad (4)$$

Color

A color analyzer (Model, BCM-110 BCM-200, Biobase, China) was employed on opposite sides of the fruits for assessing the peel color. Peel color values were denoted as L* (‘+’ values=lightness, ‘-’ values=darkness), a* (‘-’ values=green, ‘+’ values=red), and b* (‘-’

values=blue, '+' values=yellow), saturation of color [Chroma, $(C=a^{*2}+b^{*2})^{0.5}$], and angle of hue ($h^{o}=\tan^{-1} b^{*}/a^{*}$).

Ascorbic acid (AA), Titratable acidity (TA), Total soluble solids (TSS), and pH

Parameters were described by Khatun et al. (2023) with slight modification, homogenize one gram of mango pulp with 3% metaphosphoric acid, the resultant mixture was filtered, and 2, 6-dichloroindophenol dye was employed to titrate a 5 ml sample of filtrate to pink endpoints (5).

$$\text{Ascorbic acid (mg/100g)} = \frac{Titrate \times factor\ of\ dye \times prepared\ volume}{Filtrate\ volume\ taken \times weight\ of\ sample} \quad (5)$$

The TA was obtained by taking 10 ml of mango extract with 500 microliters of phenolphthalein in volumetric flask. The solution was titrated against 0.1 N NaOH until the dye changed to pink.

TSS was measured using a refractometer (1.435-1.520 nD). Fruit pulp was meshed and filtered juice through cheese cloth. A refractometer prism was used to measure two drops of filtrate in °Brix. pH was acquired with a digital pH meter (HI 2211pH/ORP, China). First, 5 ml of mango extract was collected in a 10 ml volumetric flask. The pH meter probe was then put into the mango extract, and a reading was taken.

Total phenolic content (TPC)

Filter paper (Whatman No. 1) was used to filter 1 g of 10 ml methanol-extracted fruit pulp. Add 1 ml aliquot to 0.5 ml Folin-Ciocalteu and 7.5% diluted Na_2CO_3. This held 10 ml with distilled water. Mixing samples appropriately and centrifuging at 4,000 rpm for 10 minutes after 35 min at room temperature. The One Tech, China Elisa Microplate Reader (E-19) measured 765 nm absorbance against a blank. Total phenolic was measured in mg per 100 g fruit pulp (Singleton & Rossi, 1965).

DPPH scavenging activity

Using a mortar and pestle, 1 g mango pulp and 10 ml methanol were crushed. Samples were then sieved with Whatman No. 1 filter paper. Extract and DPPH (0.3 mM) solution were mixed in a falcon tube and left to settle in darkness for 30 minutes. The standard curve was created using Trolox values from 0 to 1 µmol/g. Spectrophotometer was used to measure absorbance at 517 nm compared to blank. The result was measured in micro mole per 100 gm fruit pulp (Hossain et al., 2021).

Enzyme extraction and activity assay

0.2 g fruit pulp was mixed with 3 ml buffer of phosphate (100 mM, pH 7) and 4% PVP using a mortar and pestle. Next, samples are centrifuged at 12,000 rpm for 15 minutes. A fraction was kept at 4°C for future use. Each enzyme activity was conveyed as U/mg FW.

Polyphenol oxidase activity (PPO)

The activity of PPO enzyme was obtained by the methods of Soliva et al. (2017). 600 µl enzyme extract, 1ml phosphate buffer (100 mM, pH 7), and 600 µl catechol (100 mM) were mixed. The spectrophotometer measured solution absorbance at 410 nm in two min.

Catalase activity (CAT)

A 700 µl solution of K_2SO_4 buffer, 100 µl H_2O_2 (200 mM), and 100 µl EDTA (2.5 mM) was prepared with 100 µl of enzyme extract. A spectrophotometer measured 240 nm absorbance after two min (Aebi, 1984).

Peroxidase activity (POD)

POD activity was measured by mixing 100 µl enzyme extract and guaiacol (20 mM), 600 µl phosphate buffer solutions, 100 µl EDTA (2.5 mM), and 100 µl H_2O_2 (100 mM). A spectrophotometer recorded 470 nm absorbance for two mins (Chance & Mahely, 1955).

Shelf life

Fruit's shelf life (in days) began when their weight declined 10% while stored. Fruits' shelf lives were measured starting on the harvest date and ending when more than 50% fruits were decayed Begum et al. (2023).

Statistical analysis

The acquired data was analyzed using a completely randomized design with three replications (each containing eight fruits) and two factorial designs. Analysis of variance was used to evaluate the experimental data (ANOVA). The various coatings and storage durations led to variations. The Statistical Tool for Agricultural Research (STAR, Version 2.0.1; IRRI, Laguna, Philippines) was used for all calculations and computations. Statistical differences between mean values ($P \leq 0.05$) were calculated using the LSD test. The R statistical software (version 4.3.1; R Core Team 2023) was utilized to determine potential correlations between variables through the principal component analysis (PCA).

RESULTS AND DISCUSSION

Weight loss, respiration rate and ethylene production

As a climacteric fruit, the quick weight loss is occurred in mango fruit which causes shelf-life reduction. The weight loss improved significantly ($p \leq 0.05$) with storage days. From the Figure 1a, observed that at the end of storage, weight loss increased progressively; however, the control fruits lost maximum weight (20.03%). Loss of weight of the CTS+AVG treated fruits was the least (13.9%) among the other treatments. CTS and AVG showed weight reduction of 18% and 17.31%, respectively, after storage. Weight loss occurs in fresh fruit. Water is lost by fruit transpiration and respiration. Temperature and humidity reduce fruit weight through respiration and transpiration. Allegra et al. (2021) observed that AVG-coated apples decreased transpiration, induced weight loss and dehydration. Begum et al. (2023) found that CTS+AVG-coated mangoes lost less weight at room temperature, validating our findings.

Figure 1b shows that respiration rates gradually increased throughout storage. Nevertheless, respiration rate rose significantly in control and declined slowly on day eight (19.89 mlCO_2/kgh). However, after 12th days of storage, the coated fruits had a higher respiratory rate. Then, a declining trend became apparent. During storage, CTS+AVG-coated fruits exhibited the lowest respiration rate (11.22 mlCO_2/kg/h) when equated to control. Coating film development reduces respiration (Formiga et al., 2022). This study confirms Chauhan et al. (2015)'s findings that *Aloe vera* and chitosan function as plasticizers, retaining CO_2 in fruit tissues and limiting oxygen availability.

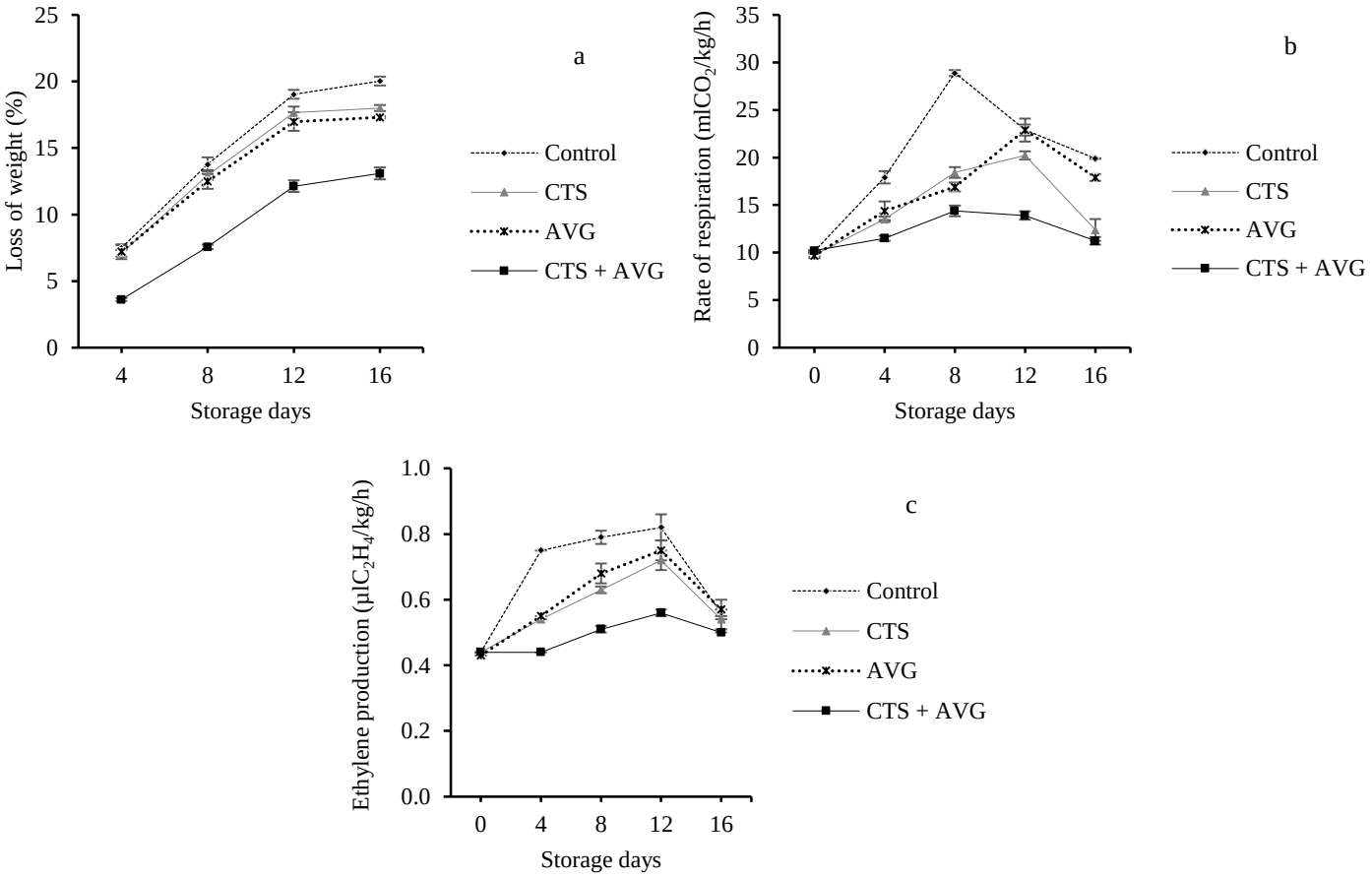

Fig. 1. Effect of different concentration of chitosan and Aloe vera gel on the weight loss (a), rate of respiration (b) and ethylene production (c) in Mishribhog mango during 16 days' storage at 25±3°C and 80–85% RH. The vertical bar shows the SE of the means (n=3). In accordance with the LSD test (P≤ 0.05), means with the same letters did not differ substantially. Control: distilled water, AVG: Aloe vera gel, CTS: 1.5% chitosan solution, CTS+AVG: 1.5% chitosan solution + Aloe vera gel, SE: Standard error

Ethylene production in mango increased gradually and then reduced as storage time increased. The initial mango ethylene production value of 0.44 µlC_2H_4/kg/h) increase dramatically up to 12th days of storage, reaching values of 0.82, 0.72, 0.75 and 0.56 µlC_2H_4/kg/h for control, CTS, and AVG and CTS+AVG, respectively. After storage, CTS+AVG-treated fruits emitted less ethylene 0.50 µlC_2H_4/kg/h compared to others treatments (Fig. 1c). Chitosan may prevent latent infection by delaying ripening and senescence. Lower internal oxygen levels may delay ethylene synthesis and respiration, causing the observed effects. Pang et al. (2024) say CTS coating is a natural covering that keeps mangoes fresh after harvest. Like our investigation, it was found that CTS and AVG reduce mango ethylene production (Shah & Hashmi, 2020).

Fruit firmness, decay incidence and color

Chitosan and *Aloe vera* gel coating had a significant impact on fruit firmness. A decreasing trend was seen throughout the time of storage. After 16 days of storing, the fruit firmness declined to 21.95 N from 58.80 N in the control group. Whereas the firmness was 21.75, 23.22 and 28.61 N in case of CTS, AVG and CTS + AVG coated fruits (Table 1). Due to metabolic breakdown of cell wall polymers such pectin, cellulose, and hemicellulose, fruits soften and lose firmness with time. Coatings prevent cell wall breakdown of fruits and may preserve fruit

firmness. Mangoes coated with CTS+AVG stayed firm during storage (Shah & Hashmi, 2020).

Table 1. Effect of different concentration of chitosan and *Aloe vera* gel on the fruit firmness (FF), decay incidence (DI) and peel color attributes (L^*, a^*, b^*, C^* and h^o) in Mishribhog mango during 16 days' storage at 25±3°C and 80–85% RH.

Treatments	Storage Periods (days) 0	4	8	12	16	Mean (Treatments)
			FF (N)			
Control	58.80±0.46^{a}	43.81±0.11^{b}	33.91±0.10^{def}	28.42±0.11^{fg}	21.95±0.22^{h}	186.89 A
CTS	57.82±0.19^{a}	46.94±0.15^{b}	34.59±0.20^{de}	28.32±0.04^{fg}	21.75±0.36^{h}	189.42 A
AVG	55.86±0.19^{a}	42.92±0.12^{bc}	37.63±0.05^{cd}	35.08±0.00^{de}	23.22±0.50^{gh}	194.71 A
CTS+AVG	52.92±0.27^{a}	44.10±0.05^{b}	41.65±0.06^{bc}	29.79±0.17^{ef}	28.61±0.34^{fg}	197.07 A
Mean (Storage periods)	225.40 A	177.77 B	147.78 C	121.61 D	95.53 E	
			DI (%)			
Control	0.00±0.00^{j}	0.00±0.00^{j}	9.64±0.31^{h}	21.31±0.13^{b}	27.64±0.27^{a}	11.72 A
CTS	0.00±0.00^{j}	0.00±0.00^{j}	0.00±0.00^{j}	13.31±0.13^{f}	17.31±0.13^{d}	6.12 C
AVG	0.00±0.00^{j}	0.00±0.00^{j}	5.31±0.13^{i}	14.64±0.31^{e}	18.31±0.13^{c}	7.65 B
CTS+AVG	0.00±0.00^{j}	0.00±0.00^{j}	0.00±0.00^{j}	12.47±0.31^{g}	13.14±0.03^{f}	5.12 D
Mean (Storage periods)	0.00 D	0.00 D	3.73 C	15.43 B	19.10 A	
			L^*			
Control	62.85±3.61^{bc}	53.24±0.52^{gh}	44.78±2.13^{j}	41.21±0.91^{kl}	37.87±0.55^{m}	47.99 C
CTS	61.28±2.99^{cd}	57.30±1.55^{ef}	50.83±1.14^{hi}	43.38±2.32^{jk}	40.78±1.34^{klm}	50.70 B
AVG	59.61±2.60^{de}	55.63±2.34^{fg}	49.82±1.15^{i}	41.72±1.84^{kl}	40.39±1.53^{lm}	49.43 C
CTS+AVG	68.97±2.16^{a}	65.26±2.96^{b}	60.81±1.93^{cd}	59.27±1.57^{de}	54.94±2.52^{fg}	61.85 A
Mean (Storage periods)	63.18 A	57.86 B	51.56 C	46.39 D	43.48 E	
			a^*			
Control	-6.62±0.84^{h}	-3.90±0.03^{f}	-1.63±0.18^{bc}	-0.28±0.14^{a}	-0.19±0.04^{a}	-2.52A
CTS	-6.62±0.83^{h}	-5.61±0.19^{g}	-3.35±0.32^{ef}	-0.97±0.06^{ab}	-1.14±0.07^{ab}	-3.54 B
AVG	-6.62±0.83^{h}	-3.95±0.17^{fghi}	-2.81±0.23^{defg}	-0.28±0.10^{ab}	-0.62±0.14^{a}	-2.96 A
CTS+AVG	-4.59±0.83^{ghij}	-3.94±0.04^{f}	-2.81±0.57^{de}	-0.83±0.04^{ab}	-0.62±0.17^{a}	-4.72 C
Mean (Storage periods)	-6.62 D	-4.86 C	-3.21 B	-1.47 A	-1.03 A	
			b^*			
Control	25.40±0.56^{gh}	29.09±0.57^{f}	28.83±1.00^{f}	24.43±1.50^{hi}	22.80±0.30^{i}	26.11 D
CTS	23.55±1.39^{hi}	23.01±0.26^{hi}	41.70±1.45^{b}	30.00±0.46^{ef}	28.33±0.59^{f}	29.32 C
AVG	24.47±0.21^{hi}	27.68±0.06^{fg}	37.54±0.36^{c}	33.14±1.43^{d}	31.81±0.16^{de}	30.92 B
CTS+AVG	23.55±1.39^{hi}	29.51±0.27^{ef}	45.75±0.33^{a}	36.03±0.46^{c}	33.36±0.97^{d}	33.64 A
Mean (Storage periods)	22.24 E	27.32 D	38.46 A	30.90 B	29.08 C	
			C^*			
Control	25.40±0.60^{gh}	29.09±0.56^{f}	28.83±1.00^{f}	24.43±0.30^{hi}	22.80±0.30^{i}	26.08 D
CTS	23.55±1.10^{hi}	23.01±0.28^{hi}	41.70±1.42^{b}	30.00±0.02^{ef}	28.33±0.59^{f}	29.59 C
AVG	24.47±0.02^{hi}	27.68±0.04^{fg}	37.54±0.35^{c}	33.14±0.14^{d}	31.81±0.15^{de}	30.92 B
CTS+AVG	23.55±1.10^{hi}	29.51±0.27^{ef}	45.75±0.33^{a}	36.03±0.02^{c}	33.36±0.98^{d}	33.97 A
Mean (Storage periods)	25.17 E	27.78 D	38.60 A	30.05 B	29.10 C	
			h^o			
Control	75.41±1.76^{a}	82.35±0.21^{c}	86.77±0.24^{efgh}	89.59±0.10^{i}	89.51±0.09^{hi}	84.72 B
CTS	74.04±2.86^{h}	76.28±0.40^{g}	85.36±0.62^{de}	88.26±0.09^{fghi}	87.68±0.15^{efghi}	82.32 A
AVG	74.86±1.99^{a}	81.88±0.37^{c}	85.71±0.39^{def}	88.52±0.17^{ghi}	88.87±0.26^{ghi}	83.97 B
CTS+AVG	74.04±2.86^{a}	78.52±0.05^{b}	83.72±0.71^{cd}	83.79±0.05^{cd}	56.30±0.22^{defg}	81.27 A
Mean (Storage periods)	74.59 A	79.76 B	85.39 C	87.54 D	88.09 D	

The mean followed by the same letter (s), is not statistically different within the columns or rows (LSD test, P ≤0.05). n=3 replications, ±SE. Control: distilled water, AVG: *Aloe vera* gel, CTS: 1.5% chitosan solution, CTS+AVG: 1.5% chitosan solution + *Aloe vera* gel.

Throughout storage, fruits covered with various coatings degraded less compared to control group. For the control and *Aloe vera* gel-treated mango fruits, the decay incidence began at 8 days of storage while chitosan alone or combination treated fruits decay incidence began at 12 days, and then increased gradually until 16 days of storing. Nonetheless, after storage, CTS+AVG coated fruits exhibited a lower mean decay incidence of 5.12% as opposed to 11.72% for the control, 6.12% for CTS, and 7.65% for AVG (Table 1). *A. vera* gel-covered hog plums resist microbial invasion and prevent fruit destruction. Nourozi and Sayyari (2020), found that AVG coatings improve storability and reduce microbial deterioration in apricots, preventing fruit decay. This study demonstrated that CTS or CTS+AVG prevented mango fruit storage deterioration.

According to the Table 1, peel brightness (L^*) showed a significant declining trend throughout storage. End of storage, fruits in the control had the lowest L^* value (32.55) out of all the treatments. Conversely, the treated fruits with CTS and AVG had lower L^* values (38.36 and 36.93), but the fruits treated with CTS+AVG coating had a higher L^* value (40.07). After the storage, the fruits coated with CTS+AVG exhibited a minor a^* value (-2.16) compared to the other treatments (-0.19, -0.62 and -1.14). The value of b^* increased during the storage period beginning on first day and reaching its maximum on the eighth day, in case of all treatments applied and then started to decline. However, fruits treated with CTS+AVG treatments had the highest b^* value (33.36) when compared to CTS (31.81), AVG (28.34), and control (22.8). The chroma value reveals 8^{th} day of storage, the color intensity was enhanced. CTS+AVG treatment had more chroma value (33.36) at the sixteenth day of storage. Following the same trend as the b* value, there are four possible angle of hue values: red (0° to 360°), yellow (90°), green (180°) and blue (270°). After initially increasing, the hue angle gradually decreased as storage progressed. The hue angle of the CTS+AVG fruits was much lower (56.30) than other treatments. As carotenoids, anthocyanins, and xanthophyll deteriorated from chlorophyll, mango turned yellow or reddish yellow after storage. The findings of the present study are consistent with the previous results by Seyed et al. (2021), who reported that the *Aloe vera* gel with chitosan coating decreased mango fruit color changes during storage, unlike the control. According to Lo'ay and Taher (2018), coating fruits prolongs their shelf life and makes them greener by preventing chlorophyll breakdown, limiting gas exchange, and lowering respiration.

Ascorbic acid (AA), Total soluble solids (TSS), Titratable acidity (TA) and pH

The AA content in Figure 2a diminished as the storage days increased, and at the conclusion of the storage, the CTS + AVG treated fruits had an advanced ascorbic acid content (14.52 mg/100g) than the other treatments (10.84, 10.71 and 11.08 mg/100g, respectively). Fruit loses ascorbic acid, a vitamin-like characteristic, as storage days' escalation. In this work, chitosan and *Aloe vera's* ability to restrict carbon dioxide and oxygen permeability on fruit surfaces was linked to its ability to reduce ascorbic acid losses. Khatri et al. (2020) found that chitosan and *Aloe vera* gel preserved tomato fruit ascorbic acid during storage.

In the Figure 2b an increasing trend was detected in case of TSS. Compared to other treatments, fruits coated with CTS+AVG had the least TSS value (17.73%), while the control had the most TSS value (22.23%). Moisture loss, starch degradation into simple sugar, and cell wall polysaccharide hydrolysis increased TSS substantially during storage (Seyed et al., 2021). Coating closes stomata and inhibits respiration, decreasing gaseous exchange, metabolic activity, and TSS content (Chavan et al., 2023). Our findings are consistent with Sree et al. (2022), who reported that CTS and AVG delayed the increase of tomato TSS during storage.

Figure 2c shows that the mean acidity 0.60%, 0.61% and 0.61% were observed in control, CTS and AVG treatments while the highest mean acidity (0.66%) was observed in case of CTS+AVG treated fruits after 16 days of storing. It was shown that acidity depends critically on the interactions between storage times and treatments. Treatment-related TA remained high. Previous studies found that CTS+AVG coatings boosted mango TA (Begum et al., 2023) supporting our findings.

The treated fruits showed considerable pH changes during storage. CTS+AVG coated fruits had the lowest pH compared to control (5.86; 7.40). On the other hand, the value of pH in case of CTS and AVG were 6.90 and 6.78 (Fig. 2d). The pH value may increase during storage because organic acid works as a component of the respiration process during fruit maturity or ripening. According to our research, CTS+AVG-coated fruit has the lowest pH. Amin et al. (2021) found that CTS+ AVG lowers mango pH, supporting our findings.

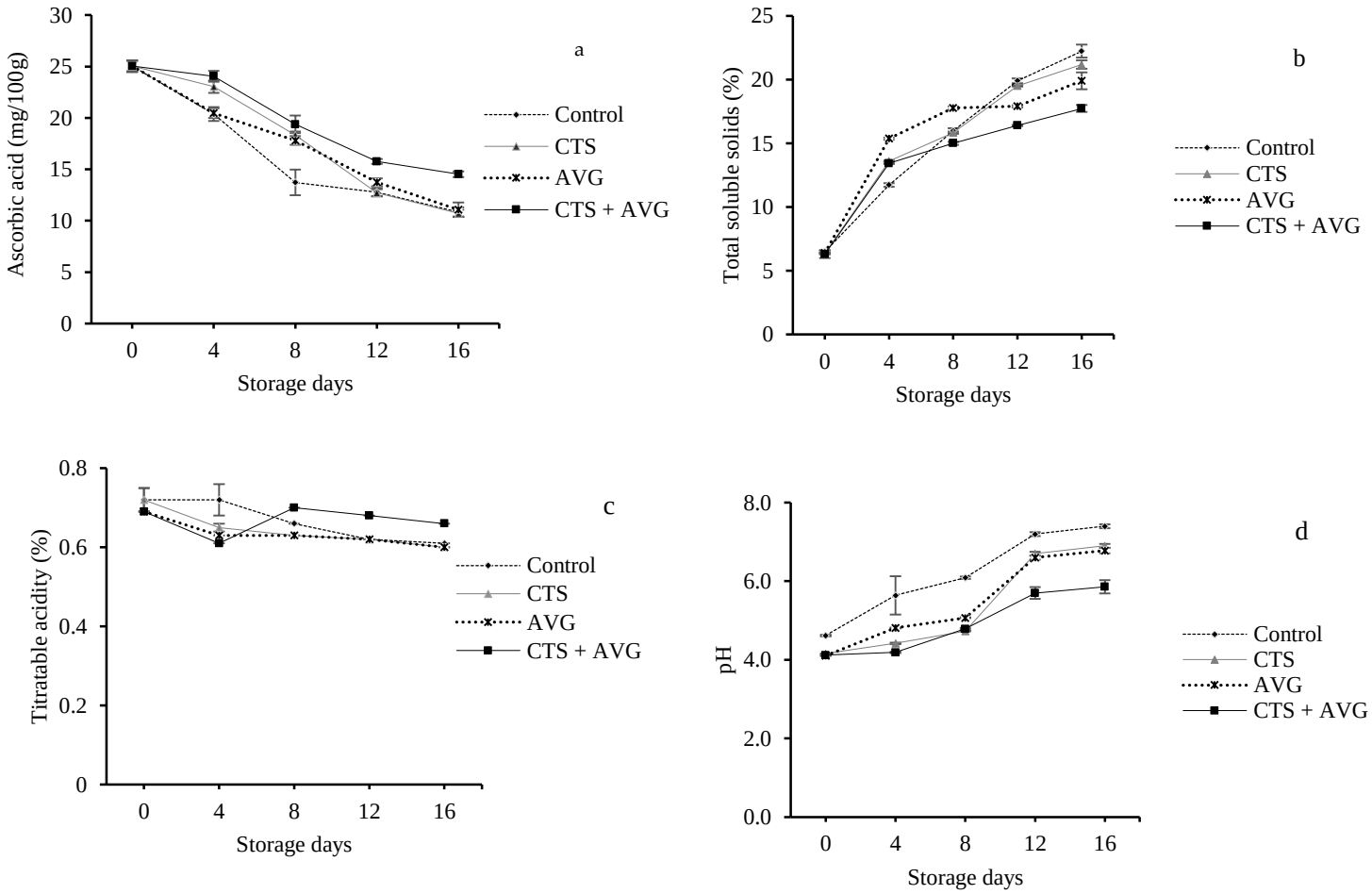

Fig. 2. Effect of different concentration of chitosan and *Aloe vera* gel on the ascorbic acid (a), total soluble solids (b), titratable acidity (c) and pH (d) in Mishribhog mango during 16 days' storage at 25±3°C and 80–85% RH.
Note: See Fig. 1.

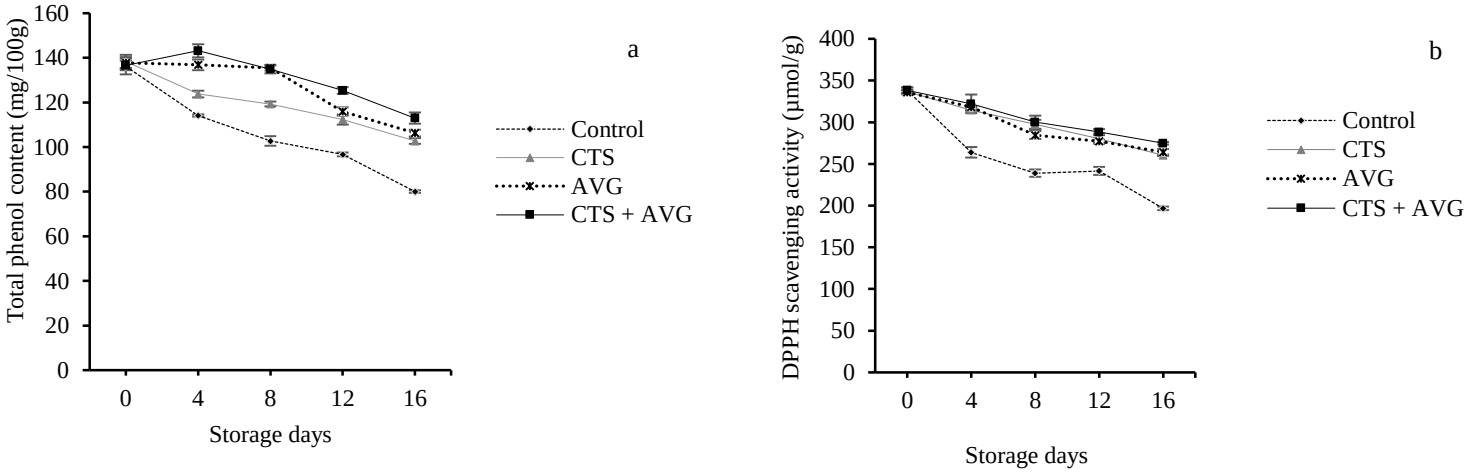

Fig. 3. Effect of different concentration of chitosan and *Aloe vera* gel on the total phenolic content (a) and DPPH scavenging activity (b) in Mishribhog mango during 16 days' storage at 25±3°C and 80–85% RH. Note: See Fig. 1.

Total phenolic content and DPPH scavenging activity

For every treatment, there was a discernible drop in the total phenolic concentration as the storage days rose (Fig. 3a). The fruits coated with CTS+AVG had the most total phenolic content of 112.99 mg/100g, whereas the lowest phenol level of 80.02 mg/100g was substantially detected in the control group. Mango phenolic compounds decreased with storage (Khaliq et al., 2019). PAL enzyme activity may increase phenolic chemical synthesis. Romanazzi et al. (2002) reported that chitosan treatment increased the activity of the grape fruit PAL enzyme, which synthesizes phenolic compounds. The current investigation found that chitosan and *Aloe vera* gel coatings improve mango phenolic retention over time, like Seyed et al. (2021).

In Figure 3b, the DPPH activity was decreased more quickly in the fruits of control compared to the fruits coated with CTS, AVG and CTS+AVG. While the fruits coated with CTS+AVG had higher DPPH scavenging activity (274.86 µmol/g) than the fruits of control (196.65 µmol/g) after the storage. All treated and control fruits lost DPPH scavenging activity during storage, and controls shriveled faster. At ripening and storage, fruits and vegetables naturally produce more reactive oxygen species (ROS), which induces oxidative stress and reduces antioxidant capacity (Rabeh et al., 2021). Coatings prevent fruit oxidation and antioxidant depletion by reducing gas permeability (Rabeh et al., 2021). Shah and Hashmi (2020) claimed that postharvest *Aloe vera* and chitosan-treated mangoes increased DPPH scavenging.

Enzyme activity

Activity of PPO was raised dramatically with the increase of storage period. As demonstrated in the figure, the fruits coated with CTS+AVG showed the lowest value of PPO activity (5.49 U/mg FW). Conversely, fruits covered with AVG and CTS also showed lower PPO activity 5.72 U/mg FW and 5.61 U/mg FW contrary to control 7.87 U/mg FW (Fig. 4a). POD (Peroxidase) activity was increased in all coated fruits. The lower POD activity was noticed in fruits of control (0.70 U/mg FW) while the coated fruits showed higher POD activity. However, AVG-coated fruits had the least POD activity (0.70 U/mg FW) after storage. Fruits treated with CTS+AVG had maximum POD activity (0.75 U/mg FW) (Fig. 4b). Increasing of

storage period shows increased CAT activity. Compared to CTS, AVG, and control (0.54, 0.54 and 0.45 U/mg FW), CTS+AVG had higher CAT activity (0.54 U/mg FW) (Fig. 4c). During ripening, genes encoding fruit antioxidant system enzymes like PPO, POD, and CAT increased, and endogenous defenses prevented ROS formation (Lo'ay & EL-Ezz, 2021). According to Adiletta et al. (2019), coatings reduced PPO activity, activated defense-related enzymes, inhibited mango browning, and extended storage. Fruit surface chitosan coating may have reduced PPO activity and enzymatic browning (Rehman et al., 2022). Edible coatings lowered PPO activity, possibly activating defense enzymes and protecting fruit. According to Zheng et al. (2024), adding CTS to sweet cherries inhibited PPO activity to varying degrees. Shah and Hashmi (2020) found that mango chitosan coating increases CAT activity by removing O_2 and H_2O. The fruits contain POD, a unique oxyradical detoxifying enzyme that reduces oxidation damage (Xing et al., 2015). Seyed et al. (2021) found that chitosan with *Aloe vera* gel preserves fruit quality and increases CAT with POD while lowering PPO activity.

The study indicated that CTS+AVG-coated fruit stored longest (16 days) and lost the least weight. However, AVG and CTS fruits lasted 16 days. Controlled fruits lasted 12 days. Chitosan coating mango fruits may improve their shelf life and maintain nutritional concentration during room temperature storage, according to Begum et al. (2023).

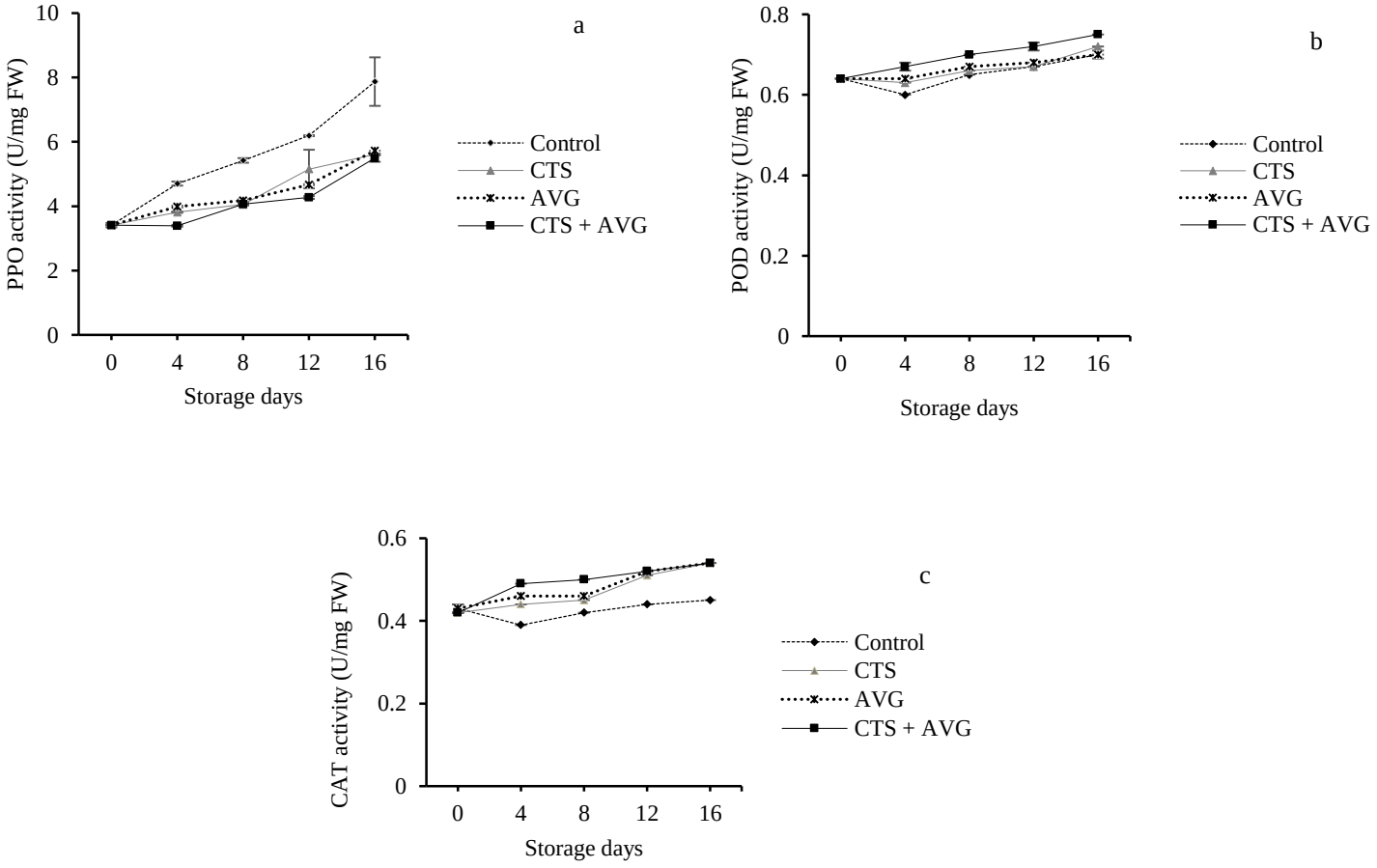

Fig. 4. Effect of different concentration of chitosan and *Aloe vera* gel on the polyphenol oxidase (PPO) (a), peroxidase (POD) (b) and catalase (CAT) (c) in Mishribhog mango during 16 days' storage at 25±3°C and 80–85% RH. Note: See Fig. 1.

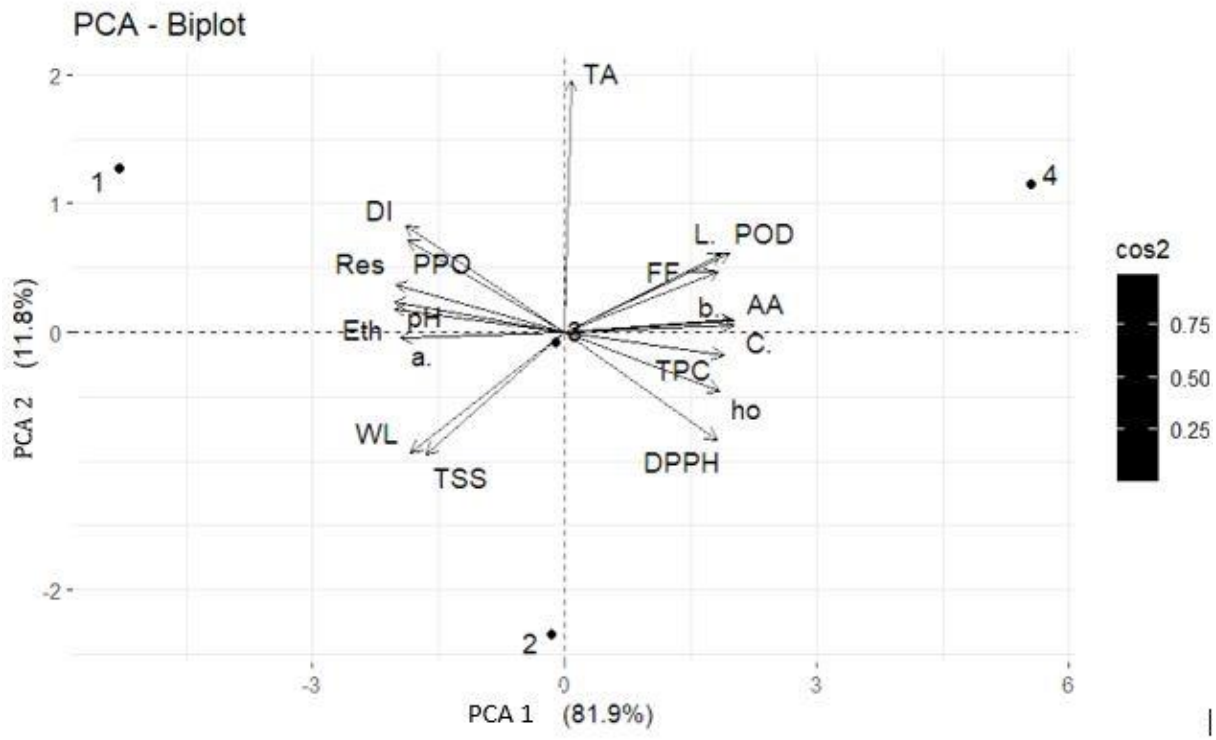

Fig. 5. Principal component loading plot of physiochemical and antioxidant enzymes activities of Mishribhog mango during storage. Here, DPPH: DPPH scavenging activity, ho: hue, TPC: Total phenol content, C: Chroma, AA: Ascorbic acid, b: b* (yellowness), FF: Fruit firmness, L: L* (Lightness), POD: Peroxidase, TA: Titratable acidity, DI: Decay incidence, PPO: Polyphenol oxidase, Res: Respiration rate, Eth: Ethylene production, a: a* (greenness to redness), WL: Weight loss, TSS: Total soluble solids.

Principal Component Analysis (PCA)

To evaluate mango fruit quality following harvest with various treatments, PCA was utilized to examine a number of biochemical limitations and antioxidant enzymes (Fig. 5). Two primary components, PC1 and PC2, represented 93.7% of variance. In the dataset, PC1 represented 82.5% of variations, whereas PC2 explained 11.2%. The antioxidant activity, color, CAT, total phenolics, chroma, ascorbic acid, firmness of fruit, POD enzymes, L*, and b* values were all found to positively correlate with PC1. Decay incidence, PPO enzyme, respiration, ethylene, pH, peel's a* value, weight loss, and TSS negatively linked with PC1. PC2 correlated negatively with TSS and antioxidant activity, while only TA showed a strong positive correlation. AVG and CTS treatments showed a substantial link with DPPH, hue, TSS, and weight loss, while CTS+AVG showed a strong correlation with L* values, POD, and hardness of fruit. Contrary to this, the control was more strongly associated with respiration, PPO, and decay.

CONCLUSION

The chitosan and *Aloe vera* coating reduced weight loss and TSS changes, improving "Mishribhog" mango fruit postharvest value and shelf life. Chitosan-infused *Aloe vera* gel preserves fruit quality such skin color, firmness, titratable acidity, and ascorbic acid after 16 days at room temperature. The coating decreases PPO and increases CAT and POD activity during storage. Compared to uncoated control, *Aloe vera* and chitosan increase bioactive substances such total phenolic and antioxidant activity. Chitosan and *Aloe vera* coatings also

greatly inhibit ethylene production and respiration, delaying fruit ripening. Given health concerns, edible coverings like chitosan and *Aloe vera* may improve mango storage quality. To commercialize mango fruit and increase storability using edible coatings, more research is required.

Conflict of interest

It is declared by the authors that there is no conflict of interest.

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Influence of pre-harvest methyl jasmonate application on the fruit quality of strawberry cv. Paros at harvest and during cold storage

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ABSTRACT

Purpose: Strawberry fruits are perishable with a short shelf life. Methyl jasmonate (MJ) is a well-known signaling molecule involved in the regulation of many processes in plants. Previous studies have addressed the effectiveness of pre- and postharvest MJ treatments on the quality of fruits. Therefore, in this study, the effect of pre-harvest MJ on the quality and physio-chemical traits of strawberry at harvest and during cold storage were investigated. **Research Method:** The effect of pre-harvest foliar spraying of MJ (0, 50, 100, and 200 µM) at three times (full flowering, production of green fruits, and the beginning of the pink stage) on quality and bioactive compounds of strawberry fruit (cv. Paros) stored at 4 °C for 12 d was studied. **Findings:** Our results showed that fruit obtained from MJ-treated plants had significantly higher firmness and APX enzyme activity than fruit from control plants. However, no significant differences in other traits were observed between control and MJ-treated fruits at this stage. During cold storage, MJ treatment significantly reduced firmness loss, physiological loss in weight, and ascorbic acid content compared to control fruits. In cold storage, MJ treatment reduced firmness loss and weight loss, while maintaining higher levels of titratable acidity, antioxidant compounds (ascorbic acid, anthocyanins, and phenolics), and total antioxidant activity. Furthermore, MJ treatment resulted in increased catalase and ascorbate peroxidase enzyme activity, as well as lowered guaiacol-peroxidase, total soluble solids/ titratable acidity ratio. Total soluble solids were not affected by MJ treatment at harvest and during cold storage. **Research limitations:** No limitations were found. **Originality/Value:** Pre-harvest application of MJ, especially 200 µM, can increase the shelf life of strawberry fruits by increasing or maintaining higher levels of bioactive compounds and antioxidant enzyme activity.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most popular fruits belonging to the Rosaceae family. It is cultivated and consumed worldwide due to its special taste, attractive color, distinctive sweetness, and nutritional value, including minerals, vitamins, phenolics, anthocyanins, amino acids, and antioxidant properties (Nguyen & Nguyen, 2021; Asgari et al., 2024; Xu et al., 2024). These bioactive compounds can reduce the risk of chronic diseases such as heart disease, type II diabetes, obesity, cancer, and neurodegenerative diseases (Darwish et al., 2021). However, strawberry is classified as a non-climacteric fruit, so they do not ripen after harvest and should be harvested at the nearly full maturity stage (Nguyen & Nguyen, 2021; Meighani & Roozkhosh, 2024). In addition, the shelf life of strawberry fruit is very limited due to its susceptibility to mechanical injury, physiological deterioration, water loss, and microbial decay (Saavedra et al., 2017, Adl et al., 2024). The shelf life of strawberry fruit is affected by various factors, such as growing conditions, harvest stage, pre- and postharvest treatments, transportation method, storage conditions, and others (Xu et al., 2024). Quality losses after harvest can be reduced by the pre and post-harvest treatments. Pre-harvest treatments not only improve fruit quality but also have positive effects on extending shelf life and preserving bioactive compounds of fruits and vegetables (Ağlar & Öztürk, 2018; Darwish et al., 2021). Many efforts have been made to improve the quality and extend the shelf life of fruits using natural compounds. One of them is the application of plant growth regulators before and after harvest.

Methyl jasmonate (MJ), as an endogenous plant hormone from the jasmonate family, plays an important role in various biological processes in plants, including growth and development, fruit growth and ripening, pigment accumulation, ethylene synthesis, disease resistance, and environmental stress (Han et al., 2019; Zuñiga et al., 2020; Asgari et al., 2024). Recent studies have shown the positive effect of pre-harvest application of MJ on the postharvest behavior of fruit, including raspberry (Shah et al., 2025), strawberry (Darwish et al., 2021), and pomegranate (García-Pastor et al., 2019). Based on previous reports, Pre-harvest application of MJ increased the biosynthesis of phytochemicals such as phenolics, flavonoids, anthocyanins, carotenoids, enzymatic and non-enzymatic antioxidants, as well as essential nutrients (Hasan et al., 2025) and activities of key enzymes involved in flavonoid biosynthesis (Zheng et al., 2024; Shah et al., 2025). Also, MJ can enhance the ripening of non-climacteric fruits by participating in anthocyanin accumulation, cell wall modification, and the biosynthesis of ethylene and jasmonates (Concha et al., 2013). In addition, García-Pastor et al. (2020) observed that pre-harvest MJ treatments increased crop yield, fruit quality, and its content of bioactive compounds at harvest and during storage. Furthermore, it has been reported that pre-harvest application of MeJA significantly preserved the volatile aroma and improved the activities of disease-resistant enzymes in kiwifruit (Yang et al., 2025).

Our idea is that the pre-harvest spraying of MJ can promote the quality of strawberry fruits at harvest and during refrigerated storage. Therefore, this study aimed to assess the effectiveness of MJ pre-harvest spraying on fruit quality attributes, bioactive compounds, decay incidence, and antioxidant enzyme activities of strawberry fruits (cv. Paros) during refrigerated storage.

MATERIALS AND METHODS

Plant material and treatment

Strawberry (*Fragaria*× *ananassa*, cv. 'Paros') transplants were cultivated in October 2023 in a plastic greenhouse located in Jiroft, southern Kerman Province, Iran. The equal-sized, healthy,

and disease-free transplants were planted over cultivation beds covered with black plastic mulch at 25 × 25 cm space between plants in a triangular planting pattern. The experimental soil was sandy-loam and irrigation was done through a drip tape system under plastic mulch. The experiment was conducted as a randomized complete block design, with four treatments and three independent replicates. About 150 strawberry plants of the 'Paros' cultivar was considered for each block.

Treatments included different concentrations of MJ (0, 50, 100, and 200 µM, Sigma–Aldrich, Germany). Distilled water was used as a control. In all treatments, 0.05% (w/v) Tween-20 was used as a surfactant. Strawberry plants were fully sprayed three times (10-day intervals) at different developmental stages: full flowering, production of green fruits, and the beginning of the pink stage (Darwish et al., 2021).

Strawberry fruits in each treatment were harvested at the 80% red color stage and immediately transported to the postharvest laboratory. Then, 40 fruits with uniform color and size, without any visual defect were randomly separated from each block and treatment, packed in polystyrene boxes with lids (750 ml, 10 fruits per box in one row), and stored at 4˚C and 90% relative humidity for 12 days. One box containing ten fruits for each block and treatment was randomly sampled after 0 (at harvest), 4, 8, and 12 days of cold storage for measurement of physio-chemical attributes and antioxidant enzyme activity.

Fruit firmness and physiological loss in weight

Fruit firmness was determined using a texture analyzer (Santam, STM-5, Iran) equipped with a flat probe (8 mm) at a constant speed of 20 mm $min^{-1}$and a penetration depth of 10 mm. The results were expressed in Newton (N) units.

For physiological loss in weight (PLW), strawberry fruits were weighed at the beginning of the experiment, after treatment, and at the end of each sampling time. The percentage of PLW was calculated using the following equation (1):

$$PLW\ (\%) = (Wi - Wf) \times 100/\ Wi \qquad (1)$$

Where Wi is the initial weight and Wf is the weight at each sampling time.

Total soluble solids (TSS), titratable acidity, and TSS/TA

Analysis of TSS, TA, and TSS/TA ratio were performed according to Saavedra et al. (2016). TSS of strawberry juice was measured at 25˚C with a hand-held digital refractometer (PDR-108-1, Taiwan) and the results were expressed as Brix degrees (˚Brix). To determine the TA, strawberry juice was diluted with distilled water (1:10 v/v), and then titrated with 0.1 N NaOH to pH 8.2. Results were calculated and expressed as the percentage of citric acid per 100 g of fresh weight (AOAC, 2000). The TSS/TA ratio was calculated by dividing the TSS value by the TA value.

Ascorbic acid content

Ascorbic acid content was determined based on the 2, 6- dichloroindophenol titration method. The amount of ascorbic acid in the strawberry fruits was expressed as mg per 100 g FW (AOAC, 2000).

Total phenolic content and total antioxidant activity

For the determination of total phenolic content (TPC) and total antioxidant activity (TAA), 2 g strawberry fruit tissue without achene was well homogenized with 10 mL of methanol (80%)

and centrifuged at 14000 rpm at 4°C for 10 min. The supernatant was used for TPC and TAA analysis.

TPC was measured using the Folin–Ciocalteau reagent according to the method of Singleton & Rossi (1965). About 250 µL of the extract was mixed with 1250 µL of 10% Folin-Ciocalteau reagent and 1000 µL of 7.5% Na_2CO_3. The samples were incubated at room temperature in darkness for 60 min, and then the absorbance of the reaction mixture was measured at 765 nm using a UV/VIS spectrometer (Lambda 25, PerkinElmer, USA). Gallic acid was used as an external standard and the results were expressed as mg gallic acid equivalents per 100 g fresh weight (mg GAE 100 g^{-1} FW).

Total antioxidant activity (TAA) was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method as outlined in Brand-Williams et al. (1995). DPPH methanolic solution (950 µL) was mixed with 50 µL of extract and kept in the dark at ambient temperature for 30 min. The solution absorbance was measured at 517 nm, and the TAA was calculated as the following equation (2):

$$TAA\ (\%) = [(Ac - As)/Ac] \times 100 \qquad (2)$$

Where 'Ac' is the absorbance of the DPPH and 'As' is the absorbance of the sample.

Total anthocyanin content

Total anthocyanin content (TAC) was determined using the pH differential method described by Darwish et al. (2021) with some modifications. Fruit puree without achenes (2 g) was homogenized in 10 mL of methanol/HCl (85/15%, v/v) and centrifuged at 14000 rpm at 4°C for 10 min. The extract was diluted in pH_1 and pH_7 solution buffers and after 30 min incubation in ambient temperature, the absorbance was measured at 510 and 700 nm. TAC was calculated as mg cyanidin 3-glucoside equivalent per kg of fresh fruit.

Antioxidant enzymes

Preparation of enzyme extract

Strawberry tissue (1 g) was homogenized in 2 mL potassium phosphate buffer (50 mM, pH, 7.0, containing 0.5 mM EDTA and 60 g/L PVPP). The homogenate was centrifuged at 14000 rpm for 15 min, and the supernatant was used as the crude extract to measure the activity of CAT and SOD enzymes (Li et al., 2023).

Catalase (CAT, EC 1.11.1.6)

CAT activity was assayed using the protocol of Li et al. (2023). Briefly, 100 µL enzyme extract was reacted with 100 µL H_2O_2 (20 mM). The absorbance of the reaction mixture was determined at 240 nm and expressed as U/mg protein. One unit of CAT activity was defined as an absorbance change of 0.01 U/min, and the CAT activity was expressed as U/mg protein.

Guaiacol peroxidase (GPX, EC 1.11.1.7)

The reaction mixture for the GPX assay consisted of 0.7 mL of phosphate buffer (50 mM, pH 5.0), 0.1 mL hydrogen peroxide (40 mM), 0.1 mL guaiacol (20 mM), and 0.1 mL enzyme extract. The absorbance of the reaction mixture was measured at 470 nm and expressed as U/mg protein. The change absorbance in 0.01 U/min is defined as one unit of GPX activity (Ali et al., 2021).

Ascorbate peroxidase (APX, EC 1.11.1.11)

APX activity was determined according to Ali et al. (2021). The reaction mixture was prepared by mixing 0.1 mL L-ascorbate (0.5 mM), 0.1 mL hydrogen peroxide (0.1 mM), 0.2 mL

phosphate buffer (50 mM, pH 5.0), and 0.1 mL enzyme extract. The absorbance of the reaction mixture was determined at 290 nm and expressed as U/mg protein. One unit of APX is defined as the amount of enzyme that can oxidize 1 µmol of ascorbate per minute.

Statistical design

The postharvest study was performed using a factorial experiment with a randomized block design, with the main factors being the MJ treatment (control, 50, 100. and 200 mM) and storage time (0, 4, 8, and 12 days). The data were analyzed with two-way analysis of variance (ANOVA) using the methods of Statistical Analysis System (SAS, version 9.1). Least significance difference (LSD) was used to compare means between applied treatments at a 5% level of probability. The values of the results were presented as means (n=3) ± SE. The graphs were prepared with the Microsoft Excel program.

RESULTS AND DISCUSSION

Firmness and physiological loss in weight (PLW)

The effect of MJ foliar spraying on the firmness and PLW of strawberry fruits are represented in Table 1. The pre-harvest treatments significantly affected firmness values ($p<0.05$). At harvest (0 d), fruits treated with MJ obtained significantly higher firmness than the untreated fruits (control). Throughout the storage, strawberry fruits regardless of treatment showed continuous declines in firmness, with a slower softening in MJ-treated fruits. At the end of the storage, the lowest and highest firmness as obtained from control (4.84 N) and 100 µM MJ treatment (5.71 N), respectively. Although, no significant difference in firmness was found between 100 and 200 µM MJ treatment throughout the study (Fig. 1A). Firmness is an important quality parameter for fresh produce. Fruit softening is a crucial characteristic of ripening in many fleshy fruits. The main factors contributing to fruit softening are cell wall disassembly and reduced cell-to-cell adhesion, primarily due to the degradation of the middle lamella. In strawberry fruit, cell wall disassembly involves solubilization of pectins, slight depolymerization of bound pectins, loss of galactose and arabinose, and reduced hemicellulosic content, which contributes to the fruit softening (Moya-León et al., 2019). Pre-harvest MJ application has been reported to increase the firmness of lemon (Serna-Escolano et al., 2019) and Fuji apple (Ağlar & Öztürk, 2018) at harvest and also maintain firmness during storage in raspberries (Shah et al., 2025) and papaya (Li et al., 2023). Several enzymes are involved in the softening of strawberry fruit. Shah et al. (2025) demonstrated that MJ application in raspberry fruit reduces the activity of cell wall-hydrolyzing enzymes, including pectin methylesterase (PME), polygalacturonase (PG), and cellulase (Cx), leading to less cell wall degradation and potentially increased fruit firmness. Furthermore, Wu et al. (2025) reported that MJ treatment can slow down the softening of blackberry fruits by increasing antioxidant contents and antioxidant enzyme activity, which is similar to the results of this study.

As illustrated in Fig. 1B, PLW in control and MJ-treated fruits showed a similar pattern of changes that involved its gradual increase with storage time. Up to 4 days of storage, no significant difference in PLW was observed between control and MJ-treated fruits, later on, MJ-treated fruits were observed significantly lower PLW compared to control fruits. At the end of storage, strawberry fruits treated with 100 µM MJ exhibited the lowest PLW (1.30%), which was significantly lower than that of the control but not that of the 50 and 200 µM MJ-treated fruits. In general, PLW during the storage of fruits is a result of increased transpiration and respiration rates (Ilea et al., 2025). The large cells and thin cell walls in the epidermis of strawberry fruits make it susceptible to moisture loss. García-Pastor et al. (2019) reported that pre-harvest application of MJ significantly reduced respiration rate in pomegranate fruits at

harvest and during postharvest storage. Also, a previous study indicated that MJ (methyl jasmonate) can delay decay and PLW in raspberry fruits by activating an antioxidant defense mechanism against free radicals and retarding membrane peroxidation (Shah et al., 2025). Therefore, the positive effect observed in MJ-treated strawberry fruit could be attributed to a combination of factors including a slower rate of respiration and transpiration, as well as its ability to enhance antioxidant levels and protect cell membranes from oxidative damage.

Table 1. Effect of methyl jasmonate pre-harvest application on some characteristics of strawberry fruit cv. Paros during 12 days of storage at 4°C.

Source of variation	Firmness (N)	Physiological loss in weight (%)	TSS (˚Brix)	TA (mg 100 g^{-1}FW)	TSS/TA	AsA (mg 100 g^{-1}FW)
Methyl Jasmonate (T)	**	**	ns	**	**	**
Storage time (ST)	**	**	**	**	**	**
T×ST	**	**	ns	ns	**	**
Storage Time (day)						
0 (harvest)	7.51±0.13 a	0.00±0.00 d	6.93±0.06 c	1.18±0.05 a	5.88±0.24 d	69.83±1.60 a
4	7.09±0.13 b	0.74±0.05 c	7.00±0.08 bc	1.11±0.05 b	6.37±0.31 c	64.30±1.25 b
8	6.55±0.26 c	0.92±0.10 b	7.06±0.06 b	0.95±0.06 c	7.53±0.52 b	58.49±2.47 c
12	5.43±0.22 d	1.72±0.37 a	7.21±0.14 a	0.81±0.06 d	9.08±0.82 a	50.50±2.89 d

Source of variation	TAC (mg 100 g^{-1}FW)	TPC (mg GAE 100 g^{-1}FW)	TAA (%DPPHsc)	CAT (U g^{-1} FW min^{-1})	GPX (U g^{-1} FW min^{-1})	APX (U g^{-1} FW min^{-1})
Methyl Jasmonate (T)	**	**	**	**	*	**
Storage time (ST)	**	**	**	**	ns	**
T×ST	ns	ns	*	*	ns	**
Storage Time (day)						
0 (harvest)	11.31±0.35 d	221.11±7.01 c	74.41±1.91 b	13.02±0.47 c	13.84±0.77 ab	41.57±1.15 b
4	12.14±0.31 c	241.50±5.12 bc	78.74±3.04 a	15.20±0.42 a	13.98±1.06 ab	50.14±2.96 a
8	12.75±0.46 b	248.79±7.67 a	80.55±2.88 a	13.91±0.59 b	14.47±1.04 a	39.12±3.22 c
12	13.14±0.50 a	238.78±9.9 b	71.79±3.35 c	13.42±0.65 c	14.09±0.72 ab	29.72±2.69 d

**, * and ns indicate significant at $P \leq 0.01$, $P \leq 0.05$ and non-significant.

Means±SE (n = 3) covered by the same letter are not significantly different ($P<0.05$).

TSS: total soluble solids, TA: titratable acidity, AsA: ascorbic acid, TAC: total anthocyanin content, TPC: total phenolic content, TAA: total antioxidant activity, CAT: catalase, GPX: guaiacol peroxidase, APX: ascorbate peroxidase.

Table 2. Effect of methyl jasmonate pre-harvest concentration on the titratable acidity, TAC, TPC, guaiacol peroxidase activity in strawberry fruit cv. Paros.

MJ concentration (µM)	Titratable acidity (mg 100 g^{-1}FW)	TAC (mg 100 g^{-1}FW)	TPC (mg GAE 100 g^{-1}FW)	GPX (U min^{-1} g^{-1} FW)
0	0.89±0.05 a	11.41±0.35 c	226.81±7.01 b	15.83±0.77 a
50	1.06±0.05 b	12.32±0.31 b	238.41±5.12 a	14.93±1.06 b
100	1.05±0.06 c	12.72±0.46 a	239.87±7.67 a	13.26±1.04 c
200	1.04±0.06 d	12.87±0.50 a	245.09±9.91 a	12.36±0.72 d

Means±SE (n = 3) within each column covered by the same letter are not significantly different ($P<0.05$). TAC: total anthocyanin content, TPC: total phenolic content, GPX: guaiacol peroxidase.

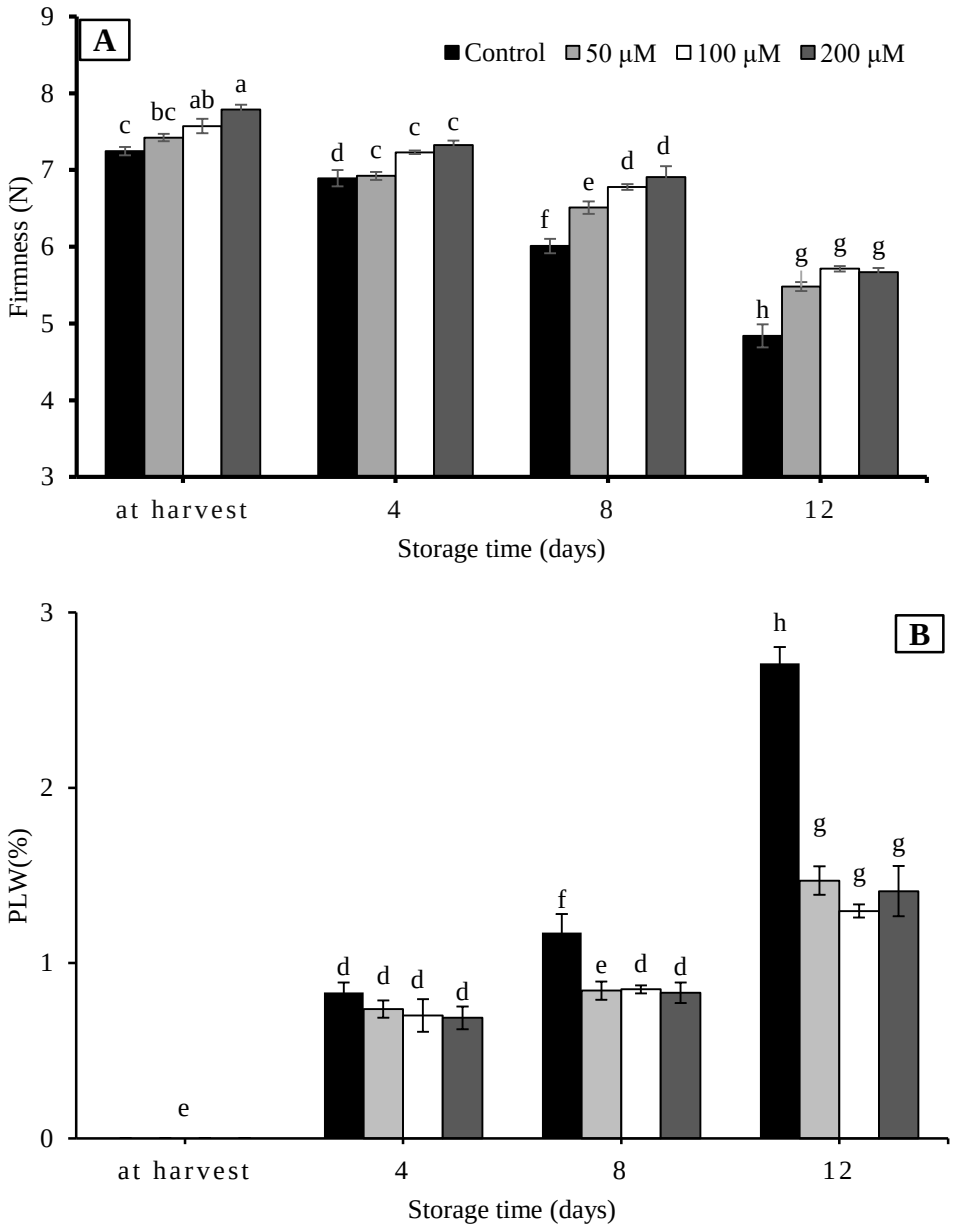

Fig. 1. Effect of methyl jasmonate (MJ) pre-harvest treatment on firmness (A) and physiological loss in weight (B, PLW) of strawberry fruit cv. Paros during 12 days of storage. Mean ± SE (n=3), followed by different letters are significantly different (P<0.05).

Total soluble solids (TSS), titratable acidity (TA), and TSS/TA ratio

The TSS and TSS/TA ratio of all treatments gradually increased throughout the storage time. In contrast, the TA values of control and MJ-treated fruits continuously decreased throughout the storage time. Storage time had a positive influence on the TSS of strawberry fruits. However, the MJ treatment and its interaction with storage time did not significantly affect TSS. The TSS value of strawberry fruit at the end of storage (7.21 ˚Brix) was significantly higher (6.93 ˚Brix) than at harvest (Table 2). The TA content of strawberry fruit was significantly affected by pre-harvest MJ treatment and storage time, but not by their interaction (Table 1). Untreated strawberry (control) exhibited significantly lower TA content (0.89 mg $100g^{-1}$ FW) than those treated with MJ, while no significant differences were observed among different MJ concentrations (Table 2). The TA content indicated a significant decline with

prolonged cold storage. The maximum and minimum TA content of strawberry fruits were recorded at harvest (1.18 mg 100 g^{-1} FW) and the end of storage (0.81 mg 100g^{-1} FW), respectively (Table 1). The TSS/TA ratio significantly increased from 5.88 to 9.08 during 12 days of cold storage (Table 1). However, at harvest and up to 8 days after storage, no significant difference was found in the TSS/TA ratio between different concentrations of MJ and the control samples. At the end of storage, control strawberry fruits showed a significantly higher TSS/TA ratio than MJ treatments, while no significant differences were observed among the different MJ concentrations (Fig. 2).

In this study, pre-harvest MJ treatment had no significant effect on TSS content, which is consistent with previous findings on lemon (Serna-Escolano et al., 2019) and blood orange (Vithana et al., 2024) fruits. On the contrary, MJ-treated raspberry fruits expressed significantly higher TSS than the control sample. TSS content in strawberry fruits increased during cold storage. The increase in TSS with the extension of storage periods has been attributed to the breakdown of complex polysaccharides and other organic acid into simple sugars and moisture loss (Wu et al., 2025). Previous studies on lemon fruits have indicated a positive correlation between PLW and TSS (Liao et al., 2022). In Our study, the TA content of all treatments gradually declined throughout the storage period, although MJ inhibited the decrease of TA in comparison with the control fruit, specifically at the end of storage, which is consistent with those obtained from raspberry (Wu et al., 2025), blueberry (Wang et al., 2019), and lemon fruits (Liao et al., 2022). The reduction in TA during cold storage could be related to a combination of factors, including metabolic changes, respiratory activity, and senescence, which these processes result in the conversion of organic acids into sugars, or the utilization of organic acids as respiratory substrates (Li et al., 2023). García-Pastor et al. (2019) reported that MJ treatment decreased TA losses during storage in pomegranate fruit by reducing respiration rate and the consumption of organic acids as respiratory substrates. In our study, the TSS/TA ratio in strawberry fruit showed an increasing trend with the extension of cold storage periods. Liao et al. (2022) reported that the TSS/TA ratio in lemon fruits increased during cold storage, and this increase was more pronounced when the fruits were treated with MJ pre- and postharvest. In general, the TSS/TA ratio is directly related to TSS and TA, and changes in these two factors affect its value. Therefore, the relative increase in TSS and decrease in TA of strawberry fruits during cold storage led to a change in the TSS/TA ratio.

Total anthocyanin content (TAC) and total phenolic content (TPC)

Significant changes and an increasing trend in the TAC of strawberry fruits were observed during the cold storage, such that the TAC at the end of storage was 16% higher than at harvest (Table 1). TAC at harvest was affected by MJ foliar spraying and was higher in MJ-treated fruits compared to the control sample. As shown in Table 2, TAC in strawberry fruits treated with MJ was higher than in the control sample. The highest TAC was obtained from 200µM MJ treatment (12.87 mg 100 g^{-1} FW) followed by 100 µM (12.72 mg 100 g^{-1} FW) and 500 µM (12.32 mg 100 g^{-1} FW) MJ treatment and the lowest from the control sample (11.41 mg 100 g^{-1} FW). Pre-harvest spraying of MJ increased the TPC of strawberry fruits at harvest. In this stage, the highest level (226.63 mg 100 g^{-1} FW) was recorded in MJ-treated fruits with 200 µM, and the lowest level (218.12 mg 100 g^{-1} FW) was found in the control sample. TPC exhibited an upward trend up to 8 days but then had a downward trend until the end of cold storage (Table 1). Strawberry fruits treated with MJ showed significantly higher TPC than control fruits. However, there was no significant difference between different MJ treatments (Table 2).

Phenolic compounds, plant secondary metabolites, play a crucial role in both enhancing the sensory and nutritional quality of fruits and inducing stress-related defense mechanisms in plants (Shah et al., 2025). Anthocyanins are a group of water-soluble pigments responsible for

the bright red color in strawberry fruits, and the predominant anthocyanins found in strawberries are pelargonidin-3-glucoside and cyanidin-3-glucoside (Wang & Li, 2000). At harvest and during postharvest storage, strawberries treated with MJ showed higher TPC and TAC than control fruits, as previously reported in raspberries (Shah et al., 2025), and 'Sabrosa' strawberry (Asghari & Hasanlooe, 2016). The increase in TPC and TAC can be attributed to the impact of MJ on phenylpropanoid metabolism. Especially, MJ can enhance the activity of key enzymes in this pathway, including phenylalanine-ammonia lyase (PAL) and shikimate dehydrogenase (SKDH), leading to increased production of phenolic compounds (Shah et al., 2025). Several studies support the idea that MJ treatment positively impacts TPC and TAC by enhancing the activity of the PAL enzyme (Cao et al., 2009; Hasan et al., 2024; Zheng et al., 2024). However, in blood orange, postharvest MJ-treated fruit pre-harvest foliar spraying

Ascorbic acid (AsA) and total antioxidant activity (TAA)

At harvest, no significant differences were observed between the control and MJ-treated fruits in AsA content. Significant variations and a downward trend in AsA content were observed in MJ-treated and control strawberry fruits during the cold storage, with this reduction being significantly less in MJ-treated fruits compared to the control. The highest (71.78 mg 100 g^{-1} FW) and lowest (42.63 mg 100 g^{-1} FW) levels of AsA were recorded from 100 mM methyl jasmonate treatment at harvest (day 0) and from the control at the end of storage, respectively (Table 3).

AsA, also known as vitamin C, is a water-soluble vitamin that decreases in levels with moisture loss and postharvest senescence (Shah et al., 2025). AsA is susceptible to oxidative and enzymatic degradation during storage, leading to the formation of dehydroascorbic acid (DHA) and eventually diketogulonic acid (DKG), which have no vitamin C activity (Hasan et al., 2025). In this study, the AsA content in strawberry fruits decreased over time in all treatments, and the MJ treatment slowed this decline compared to the control. These findings are consistent with a previous study on raspberry (Chanjirakul et al., 2006) and pomegranate (García-Pastor et al., 2019) fruits. It has been shown that pre-harvest application of MJ can increase the AsA content at harvest and maintain it during cold storage in raspberries (Shah et al., 2025) and blueberries (Wang et al., 2019) by delaying oxidation reactions and reducing moisture loss. Furthermore, Wolucka et al. (2005) reported that MJ can enhance the transcription of genes involved in the de novo biosynthesis and regeneration of AsA. Conversely, AsA levels in blood orange fruits were not significantly influenced by both pre-harvest foliar spraying and postharvest dipping treatments with MJ (Vithana et al., 2024).

MJ foliar application enhanced the TAA content of strawberry fruits. TAA, at harvest, was the highest in strawberry fruits treated by 200 µM MJ with a significant difference compared to the control. TAA in both control and MJ-treated fruits increased with storage time, reached the maximum after 8 days of storage, and then decreased sharply from days 8 to 12. At the end of storage, the highest TAA (73.33%) was observed in the fruit sprayed pre-harvest with 100 µM MJ, while the lowest TAA (60.04%) was recorded in the control samples (Table 3).

The antioxidant activity of plant tissues is attributed to some phytochemicals, such as AsA, phenolics, anthocyanins, and other flavonoid compounds (Wang & Li, 2000). Tulipani et al. (2008) reported that 55-70% of TAA in strawberry fruits is related to ASA and anthocyanins. Cao et al. (2009) observed a positive relationship between TPC and TAA. Furthermore, Wang & Li (2000), observed a linear correlation between antioxidant activity and TPC for fruits and leaves, as well as with TAC for ripe berries (blackberry, raspberry, and strawberry). Therefore, changes in the TAA of strawberry fruits at harvest and during cold storage could be due to the changes in these compounds. In the present study, a higher TAA content was recorded in MJ-treated fruits. Similarly, Wang et al. (2019) reported that postharvest application of MJ

significantly increased the antioxidant capacity of blueberry fruits by increasing the enzymatic and non-enzymatic antioxidants. Furthermore, García-Pastor et al. (2019) reported that TAA [hydrophilic (H-TAA) and lipophilic (L-TAA) fractions] was higher in arils of pomegranate fruits treated with pre-harvest MJ than in the controls at harvest and during 60 days of cold storage.

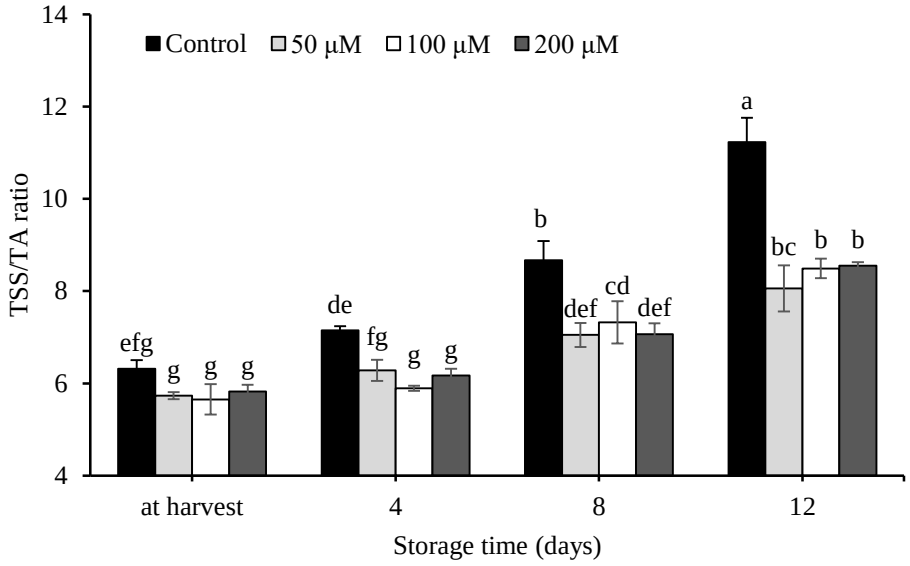

Fig. 2. Effect of methyl jasmonate (MJ) pre-harvest treatment on TSS/TA ratio of strawberry fruit cv. Paros during 12 days of storage. Mean ± SE (n=3), followed by different letters are significantly different (P<0.05).

Table 3. Effect of methyl jasmonate (MJ) pre-harvest treatment on ascorbic acid (AsA), total antioxidant activity (TAA), and antioxidant enzyme of strawberry fruit cv. Paros during 12 days of storage.

Storage time (day)	MJ (µM)	AsA (mg 100 g^{-1}FW)	TAA (%DPPHsc)	Catalase (U min^{-1} g^{-1} FW)	APX (U min^{-1} g^{-1} FW)
0 (at harvest)	0	68.56±2.31 abc	72.56±2.31 ghi	12.64±0.52 ghi	39.23±1.14 e
	50	69.27±1.44 ab	74.83±1.78 efg	12.31±0.31 hi	41.52±0.45 cde
	100	71.78±1.72 a	72.59±1.02 ghi	13.60±0.28 ef	42.91±0.37 c
	200	69.71±0.97 a	77.67±1.29 cdef	13.50±0.38 efg	42.60±1.21 cd
4	0	62.17±1.22 efg	74.08±1.29 efgh	14.25±0.36 de	42.64±0.36 cd
	50	65.37±0.59 cde	75.38±0.45 defg	15.20±0.21 abc	50.27±1.02 b
	100	63.84±0.91 def	85.63±1.28 ab	15.85±0.10 a	55.35±1.44 a
	200	65.83±1.34 bcd	79.89±2.56 cd	15.52±0.25 ab	52.27±0.71 b
8	0	53.09±1.16 h	75.65±1.72 defg	12.90±0.39 fgh	32.81±1.24 fg
	50	60.48±0.76 fg	79.03±2.74 cde	14.25±0.38 de	40.75±0.76 cde
	100	59.09±1.08 f	86.36±1.37 a	13.86±0.25 de	39.77±0.81 de
	200	61.29±1.72 fg	81.14±0.99 bc	14.62±0.33 cd	43.13±1.79 c
12	0	42.63±0.73 i	60.04±1.48 j	11.81±0.26 i	23.42±0.98 i
	50	51.81±1.10 g	68.90±0.95 i	13.44±0.21 efg	31.27±1.05 gh
	100	54.11±1.19 g	73.33±2.40 fgh	14.65±0.23 bcd	35.05±1.29 f
	200	53.46±0.30 g	69.73±2.39 hi	13.80±0.14 de	29.15±0.79 h

Means±SE (n = 3) within each column covered by the same letter are not significantly different (*P<0.05*) APX, ascorbate peroxidase.

Antioxidant enzymes activity

The results indicated that activities of catalase (CAT) and ascorbate peroxidase (APX) enzymes in strawberry fruit were influenced by the storage time, MJ treatment, and their interaction, while guaiacol peroxidase (GPX) activity was only affected by storage time (Table 1). Pre-harvest applications of MJ to strawberry plants increased the antioxidant enzyme activities of CAT and APX compared to the control at harvest, conversely, the activity of GPX enzyme in treated fruits was lower than control. During the cold storage, CAT and APX enzyme activities in control and MJ-treated strawberry fruits initially increased (up to day 4) and then decreased until the end of storage with a slower decrease in treated fruits. At the end of storage, the highest CAT (14.65 U min^{-1} g^{-1} FW) and APX (35.05 U min^{-1} g^{-1} FW) activities were observed in MJ-treated fruit with 100 µM, whereas the lowest CAT (11.81 U min^{-1} g^{-1} FW) and APX (23.42 U min^{-1} g^{-1} FW) activities were found in the control sample (Table 3). GPX enzyme activity was significantly reduced with increasing MJ concentration in strawberry fruits. The minimum GPX activity (12.36 U min^{-1} g^{-1} FW) of strawberry fruit was observed in the 200 µM MJ treatment and the maximum GPX activity (15.83 U min^{-1} g^{-1} FW) was recorded in the control sample (Table 2).

The senescence process in stored fruits is accelerated by reactive oxygen species (ROS) production, which leads to oxidative damage and a decline in fruit quality and bioactive compounds. SOD, APX, and CAT are crucial enzymatic antioxidants that play a key role in the antioxidant system, mainly by scavenging ROS and protecting cells from oxidative damage. SOD converts superoxide radicals into hydrogen peroxide, which is then converted into water by CAT and APX (Wang et al., 2019). Shah et al. (2025) reported that pre-harvest MJ treatment in raspberry fruits suppressed H_2O_2 production during cold storage, potentially due to increased activity of SOD and CAT enzymes. In our study, MJ increased the activity of CAT and APX as antioxidant and defense enzymes, which was associated with better fruit quality attributes compared to the control group. In alignment with our results, Yang et al. (2025) reported that CAT activity in kiwifruit treated with pre- or postharvest MJ was significantly higher compared to the control, while GPX activity was significantly higher in the control sample. Liao et al. (2022) also observed that the activity of APX, SOD, CAT, and POD enzymes increased during cold storage in control and MJ-treated lemon fruits, and this increase was significantly higher in the fruit treated with both pre- and postharvest MJ compared to the control fruits. Furthermore, Li et al. (2018) reported that MJ induced higher gene expression of ROS scavenging enzymes in fresh-cut pitaya fruit, leading to higher enzymatic activity in the treated fruits.

CONCLUSION

Pre-harvest application of MJ treatment showed a beneficial effect on the quality parameters of strawberry fruits. The results of the present study indicate the pre-harvest application of MJ reduces the loss of TA, ASA, firmness, and PLW at cold storage. All pre-harvest MJ concentrations displayed higher TAC, TPC, and TAA content than the control fruit at the end of cold storage. In addition, treated fruits had higher CAT and APX activities and lower GPX compared to control samples. The TSS content of strawberry fruit was not affected by pre-harvest MJ treatment at harvest time and during cold storage. The antioxidant activity of MJ-treated fruits was improved by increasing the activity of antioxidant enzymes and preserving bioactive compounds during cold storage. In conclusion, pre-harvest MJ treatment, especially at 200 µM, can be used to maintain the quality and antioxidant properties of strawberry fruits at harvest and during cold storage.

Conflict of interest

The authors declare that there is no conflict of interest.

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